HY-Sci 2022

Hyderabad's Student Centric Conference

14th May, 2022

ABSTRACT BOOK

TWENTY FIVE YEARS OF EXCELLENCE IN SERVICE AND RESEARCH

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS · CDFD ·

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CDFD, Hyderabad

Hy-Sci 2022 Schedule

Time	Session
08:30 - 09:30	Registration
09:30 - 09:40	Dr. K Thangaraj (Director, CDFD) inaugural speech
09:40 - 09:45	Dr. Murali D Bashyam (Dean, CDFD) speech
09:45 - 10:00	P Sai Uday (CCMB) Easing diagnosis and exploiting the potential of sewage-based surveillance for controlling SARS-CoV-2
10:00 - 10:15	Biswajit Samal (CDFD) Understanding the *Xanthomonas*, quorum sensing and pathogenicity
10:15 - 10:30	Flash Talks
10:30 - 10:45	Swatilekha Hazra (LVPEI) Silk fibroin for engineering human corneal endotheliumothelium
10:45 - 11:00	Pradeep Kumar (CCMB) Archaeal DTD resolves molecular conflict in organellar translation machinery of triple genetic origin in plants
11:00 - 11:20	Tea
11:20 - 11:35	Basil T (TIFR-H) Spatial heterogeneity of metabolism associated with the stages of epithelial contact inhibition of proliferation
11:35 - 11:50	Rishi Kumar (NIAB) Enhanced proinflammatory immune responses associated with resistance of native breeds of cattle to Tuberculosis
11:50 - 12:05	Sara Anisa George (CDFD) Identification of novel oncogenic targets of mutant p53 in esophageal squamous cell carcinoma
12:05 - 12:20	Flash Talks
12:20 - 12:35	Trupti Agrawal (LVPEI) RB1 null iPSC derived retinal precursors form atypical organoids with maturation and lamination defects
12:35 - 12:50	Sayantan Goswami (CDFD) Role for DNA exonuclease V resection activity of RecBCD in control of aberrant chromosomal DNA replication in *Escherichia coli*
12:50 - 13:05	Puneeta Singh (NIN) Iron and zinc homeostasis and interactions: Does enteric zinc excretion cross-talk with intestinal iron absorption?
13:05 - 16:00	Lunch and Poster
16:00 - 16:15	Binita Roy Nandi (NIAB) A cell permeable anti-inflammatory peptide for sepsis from the effector protein of the bacterial pathogen, *Brucella*
16:15 - 16:30	Hilal Ahmad Reshi (CDFD) Role of EYA proteins in cargo trafficking
16:30 - 17:10	Special talk: Dr. Sarah Iqbal Science Communication and Outreach
17:10 - 17:30	Tea
17:30 - 18:30	Panel discussion: The power and perils of research ethics
18:30 - 19:00	Prize distribution and Vote of thanks
19:00 - 20:00	Dinner

ABSTRACTS FOR ORAL PRESENTATION

ORAL_01

Easing Diagnosis and Exploiting the Potential of Sewage-based Surveillance for Controlling SARS-CoV-2

Uday Kiran[1,3], C. G. Gokulan[1], Santosh Kumar Kuncha[5], Manupati Hemalatha[2,3] Harishankar Kopperi[2,3], Athmakuri Tharak[2], S Venkata Mohan[2,3], Rakesh K Mishra[1,3,4].

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The unprecedented emergence of the SARS-CoV-2 virus as a pandemic combined with its highly infectious nature have distressed the world. The pandemic has forced all the countries to carry out large-scale testing of individuals which is very laborious and a huge economic-burden for many countries. To immediately address these issues, we developed the Dry-swab method. This method eliminates the need for VTM and RNA isolation thus making it time- and cost-efficient. While individual-centric screening is of high importance, community-centric screening could provide a better understanding of the epidemiology of the virus spread and can thereby help us to vigilantly put the control measures in place. To this end, we employed Wastewater-Based Epidemiology (WBE) as a tool for community surveillance of SARS-CoV-2. WBE holds functional advantage as an early warning system to advance the disease management measures. By isolating and quantifying SARS-CoV-2-specific RNA from the sewage collected from the sewage treatment plants (STP) we were able to effectively calculate the number of infected individuals in an area. Further, we could correlate the WBE-based estimates with clinical testing results and thereby established a concreate methodology to track the spread of infections through sewage. Overall, we present the development of methodologies for SARS-CoV-2 surveillance at both individual and community levels, which are suitable for developing and developed economies alike.

ORAL_02

Quorum sensing coordinates the *Xanthomonas* social communication and pathogenicity within host tissue

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Many microbes exhibit quorum sensing (QS) to cooperate, share and perform a social task in unison. Recent studies have shown the emergence of reversible phenotypic heterogeneity in the QS-responding pathogenic microbial population *in vitro* as a possible bet-hedging survival strategy. However, very little is known about the dynamics of QS-response and the nature of phenotypic heterogeneity in an actual host-pathogen interaction environment. Here, we investigated the dynamics of QS-response of a Gram-negative phytopathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*) inside its natural host cabbage, that communicate through a fatty acid signal molecule called DSF (diffusible signal factor) for coordination of several social traits including virulence functions. In this study, we engineered a novel DSF responsive whole-cell QS dual-bioreporter to measure the DSF mediated QS-response in *Xcc* at the single cell level inside its natural host plant *in vivo*. Employing the dual-bioreporter strain of *Xcc*, we show that QS non-responsive cells coexist with responsive cells in microcolonies at the early stage of the disease; whereas in the late stages, the QS-response is more homogeneous as the QS non-responders exhibit reduced fitness and are out competed by the wild-type. Furthermore, using the wild-type *Xcc* and its QS mutants in single and mixed infection studies, we show that QS mutants get benefit to some extend at the early stage of disease and contribute to localized colonization. However, the QS-responding cells contribute to spread along xylem vessel, and eventually within host mesophyll tissue to cause systemic infection. These results contrast with the earlier studies describing that expected cross-induction and cooperative sharing at high cell density *in vivo* may lead to synchronize QS-response. Our findings suggest that the transition from heterogeneity to homogeneity in QS-response within a bacterial population contributes to its overall virulence efficiency to cause disease in the host plant under natural environment.

ORAL_03

SILK FIBROIN MEMBRANES FOR ENGINEERING HUMAN CORNEAL ENDOTHELIUM

Swatilekha Hazra[1,2], Prerak Gupta[3], Biman B Mandal[3], Charanya Ramachandran[1]

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Corneal blindness due to endothelial dysfunction accounts for half the corneal transplantations performed each year. Engineering a monolayer of corneal endothelium (CE) for transplantation requires development of a suitable scaffold that is comparable to Descemet's membrane in transparency, mechanical strength, attachment, growth and function of cells and extracellular-matrix. This study aimed at determining the use of silk fibroin membrane as biomaterial for CE.

Silk films derived from fibroin protein of *Philosamia ricini*(PR), *Antheraea assamensis*(AA) and *Bombyx mori*(BM) were prepared. The mechanical strength, transparency and degradation properties of the scaffold were characterised. Adhesion and proliferation of CE on silk films was quantified using MTT assay and Ki67 marker. Expression of CE markers (collagen-VIII, ZO-1, N-Cadherin, Na/K-ATPase, SLC4A11 etc.) was determined at gene and protein levels using PCR and immunostaining, respectively. Barrier function of the cultured cells was measured as permeability to FITC Dextran (10kDa) in the presence or absence of thrombin. Further assessment of integrin expression (α2,α3,α5,αv,β1,β3,β6) was performed to understand the attachment of cells to the scaffold, compared to native-tissue. The ECM proteins deposited by CE cells (Collagen-I, Collagen-VIII, Laminin and fibronectin) were also determined using immunostaining and western blot. The function of cells was further assessed by performing ex-vivo experiment.

AA exhibited maximum tensile strength followed by PR and BM ($p<0.01$). BM degraded significantly (65% loss) faster than PR and AA (40% loss). MTT assay showed the cell adhesion in following order- fibronectin-collagen1 coated dish>AA≥PR>BM. CE cells formed a monolayer expressing all the specific markers and established tight barrier integrity. Integrins and ECM proteins expressed by cells on AA and PR were comparable to the native-tissue. Cells were viable and functional after 10 days of perfusion.

Our data suggests that silk fibroin could closely mimic native tissue and might offer a suitable alternative for engineering CE for transplantation.

ORAL_04

Archaeal DTD resolves molecular conflict in organellar translation machinery of triple genetic origin in plants

Pradeep Kumar[1,2], Ankit Roy[1], Jagadeesha S. Mukul[1,2], Sambhavi Pottabathini[1], Shobha P. Kruparani[1], Rajan Sankaranarayanan[1,2]

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The emergence of eukaryotes is a successful amalgamation of Bacteria and Archaea. Due to the selection pressure operated by this unification, mitochondrial $tRNA^{Gly}$ of Opisthokonta switched its discriminator base from pyrimidine to purine. Here we show that plants still kept the bacterial discriminator code of $tRNA^{Gly}$ (pyrimidine at N73) in both the mitochondria and chloroplast. This discriminator base is invariant since the divergence of plants and opisthokonts. Surprisingly, DTD1 of plants have higher activity on Gly-$tRNA^{Gly}$ with pyrimidine as a discriminator base, resulting in a lethal DTD1-discriminator base conflict. Among life forms, plants are unique in encoding an additional archaeal-derived translation surveillance enzyme- DTD2. Remarkably, unlike canonical DTD1, DTD2 does not act on achiral Gly-$tRNA^{Gly}$ hence operates by D-chiral selection. To combat the organonuclear conflict, plants must have strategically quarantined the DTD1 in cytosol and localized archaeal-derived D-chiral-specific DTD2 to both cytosol and organelles. Our results indicate that targeting of DTD1 to the organelles will deplete the Gly-$tRNA^{Gly}$ pool leading to translation arrest and hence lethality in plants. Overall, the study highlights the molecular adaptations during eukaryotic evolution where D-chiral-specific archaeal DTD2 solved the DTD1-discriminator base conflict that enabled the organellar evolution in plants.

ORAL_05

Spatial heterogeneity of metabolism associated with the stages of epithelial contact inhibition of proliferation

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In the epithelium, cell density and proliferation are closely connected to each other through contact inhibition of proliferation (CIP). Starting from a low-density state, as the cell density increases, CIP proceeds through three distinct stages, namely the free growing stage, pre-epithelial transition stage, and post-epithelial transition stage. It remains unknown whether cellular metabolism also has a density-dependent behavior. By measuring the mitochondrial membrane potential at different cell densities, we reveal a heterogeneous landscape of metabolism in the epithelium, whose length-scale depends on cell density and appears distinct in three distinct stages of CIP. Starting from a nearly random distribution of metabolic potential in the free-growing stage, epithelial cells establish a collective metabolic heterogeneity in the pre-epithelial transition stage, where the multicellular clusters of high and low potential cells emerge. However, in the post-transition stage, metabolic potential field becomes relatively homogeneous. Subsequently, to understand how metabolic heterogeneity emerges as a function of cell proliferation, we construct a system biological model of the proliferating epithelium. Finally, we show that the collective heterogeneity in metabolic state can be developed even only in presence of active contractile cell mechanics. Taken together, our results reveal a density-dependent collective heterogeneity in the metabolic field of an epithelial monolayer, which should have critical consequences on the spatiotemporal evolution of epithelial form and function.

ORAL_06

Enhanced proinflammatory immune responses associated with resistance of native breeds of cattle to Tuberculosis.

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India is the highest milk producing country in the world, in addition to being home to the largest bovine population globally. On the other hand, Tuberculosis (TB) in the bovines (known as bovine TB, bTB) in India remained a persistent threat not only affecting the agrarian farm-based economy by causing ~10% loss in milk production, but also significantly impacting the public heath scenario via acting as a major source of zoonotic TB in human. The bovine population in India constitutes a range of domestic, and crossbred varieties with a wide degree of genetic variability in terms of milk production as well as tolerance to disease, and stress conditions. The incidence of bovine TB (bTB) in India was found to be higher in the crossbred cattle compared to native indigenous breeds, however, the underlying cause is unknown. Here, we evaluated the comparative susceptibility, and immune responses to mycobacterial infection in the prominent indigenous Sahiwal dairy cattle, and crossbred- Sahiwal x Holstein Friesian cattle. We observed a significantly higher intracellular mycobacterial growth in the bovine peripheral blood mononuclear cells (PBMC) in the case of PPD negative, and TB-PCR negative crossbred cattle compared to that in the native animals. Further, comparatively higher pro-inflammatory interferon-gamma (IFN-γ), and interleukin-17 (IL-17) responses were induced by native cattle PBMCs upon exposure to mycobacterial infection, and antigenic stimulation. Overall, our findings highlight that the differential susceptibility of the native, and crossbred cattle to mycobacterial infection may stem from the differences in the innate immune cytokine responses of these breeds. This study not only elucidate a potential association of the proinflammatory cytokine responses with TB susceptibility in the bovine breeds, but also emphasises the need of appropriate crossbreeding policy to balance productivity, and diseases resistance in cattle.

ORAL_07

Identification of novel oncogenic targets of mutant p53 in esophageal squamous cell carcinoma

Sara A George[1,2], Viswakalyan Kotapalli[1], Sanjana Sarkar[1,3], Pandilla Ramaswamy[1,4], Raju Kumar[1,5], Raju SR Adduri[1,6], Murali D Bashyam[1]

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Background: Missense mutations localized to the DNA binding domain of p53 is a frequent event in Esophageal Squamous Cell Carcinoma (ESCC). In addition, patients with tumours harbouring *TP53* mutations have poorer chances of survival than those with wild-type p53 tumours. Most studies however focus on understanding the potentially oncogenic transcriptional networks regulated by frequently occurring 'hotspot' p53 mutant proteins while rarer population-specific mutants are inadequately characterized.

Methods: Gene-expression-microarray analysis was performed on ESCC tumours selected based on their p53 status to identify novel mutant p53 targets. Tumorigenic assays were performed following ectopic-expression of wild-type and mutant p53 proteins. Target-gene activation was evaluated by RT-qPCR. Chromatin affinity-purification and promoter-luciferase assays assessed the recruitment of p53 mutants to their target promoters and their ability to activate gene expression. Phenotypic assays assessed the effect of target gene knockdown on the oncogenicity of cancer cells.

Results: Twenty-seven differentially expressed genes were identified, of which ten exhibited up-regulated transcript levels in p53 mutant tumours. *ARF6*, *C1QBP* and *TRIM23* were selected for further analysis due to their previously reported association with cancer. RT-qPCR performed on the arrayed samples revealed significant correlation of *TP53* transcript levels with the transcript levels of the selected mutant p53 targets. The ectopically-expressed p53 mutants – P190T and P278L, that induced the increased expression of the targets, were localized to and activated the promoters of the target genes. Sh-RNA based knockdown of the targets resulted in a significant suppression of the tumorigenicity of cancer cells.

Conclusions: Three novel oncogenic targets of 'rare' mutant p53 proteins in ESCC were identified. The functional heterogeneity in the functioning of different p53 mutants was revealed.

ORAL_08

RB1 null iPSC-derived retinal precursors form atypical organoids with maturation and lamination defects

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Purpose: *RB1* is a tumor suppressor protein that regulates cell cycle and its loss of function results in retinoblastoma. This study aims to generate *RB1* null human induced pluripotent stem cells (hiPSCs) to understand the effects of loss-of-function on retinal development and maturation *in vitro.*

Methods: Adipose-derived mesenchymal cells from familial retinoblastoma patient was reprogrammed into iPSCs, using episomal constructs of Yamanaka factors ($RB1^{+/-}$ hiPSCs). Isogenic mutant lines were also generated by CRISPR-Cas9 gene editing of human *RB1* ($RB1^{-/-}$ hiPSCs). These iPSC lines were characterized by RT-PCR, immunofluorescence, western blotting and karyotyping. Healthy control and mutant iPSCs were differentiated into retinal lineage using established protocols.

Results: The patient specific heterozygous $RB1^{+/-}$ and CRISPR-edited homozygous $RB1^{-/-}$ iPSC mutant lines maintained their stemness, pluripotency, genomic integrity and formed embryoid bodies comprising of all three germ layers. Upon differentiation into retinal lineages, the $RB1^{+/-}$ hiPSCs formed normal eye-fields in 2D cultures at 4 weeks [30-40 eye-field primordial clusters (EFPs) per million cells; n=4], comparable to that of the healthy control hiPSC line. However, the differentiation of $RB1^{-/-}$ hiPSCs resulted in about 25 folds reduction in eye-field numbers per million cells. The eye-field cells of all mutant lines expressed the early neuro-retinal precursor markers and also formed retinal pigmented epithelial patches, suggesting normal retinal lineage commitment. When EFPs were excised and grown in suspension cultures, the retinal progenitors underwent self-organization and formed well laminated neuro-retinal cups in control and $RB1^{+/-}$ hiPSCs. However, the rare EFPs in $RB1^{-/-}$ hiPSCs formed only atypical retinal clusters with severe lamination and maturation defects.

Conclusion: The iPSC models confirm that loss of RB1 does not affect their stemness, pluripotency or retinal lineage commitment. However, one copy of RB1 is necessary for proper differentiation, post-mitotic maturation and lamination of neuro-retinal precursors.

ORAL_09

Role for DNA exonuclease V resection activity of RecBCD in control of aberrant chromosomal DNA replication in *Escherichia coli*

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Replication of the circular chromosome in most bacteria is initiated from a single locus *oriC* with the aid of an unstable essential protein DnaA. One approach to identify factors that act to prevent aberrant replication initiation has been that to obtain mutants which survive in absence of DnaA. Previous studies in *Escherichia coli* have demonstrated that at least four single strand (ss)-DNA exonucleases Exo I, Exo VII, SbcCD, and RecJ act redundantly to control aberrant initiation of chromosomal replication. Here we show that a Δ*recD* mutation (which eliminates double strand-DNA exonuclease V activity of RecBCD) can rescue Δ*dnaA* lethality in two alternative situations: (i) in absence of RecJ (but not of any other ss-DNA exonuclease), or (ii) when two or more two-ended DNA double strand breaks (DSBs) are generated either by ectopic expression of a site-specific endonuclease I-SceI, or by radiomimetic agents such as phleomycin or bleomycin. With two-ended DSBs in the *recD* strain, Δ*dnaA* viability was retained even after linearization of the chromosome. Data from genome-wide DNA copy number determinations in populations of Δ*dnaA recD* cells that had suffered two or more site-specific two-ended DSBs have lent support to a model that exonuclease V activity is required to prevent rolling-circle over-replication when convergent replication forks merge and fuse; such events would occur during normal replication at the chromosomal terminus region, as well as during repair of two-ended DSBs following "ends-in" replication. Further we show that the ribosomal RNA operons constitute significant barriers to progression of aberrant *oriC*-independent replication in *E. coli*.

ORAL_10

Iron and Zinc Homeostasis and Interactions: Does Enteric Zinc Excretion Cross-Talk with Intestinal Iron Absorption?

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Iron and zinc deficiencies likely coexist in general populations. Phytic acid-rich in plant foods inhibits the absorption of both iron and zinc. Therefore, populations consuming a phytate-rich diet with high prevalence of anaemia are also expected to be at risk of zinc deficiency. Supplementation of both iron and zinc together is a simple therapeutic alternative, however their combined supplementation in humans showed negative interactions, but the magnitude and direction of these interactions are conflicting. In particular, the interactions are evident only when iron and zinc are supplemented in solution but not in food. Though competitive interaction at a transport protein level during intestinal absorption is hypothesized, the underlying mechanisms remained elusive). Studies in animal models indicated accumulation of iron, reduction in haemoglobin during zinc deficiency. On the other hand, cross sectional studies found higher prevalence of anaemia and iron deficiency in subjects with zinc deficiency. A positive association of serum zinc levels with haemoglobin and markers of iron status was also observed in humans. Interestingly, alternate supplementation of iron and zinc significantly improved the Hb and iron status compared to iron alone supplementation. Zinc supplementation has been shown to induce erythropoiesis in rats, while zinc deficiency reduced the erythroid cell mass in mice. These observations together suggest that zinc interacts at multiple sites of iron metabolism including intestinal absorption and its utilization via regulating the DMT1 and FPN1 levels. Therefore, it is interesting to understand the homeostasis of zinc and iron at the level of intestinal absorption and tissue mobilization.

ORAL_11

A cell permeable anti-inflammatory peptide for sepsis from the effector protein of the bacterial pathogen, *Brucella*.

Binita Roy Nandi, Dr. Girish K. Radhakrishnan

NIAB

Sepsis is a serious medical condition caused by aberrant activation of Toll-like receptors (TLRs) resulting in wide-spread inflammation that leads to organ failure, septic shock and death. Inappropriate activation of TLR4 and TLR2 by microbial components leads to secretion of pro-inflammatory cytokines and whole-body inflammation. Therefore, inhibition of these TLRs could re-establish immune homeostasis and dampen inappropriate inflammatory responses. Therefore, drugs targeting TLR4 and TLR2 are promising therapeutic agents to treat sepsis. However, there are no specific therapeutic agents that are currently approved for sepsis treatment. The intracellular bacterial pathogen, *Brucella*, encodes a cell permeable anti-inflammatory protein, TcpB, that efficiently attenuates TLR4 and 2-mediated production of pro-inflammatory cytokines. TcpB has been reported to promote enhanced ubiquitination and degradation of the TLR2/4-adaptor protein TIRAP to block the signalling cascade from these receptors. Therefore, TcpB could serve as an ideal candidate for developing specific therapeutic agents for sepsis. Towards this objective, we identified the peptides from TcpB that confer cell permeability and anti-inflammatory properties. Subsequently, we generated a chimeric peptide (TB4-BBL2) of 29 amino acids by fusing the cell permeable and anti-inflammatory peptides. The TB4-BBL2 peptide showed excellent cell permeability, which was dependent on cholesterol-rich lipid rafts on the plasma membrane. The TB4-BBL2 peptide did not induce any cytotoxicity and it suppressed TLR4-mediated secretion of pro-inflammatory cytokines by macrophages. *In vivo* studies indicated that the TB4-BBL2 peptide could efficiently attenuate production of pro-inflammatory cytokines in mice that were treated with the TLR4 ligand, lipopolysaccharide. Our studies indicate that TB4-BBL2 could serve as an ideal therapeutic agent to suppress the production of pro-inflammatory cytokines in sepsis.

ORAL_12

Role of EYA Phosphatases in cargo trafficking

Hilal Ahmad Reshi and Dr. Maddika Subba Reddy
CDFD

The ever-changing cellular environment demands a quick and efficient response for which a cell relies mainly on the recycling of proteins rather than their de-novo synthesis. A well-known mechanism employed by the cells for recycling is called retrograde trafficking in which a protein moves from the plasma membrane back to the centre of the cell. Early endosomes act as sorting stations from where proteins can be trafficked back to the plasma membrane, sent for lysosomal degradation or moved to the trans-Golgi apparatus for recycling purposes. The movement to the trans-Golgi is mediated by a protein complex called retromer that tethers to the early endosome, complexes with the cargo and hands it over to the destination Golgi membrane. Retromer thus acts as a conveyer belt where it receives the cargo and delivers it to the Golgi. The accepting arm of this belt, however, is not well characterized. We have identified a protein complex, other than the retromer, that links the endosomes/retromer with Golgi. It comprises four members of the EYA phosphatase family (EYA1-4). Any disruption in the formation of this complex severely affects the recycling of a specific cargo called *wntless.* Patients with mutations in EYA1-4 genes suffer from Sensineuronal hearing loss. We believe this can be ascribed to the formation of a dysfunctional EYA complex. The trafficking pattern, molecular mechanism and clinical significance will be discussed.

ABSTRACTS FOR POSTER PRESENTATION

Poster_01

Biochemical characterization of putative lipase from pathogenic *Leptospira*.

Aayushi Kale

Leptospirosis is a zoonosis caused by the pathogenic spirochaete bacteria of the genus Leptospira, affecting humans and animals worldwide by causing more than 1 million infections with 60,000 deaths per year. Humans may be infected by direct contact with urine or reproductive fluids from infected animals, through contact with urine-contaminated water or soil, or by consuming contaminated food or water. Commensal and pathogenic bacteria hydrolyze host lipid substrates with secreted lipases and phospholipases for nutrient acquisition, colonization, and infection. Bacterial lipase activity on mammalian lipids and phospholipids can promote release of free fatty acids from lipid stores, detoxify antimicrobial lipids, and facilitate membrane dissolution. In our study, we have cloned, expressed and purified one of the predicted outer membrane putative lipases from pathogenic Leptospira. The in-silico study shows the conserved lipase motif and catalytic residues which helped us to biochemically characterize this protein. Ester hydrolase activity has been determined by using the substrate para-nitrophenyl (pNp) palmitate. Altogether, these studies will provide insight into an adaptive trait that helps in pathogenesis.

Poster_02

Optimization and Characterization of human limbus-derived mesenchymal stem cell in Xeno-Free medium.

Abhishek Sahoo, Jilu Jaffet, Mukesh Damala, Sayan Basu, Vivek Singh

Center for Ocular Regeneration, Prof. Brien Holden Eye Research Center, LV Prasad Eye Institute, Hyderabad, Telangana

Purpose: This study aims to optimize and characterize method of expanding hLMSCs *in vitro* in xeno-free medium and compare with standard serum based medium (as control).

Methods: Limbal tissues were excised from the therapeutic grade corneoscleral rims and the primary cells were expanded till tertiary culture. The cells were characterized for the expression of MSC specific markers using FACS, immunofluorescence as recommended by International Society for Cellular Therapy (ISCT). hLMSCs cultured were also checked for tri-lineage differentiation, cell viability and population doubling time. All the assays were performed in biological triplicates.

Results: The xeno-free medium supported growth of primary hLMSCs, retaining an average doubling time of 23 hours. Cells retained their morphological and phenotypic characteristics as evident by microscopic images. hLMSCs cultured in STEM MACS XF had comparatively lesser PDT as that of serum based medium (**p=0.0042; p<0.005). FACS analysis showed that ≥90% hLMSCs were positive for *CD90*$^+$, *CD73*$^+$, *CD105*$^+$, and ≤5% were positive for *CD45*$^-$, *CD34*$^-$ *and HLA-DR*$^-$. Immunofluorescence analysis showed higher expression of Limbal Stem Cell specific markers (*CD90*$^+$, *CD73*$^+$, *CD105*$^+$, *ABCG2*$^+$, *PAX6*$^+$, *ABCB5*$^+$ *and P63α*$^+$*)* markers and no expression of negative markers (*CD45*$^-$, *CD34*$^-$ *and HLA-DR*$^-$*)*, as compared to cells in serum containing medium. hLMSCs in xeno-free medium differentiated into Osteocytes, Chondrocytes and Adipocytes which was evident by Alizarin Red, Alcian Blue and Oil Red O stain respectively. The quantification was done by eluting the stain and normalizing with the control.

Conclusions: The findings of our study suggest that the hLMSCs can successfully be cultivated in serum deprived media to prevent xenogeneic contamination, enabling them to be utilized in a clinical trial in their full potential, overcoming the regulatory issues.

Poster_03

MOLECULAR MECHANISMS INVOLVED IN THE DNA DAMAGE RESPONSE IN XERODERMA PIGMENTOSA PATIENTS

Akepogu Jacquelyn[a], Sunita Chaurasia[b], Charanya Ramachandran[a]

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b. The Cornea Institute, L V Prasad Eye Institute, L V Prasad Eye Institute, Hyderabad, India

Purpose: To study the mechanism involved in repair of ultra-violet (UV) induced DNA damage in the corneal tissues of Xeroderma pigmentosa (XP) patients.

Methods: XP patient corneas were procured from the pathology department at the L. V. Prasad Eye Institute (n=5) after obtaining IRB approval. Healthy corneas (n=3) were obtained from Ramayamma International Eye Bank and served as controls. Immunohistochemistry was performed on sections from these samples using specific antibodies against proteins involved in DNA repair including XPA (Xeroderma pigmentosa group A), phospho-ataxia telangiectasia mutated (ATM), -ataxia telangiectasia and Rad3-related protein (ATR), -breast cancer 1 (BRCA1), -checkpoint kinase 1&2 (Chk1&2), -Histone H2A.X, -p53, anti-thymine dimer antibody [H3] and anti-rabbit/mouse secondary antibodies. Confocal microscope was used for imaging the sections.

Results: Control corneas showed positive expression of thymine dimer and H2AX only in the epithelial layers unlike XP samples wherein the keratocytes and endothelial cells also showed positive expression. Unlike control corneas, positive expression of specific markers of the DNA repair pathway were noted in all cell layers indicating accumulation of DNA damage that affected the deeper corneal layers. XPA (Nucleotide Excision Repair pathway protein) was seen to co-localise more with ATM than ATR suggesting a stronger involvement of the ATM pathway in XP patients. More cells expressed Chk1 as compared to Chk2 protein which suggests that the double stranded and single stranded breaks led to the phosphorylation of BRCA1 through activation of ATM/ATR which ultimately led to apoptosis of these cells as evidenced by the positive staining for p53.

Conclusion: The study shows conclusively the presence of DNA damage in all cell layers of the cornea in XP patient samples and the impaired repair mechanism likely result in their death.

Poster_04

Whole exome sequencing reveals novel nonsense variant in GLI3 gene for Greig cephalopolysyndactyly syndrome

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Greig cephalopolysyndactyly syndrome is characterized by a peculiar skull shape in the form of expanded cranial vaults leading to a high forehead and bregma with syndactyly in hands. In this study, we conducted whole-exome sequencing in the proband born out of incest. 6 months old female child presented with frontal bossing, pre and post-axial polydactyly with syndactyly, and polydactyly of toes with syndactyly. WES revealed the presence of a novel homozygous nonsense variant in the GLI3 gene. Variant Gln1292Ter present in transcription activator domain 1 (TA1) of GLI3 leads to truncation of the protein. Gln1292Ter affects TA1 and TA2 which might lead to reduced or abnormal processing. GLI3 is a transcription factor under Hedgehog signalling. Human GLI3 functional domains comprise N terminal transcriptional repressor and C terminal transcriptional activator, where the majority of pathogenic variants are present in ZN (Zinc finger repeats: DNA binding domain) and CBD binding domain. Deleterious variants in GLI3 show allelic heterogeneity, they have been associated with Greig cephalopolysyndactyly syndrome, Pallister-Hall syndrome, preaxial polydactyly type IV, and postaxial polydactyly type A1 and B. These disorders follow autosomal dominant inheritance pattern. Only one report of a homozygous variant in GLI3 in the ZN domain was reported earlier. This is the first report on the homozygous nonsense pathogenic variant in the C terminal transcriptional activator region leading to Greig cephalopolysyndactyly syndrome. Further functional analysis is required to establish the pathogenicity and pathophysiological mechanism of this variant.

Poster_05

A novel histone lysine demethylase of the KDM7 family regulates depression-like phenotype in mice with possible involvement in dentate gyrus neurogenesis

Annapoorna P K

Depression is a complex neuropsychiatric disorder with varied symptoms including low mood, inability to feel pleasure, and comorbid cognitive impairment. Chronic psychological stress is a major risk factor for depression as it affects several brain regions involved in the pathology of depression. One such brain region is the hippocampus. It is unique in that it has a niche called the dentate gyrus (DG) where adult neurogenesis occurs. The effects of chronic stress are oftentimes mediated by epigenetic mechanisms in the brain. Histone modifications and the enzymes that regulate them play a major role in this. Among these, Histone Lysine Demethylases of the KDM7 family are notable due to their involvement in neurodevelopment and neural differentiation. However, their exact role in mediating the effects of stress on the hippocampus, specifically on neurogenesis, which may contribute to depression-like phenotype and comorbid cognitive impairment, is not yet known.

To investigate this, we used chronic social defeat stress (CSDS) mouse model. We found that the expression levels of the KDM7 family genes were dysregulated in the hippocampus including the DG of the defeated C57bl/6 mice. A putative demethylase of this family, 49…Rik, that got picked in a previous high-throughput ChIP-ChIP study from the mouse brain, was also found to be dysregulated in the DG of defeated mice. On probing in vitro on cultured neural stem/progenitor cells derived from the DG, results indicate its involvement in neural stem/progenitor cell differentiation. Interestingly, upon its overexpression in the mouse DG using Adeno Associated Viral approach, the animals showed depression –like phenotype without CSDS. We, for the first time report the involvement of this novel demethylase in depression and have also identified a few of its tentative histone lysine methyl targets.

Poster_06

Examining the role of IP7-mediated pyrophosphorylation in nucleolar function

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The inositol pyrophosphate IP_7 is an energy rich water-soluble small signalling molecule, containing five monophosphate and one pyrophosphate moieties. IP_7 is synthesized from IP_6 by IP_6 kinases, and specific phospho-hydrolases convert IP_7 back to IP_6. IP_7 regulates protein function either by direct binding to proteins or by protein pyrophosphorylation, a unique post translational modification where the β-phosphate moiety of IP_7 is transferred to a pre-phosphorylated serine residue lying in an intrinsically disordered region (IDR) of the substrate protein. Analysis of the IP_7 interactome revealed an enrichment of several nucleolar proteins, including TCOF1, NOLC1, NCL, NPM1, DDX21, DKC1, NOP58 and UBF1. Using radiolabelled IP7, we observed that TCOF1, NOLC1, NCL and UBF1 undergo in vitro pyrophosphorylation. Our sequence analysis revealed that these nucleolar IP_7 interactors have potential pyrophosphorylation sites in their IDRs. As IDRs are known to drive liquid-liquid phase separation (LLPS) of proteins, we hypothesized that pyrophosphorylation of these nucleolar proteins by IP_7 may regulate their LLPS behaviour, thereby regulating nucleolar assembly and the maintenance of nucleolar architecture and function. While deciphering the role of pyrophosphorylation in the nucleolus, we found IP6K1, the enzyme that synthesizes IP_7, colocalizes with UBF1, a nucleolar fibrillar centre marker, in an rDNA transcription-independent manner. We observed that IP6K1 co-condenses with DDX21 and UBF, suggesting a role for DDX21 and UBF in the localisation of IP6K1 to the nucleolus. We found that loss of IP6K1 leads to an increase in nucleolar number, decrease in nucleolar volume and reduced rDNA transcription, indicating a role for IP6K1 or its product IP_7 in rDNA transcription and nucleolar assembly. We are currently investigating the effect of pyrophosphorylation on the LLPS behaviour of these nucleolar proteins in cells with varying level of IP7 and its contribution towards nucleolar assembly and function.

Poster_07

Gas electro fermentation syngas for fatty acids, ethanol synthesis in single chambered Microbial Electrochemical System: conductive material synergy with carboxydotrophs

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Single-chambered microbial electrosynthesis systems (MESs) with graphite as counter electrode and carbon cloth (CC-MES)/Fluidized granular activated carbon (FGAC-MES) as working electrodes were fabricated and evaluated for the gas electro-fermentation by active biocatalyst. Continuous syngas sparging near to the working electrode (WE) environment enables the growth of endo electrogenic bacteria by availing the inorganic carbon source. Applied potential (-0.5 V) on working electrodes facilitates to the reduction of the supplemented carbon source leading to the synthesis of fatty acids. Higher the acetic acid titter of 4.3 g/L in FGAC-MES whereas in CC-MES 3.8 g/L was achieved with actively grown electro-microbiome. Concentrations of ethanol were depicted in electrolyte of both MES systems revels the solventogenic metabolism of electro active culture in the system. FGAC acts as intermediate electron shuttler between the inert electrode and microbiota enhances the fermentation products in MES. Along with bioprocess parameters (VFA, Substrate and PH trends), the electrochemical properties of biocatalyst will be evaluated with electrochemical techniques like cyclic voltammetry (CV), Chrono amperometry (CA), electrochemical impedance spectroscopy (EIS), and electrode kinetics. Abundance developed biofilm and applied potential triggers the chemolithoautotrophic metabolic activity for efficient syngas conversions into the short-chain carboxylic acids.

Poster_08

Molecular cloning, Expression, Purification and Immunological characterization of surface protein OmpL1 of *Leptospira*

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Leptospirosis is a severe bacterial infection caused by pathogenic spp of genus *Leptospira* that affects both humans and animals. It is now known that this is only one of more than 200 pathogenic serovars found in 7 species and 23 serogroups around the world. *Leptospira* Outer membrane or surface proteins, play an important role in pathogenesis, and several proteins have been identified to date. These proteins can bind a diverse range of extracellular matrix components like fibronectin, collagen, laminin, and elastin, complement regulators such as like Factor H, C3a and C4a. Some of the surface proteins have been shown to be ligand of innate immune receptors like toll-like receptors, specifically TLR2 or TLR4, which activate innate immune cells, like macrophages, dendritic cells and neutrophils. Identification and characterization of outer membrane or surface exposed lipoproteins that are involved in immune modulation particularly innate immune activation is crucial for understanding *Leptospira p*athogenesis and devising strategies to combat this zoonosis. These proteins could also serve as potential subunit vaccine candidate. In order to identify and characterize surface proteins which are involved in immune activation, literature review was done and few surface exposed lipoproteins were identified like *Leptospira* immunoglobulin like proteins (LigA and LigB), LipL32, LipL21, LipL41 and ompL1 that are involved in bacterial pathogenesis and host response. Out of these proteins, ompL1 was selected to clone, express and purify in recombinant form and then test its innate immune activity. The gene coding for ompL1 was PCR amplified and cloned in pET28 vector. The pET28-ompL1 was transformed in *E.coli* BL21 cells and expressed and purified in soluble form through Ni-NTA column. The purity of recombinant ompL1 was checked by SDS-PAGE and concentration was estimated by Bradford assay. The innate immune activity of recombinant ompL1 was evaluated by its ability to induce production of pro-inflammatory cytokine IL-6 and TNFα in mouse macrophage cell line, RAW264.7. Our result shows that ompL1 was successfully cloned, expressed and purified in soluble form with expected molecular weight of 48KDa. The pure recombinant protein was able to activate mouse macrophages as indicated by production of IL-6 and TNFα in dose dependent manner. These results indicate that ompL1 is surface protein of *Leptospira* which can activate mouse macrophages and might be involved in innate immune modulation.

Poster_09

Tear Profiling for Early Dry Eye Disease Biomarker Identification using Proteomics Approach

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Purpose: Dry Eye Disease (DED) is a multifactorial condition of the tear film and ocular surface where its aetiology is not clearly understood. Therefore, this study aims to identify early dry eye disease biomarker for diagnosis and treatment by proteomics approach.

Method: The study was approved by Institutional Review Board of LVPEI (Ref-no LEC 09-19-327) with informed consent. Tear samples were collected from DED patients (n=12) along with age & gender matched controls (n=12) by Schirmer's method and analysed by Ultimate 3000 RSLCnano system coupled with an Orbitrap Eclipse followed by database search using SEQUEST and AMANDA. For Sequest search, the precursor and fragment mass tolerances were set at 10 ppm and 0.8 Da, respectively. All the statistical analysis performed using, MBQN and LIMMA libraries in R environment. Gene ontology studies was also performed further.

Results: The total tear proteins identified were ~1733 along with 25 and 244 proteins unique proteins were identified in control and patient resp. The % variable was more than 80%. T-test significance revealed in total 559 proteins out of which 247 proteins were upregulated which include compliment C3, Albumin, Apolipoprotein, Annexin A11 and A5, Hsp-1 ($p<0.0001$) and 241 proteins are down-regulated which are lactoferrin, Lipocalin, Prolactin, Hsp-70, Microphage capping Protein($P<0.001$). Gene Ontology studies showed involvement of innate immune system proteins which include Carboxypeptidase, Abl interactor 1, Poly(rC)-binding protein 2 ($P< 0.0003$) and neutrophil degranulation pathway proteins; Alpha-1-antitrypsin, Beta-mannosidase, Pro-cathepsin H, Cathepsin X, Cystatin-B ($P< 0.00005$).

Conclusion: The tear profiling revealed the differential expression of tear proteins in patients with DED which will be valuable for understanding of the molecular mechanisms of DED and identify an early disease biomarker for diagnosis and treatment.

Poster_10

Newcastle disease virus W protein – a death inducing accessory viral protein

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Newcastle disease virus (NDV), a highly infectious avian virus belongs to the family *Paramyxovridae*. Few strains of NDV are shown to have oncolytic properties and are explored for applications in the treatment of several cancers. There are reports of different structural genes of NDV contributing to apoptosis in NDV. Here we studied the role of the accessory viral protein, W, as a contributor to the oncolytic nature of NDV. The effect of NDV on cellular DNA was studied by infection of various cell lines with the NDV Komarov strain (NDV K strain), where a typical laddering of the cellular DNA (a feature of caspase-activated DNA damage during apoptosis) was observed upon infection. The DNA laddering observed upon NDV infection could be replicated by over expression of W protein in DF1, MCF-7 and A549 cell lines. Further, we observed various changes in the nuclear morphology such as nuclear condensation, fragmentation, blebbing and formation of apoptotic bodies starting from 24 hours post transfection with W plasmid. Our quantification studies revealed that around 30 – 40% of the transfected DF1 and A549 cells carried abnormal nuclei by 24 hours post transfection. The presence of active caspase 3 suggesting induction of apoptosis was observed upon overexpression of W protein. To further substantiate the activation of apoptosis, we observed the cleavage of caspase 3, 7 and Poly (ADP-ribose) polymerase (PARP-1), all the hallmarks of apoptotic cell death, upon transfection of W protein in 293T cells. A flow cytometry analysis of DF1 cells transfected with W plasmid showed ~25% increase in apoptotic cells in comparison to cells transfected with only backbone plasmid as a control. A similar, time point-based increase in apoptotic cells was observed upon infection of DF1 cells with NDV K strain. Further, apoptosis induction by W protein was confirmed by TUNEL assay in DF1 cells transfected with W plasmid. Our data suggests a strong co-relation between the over expression of W protein and induction of apoptosis.

Poster_11

Developing therapeutic strategies for targeting fungal protein SUMOylation

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Protein SUMOylation is a post-translational modification that plays critical regulatory roles in diverse cellular processes including gene transcription, cell cycle regulation, DNA replication and repair. The SUMO (Small Ubiquitin like Modifier) protein belongs to a group of ubiquitin-like modifiers, present in organisms ranging from yeast to humans, and is covalently attached to other proteins to modify their function. SUMOylation is a reversible process with deSUMOylating enzymes removing the SUMO from target proteins. In addition, polySUMOylation, wherein SUMO chains are added to the target proteins plays a significant role in protein homeostasis. These polySUMOylated proteins are further ubiquitinated by a group of ubiquitin-ligases and are targeted for proteosomal degradation. SUMO has been identified in pathogenic fungi, and studies suggest that it is important either for survival and/or pathogenicity. In *Candida glabrata*, we had earlier identified the SUMO conjugation pathway and demonstrated that perturbation of SUMOylation reduces the virulence of *C. glabrata*. Based on sequence homology, we have now identified the components of the SUMO conjugation pathway in multiple pathogenic fungi. Further, comparing with the human and plant SUMO conjugation enzymes, identify Ulp2, a conserved deSUMOylating enzyme as a potential target for intervention. We have characterized further the role of Ulp2 in protein homeostasis and have performed additional structural studies to design small molecule inhibitors of Ulp2.

Poster_12

Defects in cone photoreceptor development and progressive retinal dystrophy in *rd3* and *abca4b* knockout stable zebrafish models.

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Purpose: To knockout zebrafish *rd3 and abca4b* genes linked to retinal dystrophies and to study the effects of loss of function on retinal development and visual functions.
Methods: Zebrafish *rd3* and *abca4b* gene knockout models were generated by CRISPR-Cas9 based genome editing. The edits in the founder animals (F_0) was confirmed by T7 endonuclease assay followed by Sanger sequencing. Homozygous mutants were generated by interbreeding of heterozygotes. The wild type, *rd3*$^{-/-}$, *abca4b*$^{-/-}$ mutant fishes were euthanized at 3, 6 and 12 months of age and their retinal morphology was evaluated by immunohistochemistry. Visual assessment of these animals was performed using different functional test paradigms.
Results: We report that the retina of *rd3*$^{-/-}$ mutants at 3 months have underdeveloped cones, with missing outer limiting membrane and lamination defects in the outer nuclear layer. Both rods and cones degenerate at later time points (6 and 12 months), with major loss in cone subtypes. In *abca4b*$^{-/-}$ mutants, a marked reduction in the cone nuclear layer and a corresponding increase in the number of rod nuclei suggest significant defects in cone precursor cell differentiation and maturation. The single blue cones are completely absent and the UV cones are underdeveloped with rudimentary inner and outer segments. The double cones develop normally, but undergo degeneration in older animals. In experimental paradigms to assess the feed capture response under normal photopic conditions (n=10), the mutant fishes failed to approach their feed/prey and displayed significantly higher latency than the wild type fishes. This visual behavioral deficit corroborates with the cone photoreceptor-specific developmental anomaly and retinal degeneration in mutant fishes.
Conclusion: The zebrafish *rd3*$^{-/-}$ and *abca4b*$^{-/-}$ mutants show cone developmental defects and degenerative phenotypes that resemble the human disease. Defects in photoreceptor precursor cell differentiation, maturation and lamination indicates possible role(s) for these genes in the early retinal development.

Poster_13

Effect of Light intensity on Growth, Photosynthetic Performance and Biochemical Composition of *Monoraphidium* sp.

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In this study, *Monoraphidium sp. SVMIICT6 s* was cultivated in Bold's basal medium under blue LED at different light intensities (50 - 300 µmol photon $m^{-2}s^{-1}$) in photoautotrophic condition. The effect of wavelength showed a marked influence on photosynthetic efficiency, that augmented the growth, pigment accumulation, protein, and carbohydrates which cooperatively prevented Photosystem II from damage observed under light stress. The electron transport rate (ETR) was improved along with OJIP fluorescence transients, and quantum yield under 100 µmol photon $m^{-2}s^{-1}$ indicating efficient photosynthetic machinery. The maximal photochemical yield (Fv/Fm-0.547) and non-photochemical quenching (NPQ-0.348) with self-protection were observed at 300 µmol photon $m^{-2}s^{-1}$. The Chlorophyll a content of 16.84 mg g^{-1} and carotenoid content of 12 mg g^{-1} was recorded. Biochemical profiling of the pigmentation phases was performed revealing their photosensitivity in the biosynthesis of protein (12 mg g^{-1}), and carbohydrate (18 mg g^{-1}) was obtained under blue light. Thus, the study provides an insight into blue light assessing its growth response while synthesizing photosynthetic pigments and accumulation of biomolecules.

Poster_14

Associating with the 'cool' crowd: role of MLL and MLL fusion proteins in ribosomal RNA transcription

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MLL (Mixed lineage leukemia) protein is a Histone 3 lysine 4(H3K4) methyl transferase, involved in transcriptional activation of development genes. *MLL* gene often gets translocated and fuses with various random genes resulting in the formation of in-frame chimeric MLL fusion proteins. MLL fusion proteins drive aberrant transcription of various genes and this is believed to cause acute myeloid leukemia (AML) or acute lymphoid leukemia (ALL). MLL plays an important role in the RNA polymerase (Pol) II mediated transcription. However, the role of MLL and MLL fusion proteins in other RNA polymerase (I & III) associated transcription is not known. Current studies from our laboratory have shown that MLL family members and MLL fusion proteins affect the transcription of repeat regions of the genome. Ribosomal DNA is one of the tandemly repeated regions present on five acrocentric chromosomes. Ribosomal RNA transcription mediated by RNA Pol I leads to the formation of precursor RNAs required for ribosomal biogenesis. Here we discuss specifically how MLL family members/MLL fusion proteins are regulating the ribosomal RNA transcription and the association of MLL family members (MLL1 and Set1A), and MLL fusion protein (MLL-AF9) with various components of RNA Pol I transcription machinery. The implications of our finding on MLL-rearranged leukemia will be discussed.

Poster_15

Paternal control of seed development in Arabidopsis

Ginkuntla SaiKiran Goud

Endosperm development is the place where the postzygotic barrier is established via imprinting in flowering plants. Endosperm with 2 maternal and 1 paternal genomes helps in driving the normal seed development. Wherein any deviation from the maternal and paternal ratio leads to abnormal endosperm development with consequences for seed development, including possible lethality due to the maternal and paternal genome conflict. We use Arabidopsis as a model for studying the endosperm development. Seeds developed from BHRIGU mutations result in bigger seeds, which is common in paternal excess seeds. There are studies reporting that mutations in certain genes can rescue the paternal excess lethality. We found a few of those genes' mutations can suppress the bhr seed size. Here we show BHRIGU is expressed in the paternal lineage to control the seed size. We are further studying the role of BHR in endosperm development.

Poster_16

Uncovering the role of inorganic polyphosphate in mitochondrial physiology

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Inorganic polyphosphate (PolyP), a biopolymer found in all living organisms, is composed of long chains of phosphate residues linked by phosphoanhydride bonds. In mammals, PolyP is present at low levels, playing a regulatory role in blood coagulation, inflammation, and mitochondrial function. The enzymes responsible for polyP synthesis have been identified in prokaryotes and unicellular eukaryotes, but the machinery involved in polyP biosynthesis in mammals is unknown. PolyP synthesis in budding yeast is allosterically activated by the inositol pyrophosphate IP7, a derivative of inositol substituted with one diphosphate and five monophosphate moieties. We have reported that mice lacking the IP7 synthesising enzyme IP6K1, have lower levels of platelet polyP and delayed blood coagulation, indicating that IP7 also upregulates polyP synthesis in mammals. To further explore the relationship between IP7 and polyP in mammals, we identified proteins from HEK293T cells that interact with IP7 or polyP immobilised on agarose beads. Interestingly, the IP7 and polyP interactome contain several mitochondrial proteins, suggestive of a cross-talk between IP7 and polyP in the regulation of mitochondrial energy metabolism. Mitochondria isolated from human cell lines or mouse liver depleted for IP6K1 show reduced PolyP levels compared to their respective wild type controls. We are conducting assays to examine the dependence of mitochondrial polyP synthesis on orthophosphate, ATP, and IP7 levels. These investigations may also reveal the direct and indirect phosphate sources for polyP synthesis in mammalian mitochondria. We observed that the mitochondrial membrane potential is reduced IP6K1 knock out cell lines. We are presently exploring the effect of altering intra-mitochondrial levels of IP7 or polyP on mitochondrial function.

Poster_17

Human Lacrimal Gland Mesenchymal Stem Cell Characterization to Understand Its Role in Dry Eye Disease

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Purpose: Mesenchymal stem cells (MSCs) from lacrimal glands (LGs) have been evaluated in murine models to treat dry eye disease (DED). We herein aim to identify, isolate and characterize the LG-MSCs from normal and DED patients.

Methods: The study was approved by IRB of LVPEI (Ref.No.LEC BHR-P-04-21-622) and the informed consent was obtained from each participant. Normal human LGs (n=6; mean age=54.6±19.6) from individuals undergoing LG debulking surgery and LG biopsies from patients with severe DED (n=3) were cultured and p3 generation cells were characterized. Also, the differential expression of genes in the LG tissues of normal and SJS patients were analyzed by q-PCR. Limbal stromal cells were used as controls.

Results: The normal LG cultures demonstrated spindle shaped cells in sheets and colonies similar to the limbal MSCs. The immunophenotyping of normal lacrimal and limbal cells revealed expression for CD105 (99.15%, 98.38%), CD73 (99.19%, 97.57%), CD90 (98.60%, 97.68%) and no expression for CD45 (2.60%, 0.03%), CD34 (2.00%, 0.03%) and HLA-DR (1.76%, 0.06%) respectively. Trilineage differentiation into osteogenic, adipogenic and chondrogenic lineages were noted similar to control cells. In contrast to the normal LGs, the tissues from SJS patients showed no growth. However, upregulation of inflammation related genes i.e., IFN-β1 (14-fold), CCL5 (5-fold), CCL2 (8-fold), Ro60 (4.5-fold) and downregulation of lactoferrin was noted in LGs of DED patients.

Conclusions: The study establishes the finding of stromal cells from human LG cultures with characteristic features of MSCs in terms of phenotype and trilineage differentiation. We also provide data for altered gene expression profile in DED patients which warrants further studies with more patient samples to explore its role in DED.

Poster_18

Whole Exome Sequencing helped in identification of novel homozygous deletion in PCNT gene as a cause of Microcephalic Osteodysplastic Primordial Dysmorphism Type II

K. Mohini Annapurna

Rare genetic disorders in consanguineous families are common in India. MOPD (Microcephalic Osteodysplastic Primordial Dwarfism) is one of such rare genetic disorder where dwarfism begins in early stages of intrauterine life (primordial stages). We are reporting a rare case of primordial dwarfism in a 9yrs old girl with low birth weight, short stature since birth, facial dysmorphism and multiple bone deformities, who was born to 3rd degree consanguineous parents. The child had straight legs till 2yrs of age and then deformity in legs and hands has started. X-ray of limbs shows shortening and bowing of radius-ulna and tibia-fibula. Both knee joints had lateral displacement of the tibia with respect to the femur. Child has no history of having walking difficulty but has difficulty in holding objects due to short fingers. Whole Exome Sequencing was performed in the proband. Whole exome sequencing identified a homozygous deletion in PCNT gene spanning Exon-Intron junction. Further genetic analysis in proband and parents using sanger sequencing proved the presence of deletion.

Poster_19

Pulling the tricks out of the cap - Identification and characterization of mRNA capping machinery in Toxoplasma gondii

Kalyani

Toxoplasma gondii is an eukaryotic, unicellular apicomplexan parasitic protist causing Toxoplasmosis. It is one of the most persuasive and prevalent infection in humans and livestock. Infection, pathogenesis and transmission are of utmost importance for the survival of the parasite in a wide range of hosts and across across 3 different developmental stages (actively replicating-Tachyzoite, dormant stage-Bradyzoite and sexual stage-Oocyst). Thus, the parasite must timely respond to the regulatory requirements in order to process through its life cycle via tight epigenetic, transcriptional, post transcriptional mechanisms. Over the last decade, the modification of mRNA has emerged as another important layer of gene regulation. mRNA capping, is one such fundamental process that co- transcriptionally caps the nascent mRNA at 5'end and confers stability to the mRNA. Despite the biological relevance of this process, it remains unexplored in the parasite. This study reports our findings in T. gondii of a mRNA capping machinery wherein we identify 3 core mRNA capping enzymes which are divergent from the human capping machinery. We show the existence of these enzymes in both the asexual forms of the parasite We bring in the specificity of these enzymes to establish 7-methylguanosine (m7G) mark on 5' end of mRNA through biochemical studies. We show that these 3 enzymes act sequentially to incorporate cap at the 5'end of in vitro transcribed RNA through colorimetric, radiometric and fluorescent based approaches. Taken together, our findings highlight an essential process in T. gondii that undertakes 5' end processing of mRNA which could pave way to development of specific inhibitors for novel anti-toxoplasma strategies.

Poster_20

Domain driven design for microscopy

P S Kesavan, Darshika Bohra, Aprotim Mazumder

The meaning of microscopy has evolved from being a standalone instrument to a critical research technology that has diverse roles in the overall research ecosystem. Across domains, imaging specialists use various software to record physical information of the sample and process it. However, in widefield microscopy, the current instrument control software that records data of the sample in runtime, is running blind with manual input. The imaging software is designed for adjusting device parameters in sequence, however the task of perception involving identifying regions of interests suitable for imaging is left to the human. We propose a solution that automates this process, with layers of abstraction to compartmentalize real-life computation problems associated with microscopy into modules. Our imaging pipeline has object detectors, which can detects objects as the images are captured, and can provide the necessary information about the sample in runtime. Using such a tool, we have demonstrated high yield of data from routine microscopy experiments.

Poster_21

Understanding the mechanisms in ocular surface manifestation of chronic Stevens-Johnson syndrome patients.

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Purpose: The chronic sequelae of Stevens-Johnson syndrome (SJS) can progress to various ocular complications and lead to visual impairment. The exact mechanism of the interplay of protein molecules in chronic ocular surface inflammation is not clearly understood. Therefore, this study aims to explore the underlying molecular signaling pathways to understand the disease process and mechanism of ocular damage using a proteomic approach.

Methods: Tear samples were collected from chronic SJS (n=6) with age-gender matched controls (n=6) and analyzed by liquid chromatography-mass spectroscopy for protein profiling and label-free quantification of tear proteins. Highly differentially regulated proteins were selected by z-score normalization followed by t-test significance using abundance values. The candidate proteins were shortlisted based on their presence in at least five patients with exclusive extracellular expression. Also, performed Ingenuity pathway analysis (IPA) to understand the differentially regulated canonical pathways in chronic SJS patients.

Results: The total tear proteins identified were ~1760, of which the t-test revealed 249 were differentially regulated proteins in chronic SJS tears. The results underline that 63 proteins were identified based on the extracellular expression, of which 33 proteins were significantly upregulated, including Neutrophil elastase ($p<0.0001$), Protein S100-A7 ($p<0.0004$), Neutrophil collagenase ($p<0.0016$), Myeloblastin ($p<0.004$), Myeloperoxidase ($p<0.003$), Matrilysin ($p<0.04$). And other 30 proteins were significantly downregulated, mostly related to lacrimal gland secretions. The IPA analysis of the significantly differentially regulated proteins revealed the IL-8 signaling pathway (p-value 1.24E-06) and inflammatory response (p-value 2.64E-04) as a major player in chronic SJS tears and could be correlated with disease conditions. Additionally, 33 proteins were uniquely expressing including Interleukin-36γ in SJS tear.

Conclusion: The findings of this study will contribute to a better understanding of the molecular mechanisms of chronic SJS ocular surface disease and may unravel new biological insights that help identify a potential therapeutic target for an effective treatment strategy.

Poster_22

Role of inorganic polyphosphate in mammalian granule biology

Manisha Mallick and Rashna Bhandari

Inorganic polyphosphate (polyP) is a polymer of orthophosphate residues linked by high energy phosphoanhydride bonds. This bio-polymer has been reported ubiquitously in all taxonomic kingdoms, and its abundance is highest in prokaryotes and unicellular eukaryotes. The polyP is involved in bio-energetics, cell growth, stress response, virulence, pathogenicity, cell signalling, seed germination, blood coagulation and immune response. In Bacteria, Protista and Fungi polyP synthesis, function and storage are well explored and understood. In mammals, polyP is stored in lysosome-related organelles (LROs) but its synthesis machinery and its regulation remain enigmatic. PolyP synthesis in budding yeast is allosterically regulated by diphosphoinositol pentakisphosphate (IP7). Our lab has shown that mice lacking the IP7 synthesizing enzyme IP6K1, show low platelet polyP levels compared to wild-type mice. In addition, we observed slower platelet aggregation, lengthened plasma clotting time and altered clot ultrastructure. The clot ultrastructure was rescued upon the addition of exogenous polyP. To further study the relationship between IP6K1 and polyP, we selected a rat mast cell model that is rich in LROs. We detected polyP and found its co-localisation with granule mediators. Our goal is to study the synthesis and storage of polyP inside LROs and to see how IP6K1 or IP7 tunes polyP biogenesis in mammals. Additionally, protein polyphosphorylation has been recognised as a post-translational modification (PTM) in eukaryotic systems. This PTM affects the localisation and interaction of its target proteins in yeast. We have generated a list of human proteins that interact with polyP and possess potential sites for polyphosphorylation. We plan to investigate polyphosphorylation on granule proteins. Overall, this study will highlight the proteins involved either in the synthesis or in the storage of polyP. Furthermore, it will add granule protein targets of polyphosphorylation highlighting its effect.

Poster_23

A Boolean network analysis of Parkinson's Disease genes and mitochondrial metabolic factors influencing Oxidative phosphorylation

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Background: Loss of dopaminergic neurons (DNs) is the primary cause of Parkinson's disease (PD). DNs have high energy demand, and oxidative phosphorylation pathway (OxPhos) is responsible for most of the ATP production, which is intricately linked with a few familial PD genes. Alterations in these genes promote SNCA aggregation and affect mitochondrial metabolic factors (MMFs). These MMFs are considered as key players in determining the functioning of OxPhos.

Objective: The detailed mechanism of regulation of the MMFs by PD-related genes involved in OxPhos is yet to be unveiled. In this study, we constructed a Boolean network that explains the role of these PD genes on OxPhos, MMFs, and the participation of intermediary components.

Methods: We performed GO analysis and literature survey that gives a list of familial PD genes which are implicated in OxPhos. The mechanisms of action of these genes and their interactions have been studied using Boolean network analysis.

Results: The Boolean model demystifies these PD genes' normal and pathological function and their effects on MMFs. It also explains probable mechanistic detail of the compensation pathway of a PD gene upon dysfunction of another PD gene in OxPhos. The model also suggests essential PD genes (DJ1, PARKIN, MNRR1, and LRRK2) and Ca^{2+} ions concentration as most crucial MMFs, whose pathological state will perturb the network substantially.

Conclusion: The function of these genes is interlinked, and changes in the PD genes affect MMFs which dysfunctions OxPhos that leads to Parkinson's disease.

Poster_24

Identifications and in-silico analysis of a spectrum of SLC4A11 mutations in familial and non-familial cases of congenital hereditary endothelial dystrophy in Indian families

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Background/Aims: Congenital Hereditary Endothelial Dystrophy (CHED) is a rare form of corneal dystrophy and is known to be caused by SLC4A11 gene mutation. In this study, familial and non-familial cases of CHED were analyzed for the presence of SLC4A11 mutations, followed by their in-silico characterization and genotype-phenotype correlations.

Methods: Two familial cases of CHED, n=3 and n=2 affected members, respectively, and five non-familial cases with a single affected member were screened for all coding exons with flanking intronic region of SLC4A11 gene using the direct sequencing method. Identified mutations were excluded from a large number of controls (n=80) and analyzed in-silico using homology-based protein modeling and pathogenicity prediction tools.

Results: Three affected members of the first CHED family were identified with novel hom. c.1514C>G (p.Ser489Trp) mutation while second family had compound heterozygous, het c.529A>C (p.Arg161Arg) + c.2461insT (p.Val805fs) mutation. Among five non-familial cases, two had novel changes- hom. c.1487G>T (p.Ser480Ile) and hom. c.620-2A>G respectively, while the other one showed previously reported hom. c.2653C>T (p.Arg869Cys) mutation. The remaining two cases did not reveal the presence of CHED-related pathogenic mutations. Homology modelling based in-silico analysis predicted change in protein stability, protein local flexibility and hydrogen bond interactions caused due to such mutations.

Conclusion: Our study demonstrated a spectrum of mutations ranging from coding, non-coding, homozygous, and synonymous compound heterozygous in CHED cases. The identified mutations demonstrated different degrees of pathogenic effects based on in-silico protein modeling analysis. In addition, two non-familial cases could not be identified with pathogenic mutation emphasizing the involvement of other genes, genetic mechanisms or clinical diagnosis for such cases.

Poster_25

A stringently regulated lytic transglycosylase, MltD contributes to expansion of peptidoglycan in *Escherichia coli*

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Peptidoglycan (PG) is an essential shape defining constituent of most bacterial cell walls. It is a sac-like exoskeleton that surrounds the cells, protecting the cells from lysis due to internal osmotic pressure. The PG is composed of linear glycan strands crosslinked by short peptide stems forming a mesh-like sacculus. Since the PG encases the cytoplasmic membrane, the growth of a bacterial cell is tightly coupled to the expansion of PG. We had earlier identified redundant cross-link cleaving endopeptidases, MepS and MepM that are required for crosslink cleavage of the PG for incorporation of new material thus helping in PG expansion. Here, we find that overexpression of MltD, a lytic transglycosylase that cleaves the glycan strands can compensate for the absence of MepS and MepM. By means of genetic and biochemical approaches, we establish that MltD contributes to PG enlargement. Using pulse-chase experiments and *in-vitro* degradation assays, we show that MltD is regulated post-translationally by a periplasmic proteolytic machinery comprised of an adaptor-protease duo, NlpI-Prc. Our results collectively suggest a coordinated cleavage of glycan strands and peptide crosslinks that opens the PG mesh for the insertion of nascent PG material for successful expansion of bacterial cell wall.

Poster_26

Structural and functional analysis of putative HtrA like proteins in *Leptospira interrogans*

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Leptospirosis is a zoonotic infection caused by the pathogenic spirochete *Leptospira interrogans*. Humans acquire this infection by contact with the urine or other fluids of infected animals. Human-to-human transmission is rare. Developing countries carry the major burden of the disease, with half a million cases reported yearly and a mortality rate ranging from 5 to 10%.

Leptospira survives under stress conditions like high temperatures, and low nutrient availability, as well as protects itself against antibiotics. Such stress conditions tend to induce an increase in the levels of misfolded proteins and to ensure the survival of the organism, these proteins are either repaired or degraded. HtrA proteins are known to exhibit protease activity and act as a chaperone. Therefore, we studied putative HtrA (High-Temperature requirement A proteases) like proteins that have up to 36% identity with *E. Coli* DegP and help survive high-temperature conditions. They contain a Serine protease domain (with a catalytic triad His-Asp-Ser) and a carboxy-terminal PDZ domain. In this study, we are trying to understand the structure using *in-silico* tools, function through genetic complementation with *E. coli*, and biochemical characterisation by proteolytic substrate degradation, to decipher its role in bacterial survival. The results show that HtrA is structurally homologous to DegP and forms an oligomeric state in solution as well as can cleave casein. These proteins can further be used as a target for vaccine or drug development.

Poster_27

Interaction of bovine ephemeral fever virus (BEFV) with cell surface protein(s) during initiation of infection

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Bovine ephemeral fever virus (BEFV) is the type species of Genus *Ephemerovirus* under Family *Rhabdoviridae*. BEFV carries a single stranded, negative sense RNA genome of 14.6-14.9 kb. Much is known about the life cycle of related rhabdoviruses, viz., rabies virus and vesicular stomatitis virus, but not much is known about the interaction of viral surface proteins with cellular proteins during entry of BEFV. However, neither an Indian virus isolate nor commercially available tools and reagents are available. In order to explore virus-host protein-protein interactions during entry of BEFV, we generated tools and reagents. The expected viral entry mediator, the BEFV glycoprotein, was expressed through baculovirus system, and polyclonal antibodies were produced in rabbits. On the other hand, eukaryotic expression plasmids and recombinant adenoviruses were also generated. The functionality of the systems was verified by ELISA, western blotting and immunofluorescence using the reagents generated. Parallely, full genome of virus was amplified through PCR amplification and joining the fragments and the regulatory elements to develop a construct for generating infectious virus using reverse genetics system. In addition, heterologous pseudo and chimeric viruses encoding the G protein are being envisaged to study BEFV entry mechanisms, including interaction with cellular proteins to initiate infection as well as bud out of cells through various biochemical and cell biological assays.

Poster_28

Matrix stiffening promotes perinuclear mitochondrial localization

Piyush Daga

Mechanical signals from the cellular microenvironment modulate various cell functions via cytoskeleton remodelling and actomyosin contractility. Although these processes consume energy and alter metabolism, it is unknown how the form and function of the main energy-producing organelle, mitochondria, adapt to meet such mechanical demands. Here, we show that the stiffness of the extracellular matrix alters mitochondrial morphology, subcellular localization, and dynamics. Matrix stiffening causes an increase in fragmented and perinuclear mitochondrial populations whereas a soft matrix has elongated and homogeneously distributed mitochondria. We identify stiffness-sensitive perinuclear localization of an actin binding protein, Filamin A as the key mechano- sensory feature responsible for the observed mitochondrial morphology and subcellular localization. Using photo-conversion labeling and FRAP studies, we further show two mitochondrial populations- perinuclear and peripheral- which differ in their motility on soft matrix and their morphologies on stiff matrix. Subsequently, maintenance of the perinuclear mitochondria on a stiff matrix is crucial for priming human mesenchymal stem cells towards osteogenesis. Taken together, our results discover an unknown mitochondria-centric mechanism that enables a cell to adapt to its microenvironment.

Poster_29

The Fenfluramine Dravet Story

Pooja Ram

Dravet syndrome also referred as severe myoclonic epilepsy of infancy (SMEI) is a rare form of childhood epilepsy characterized by prolonged, persistent, and pharmacoresistant seizure leading to severe intellectual disability. Sudden unexpected death in epilepsy (SUDEP) is three times higher for Dravet's as compared with other causes of epilepsy. About 85% of the DS patient have mutation in Nav1.1 (SCN1A), a voltage gated sodium channel and 90% cases are de novo i.e., mutation is not inherited from parent. Dravet patients experience generalized or focal seizures in first year of age later have other comorbidities like behavioural and developmental delay, speech impairment, hyperthermia, crouched gait, etc. Available treatments for Dravet (anti-epileptic drugs) are limited to suppressing seizures. Zebrafish model system recapitulate human disease and are used for drug discovery and drug repurposing testing. Zebrafish, scn1lab gene is human ortholog to SCN1A, shares 77% identity and expressed in central nervous system. Zebrafish scn1lab function has been validated by various approaches like chemical mutagenesis, CRISPR/Cas9 and antisence method and studies suggest zebrafish mutant's mimics similar epileptic like phenotypes as seen in DS. Screening of Fenfluramine, the 5HT receptor agonist in zebrafish mutant reduced epilepsy, locomotor and brain activity. The molecule was further tested in a randomized, double blind placebo-controlled trials, the results from this trail show a 74% efficacy in Dravet Syndrome patients 2 to 18 years of age with refractory seizures. On 25th June 2020 US FDA has approved Fintepla (fenfluramine) an anrorectic drug which has been repurposed for Dravet Syndrome. While the diagnosis, treatment and management of Dravet is devastating, more research is needed to find a cure.

Poster_30

Characterization of spliceosome-associated NineTeen complex (NTC) proteins, Prp19 and Cdc5 in *Toxoplasma gondii*

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Spliceosome, a highly dynamic macromolecular machinery is required to remove the non-coding introns from pre-mRNA. This multiprotein complex comprises of small nuclear ribonucleoproteins subunits (snRNPs) and several other non snRNP proteins. Conformational changes or rearrangements of the proteins in the complex play utmost important role to drive the splicing mechanism and activate the spliceosome. These conformational changes are brought about by a group of proteins called NineTeen complex (NTC), of which Prp19 and Cdc5 form the catalytic core. NTC complex is diverse, when compared in eukaryotes and sometimes in closely related organisms. Toxoplasma genome is highly intron rich with 80% genes containing high number of introns compared to other eukaryotes. Several splicing factors show low similarity to human or yeast counterparts implicating specialized evolution of splicing in the Toxoplasma. With the help of Prp19/Cdc5 complex, we try to understand the splicing repertoire in *T.gondii*. We identified the putative TgPrp19 and TgCdc5 in *T.gondii* and confirmed their presence by loss of function complementation in yeast. Endogenously tagging TgPrp19 and TgCdc5 helped to pulldown the proteins in the complex and identified co-purified protein by mass spectrometry. As TgCdc5 is catalytic component of NTC, its pulls down two of the important U2 and U6 snRNPs, apart from other splicing factors. These interactions are also demonstrated via invitro assays. TgPrp19 also pulls down many other splicing proteins, along with TgCdc5. Both the proteins co localize in nucleus and reciprocal Co-IP confirms their interaction. We tend to study the role of Prp19/TgCdc5 related complex in splicing with *invitro* splicing assay. We also propose conditional knockdown of TgPrp19 and TgCdc5 to asses role of these proteins in splicing by observing phenotypic anomalies and RNA seq confirming the accumulation of pre-mRNA. Together our study steps towards achieving a more comprehensive understanding of core spliceosome structure and function.

Poster_31

Role of WWP2 in regulation of ARID1B

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Background: Mammalian SWI/SNF complex protein ARID1B is a well-known tumour suppresser in multiple cancers. Deregulation of ARID1B protein level by mislocalisation or degradation leads to tumor formation. Till date multiple studies have been performed on ARID1A, mutually exclusive paralog of ARID1B, to understand proteins involved in its regulation and localisation. In this study we focused on novel interacting protein partners involved in regulation of ARID1B.

Methods: Affinity precipitation followed by mass spectrometry study was performed to understand the interacting partners of ARID1B. Proteins involved in ubiquitin mediated proteasomal degradation were sorted from the obtained mass spectrometry list. Interaction of selected protein with ARID1B was validated by overexpression followed by affinity precipitation. Region at which selected protein interact with ARID1B was detected by co-expressing deletion constructs of ARID1B with those proteins. Cycloheximide chase assay was performed to understand change in ARID1B protein level upon over-expression of selected protein. Proteasomal inhibitor MG132 treatment was performed to confirm proteasome mediated degradation of ARID1B by the selected protein.

Results: NEDD4 family ubiquitin ligase WWP2 was among the top interacting partners involved in protein regulation and stability. Interactions of WWP2 with ARID1B was confirmed by affinity precipitation. WWP2 interacts at the N- terminal domain of ARID1B. Increasing expression of WWP2 leads to decrease in ARID1B protein level. Wild type WWP2, but not the ubiquitin ligase dead form of WWP2 (mutant WWP2), showed change in the ARID1B protein stability. Treating with MG132 inhibits proteasome mediated degradation of ARID1B by WWP2.

Conclusion: WWP2, a novel interacting partner of ARID1B, regulates its stability by proteasome mediated degradation which may play a role in tumor formation.

Poster_32

Clinical Exome Sequencing Identification of Novel Nonsense Variant in DAAM2 Gene in siblings with Steroid-resistant Nephrotic Syndrome

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Steroid-resistant nephrotic syndrome (SRNS) occurs in few of the patients that undergo steroid therapy for nephrotic syndrome which may lead to end-stage renal disease. It is characterized by edema, proteinuria, hypoalbuminemia and hyperlipidemia. In the current study, we conducted clinical exome sequencing in the proband born to third degree consanguineous parents. The 14 year old male presented with recurrent episodes of steroid-resistant nephrotic syndrome phenotype along with short stature, myopia, atlanto-axial joint instability, and facial dysmorphisms such as midface hypoplasia, low set ears with overfolded helices, retrognathia, hypoplastic toe-nails. Renal biopsy revealed immune-complex mediated membranoproliferative glomerulonephritis. Skeletal survey showed increased cortical thickness in the skull, sloping ribs and mild osteopenia. The proband has an affected sibling with the similar phenotypes. The initial CES analysis done in 2018 could not identify causative pathogenic/likely pathogenic variant for the reported phenotype as the gene-phenotype relationship was not established. However, reanalysis done in 2022 revealed a novel homozygous nonsense variant in the formin encoding gene *DAAM2* (p.Arg66x), which caused Nephrotic syndrome type 24 in both siblings. This variant introduces a stop codon at position p.Arg66 and it is mapped to N-terminal GTPase binding domain (GBD). Nephrotic syndrome type 24 follows an autosomal recessive inheritance pattern. Only one homozygous nonsense variant (p.Arg445x) is reported and it is mapped to the region after GBD/FH3 (DID) domain, apart from missense variants. Other clinical features in the patient could be part of same genetic condition or due to coexisting genetic etiology. Further genetic evaluation is planned with whole-exome or whole-genome sequencing for complete genetic diagnosis. This case exemplifies the importance of periodic reanalysis of unsolved cases for establishing molecular diagnosis for recently identified monogenic conditions.

Poster_33

Role of protein homeostasis in the diversification of flowering plants

Prakash Sivakumar

Current understanding of the origin of flowering plants dates back to the early Cretaceous/late Jurassic period, which is around 130 Mya. Given that 80 - 90 % of plants are flowering plants, land acquisition by them is massive in the recent geological time frame. The sudden appearance and diversity of dicot flowering plants (75 % of flowering plants are dicots) on earth during the Late Cretaceous, as noted by Charles Darwin is still an “abominable mystery”. The earliest known dicot plants are identified in fossils by their pollen. Pollens are one of the flowering plants' innovations and are tiny haploid organs present inside the diploid flowers. Variation in pollen morphology, number of pollen, rate of germination of pollen on the stigma, and paternal imprinting are a few of the pollen features which can potentially contribute to reproductive isolation in flowering plants. We study the gene SHUKR (SKR) which modulates protein homeostasis to control pollen development in Arabidopsis. We show that SKR acquired control over recently formed F-box genes - a family of E3 ubiquitin ligases. Phylogenetic analyses of the SHUKR gene family suggested that they are actively changing their amino acids (also known as positively evolving) in major plant families like the sunflower and mustard families. Incidentally, these two plant families show a heightened speciation rate among the land plants. Based on the above-mentioned evidence, we hypothesize that modulation of the pollen proteome would have positively contributed to the diversification of flowering plants.

Poster_34

Growth inhibitory and anti-biofilm activity of caprine gut probiotic bacteria against ESKAPE Pathogens.

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The livestock sector contributes to ~ 40 percent of the global agricultural production making it as one of the fastest growing agricultural sub-sectors in the low to middle –income countries. At the same time, non-judicial use of antibiotic growth promoters (AGPs) in the organised, and large scale livestock, and poultry farming has contributed to the emergence of antimicrobial resistance (AMR), and enhanced the risk of transmission to human. Owing to the beneficial influence on overall health of livestock animals, probiotic therapy is emerging as a desirable component of prophylactic, and therapeutic strategies for prevention, and treatment of not only enteric infections but also as alternative to antibiotic treatment for bacterial infections of other organ systems. In this study, we have isolated and characterized a number of Lactic Acid Bacteria (LAB) from the goat intestine, and evaluated their growth inhibitory, and anti-biofilm activity against ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa, and Enterobacter spp) group of pathogens. In addition to exhibiting the biochemical, and bacteriological properties of probiotic bacteria, majority of the LAB isolates exhibited considerable inhibition of pathogenic bacterial growth in the in vitro cultures. In addition, the secretory fraction from the culture supernatants of the LAB isolates prevented biofilm generation by the ESKAPE group bacteria indicating production of antibacterial metabolites or peptides. Altogether, our study highlights the probiotic potential of the livestock derived LAB isolates, and substantiate their usage as an alternative to antibiotics against pathogenic bacterial infections.

Poster_35

Generation of tools to study bovine ephemeral fever virus as model to understand ephemerovirus biology

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Bovine ephemeral fever virus (BEFV) belongs to Genus *Ephemerovirus* of Family *Rhabdoviridae*. Its genome is a single stranded, negative sense RNA of 14.6-14.9 kb. While much is known about the life-cycle events for the related viruses, vesicular stomatitis virus (VSV) and rabies virus (RABV), not much is known about the biology of BEFV. Recent studies with VSV and RABV have suggested the involvement of lipid rafts in their life-cycle, and we hypothesized that the same would be true for BEFV entry and egress. Since no tools are available to study BEFV biology, we initiated virus genome assembly to set up a reverse genetic system as well as generating reagents. The full genome of the virus was amplified and assembled through PCR amplification and joining of small fragments while a minigenome construct containing regulatory elements to generate infectious virus was designed and obtained synthetically. Matrix (M) and nucleocapsid (N) proteins were expressed in bacterial system and polyclonal antibodies were raised against each in rabbits. Eukaryotic expression plasmids as well as recombinant adenoviruses were generated for both the viral proteins, and expression was confirmed by using the rabbit polyclonal antibodies. On the other hand, cell fractionation is being standardized to purify lipid rafts. Using these tools and protocols, the interaction of M protein and its domains with lipid rafts will be studied at various stages of BEFV life-cycle, using various methods. This is expected to contribute to the understanding of the molecular events during BEFV entry and egress.

Poster_36

Role of a long non-coding RNA during cardiac hypertrophy

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Non-coding RNAs are emerging as lucrative targets for therapies of various disorders. In a genome wide screening for deregulated long non-coding RNAs in heart failure, we identified a conserved long non-coding RNA (lncRNA) whose expression is correlated functionality of the heart. We are studying this lncRNA in the context of heart failure using cardiac hypertrophy as a disease model. Cardiac hypertrophy is a condition that starts as a compensatory mechanism for the better functioning of the heart in response to pressure overload. However, continuous pressure overload causes the heart to progress towards failure. Using cell culture and animal models, we show that this lncRNA is upregulated during cardiac hypertrophy and its expression is regulated by pro-hypertrophic transcription factors. Loss of function and gain of function studies further confirm its involvement in cardiomyocyte hypertrophy and failure.

Poster_37

S100A12 in *Pseudomonas aeruginosa* keratitis

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RATIONAL: Bacterial keratitis is one of the leading causes of corneal blindness and opacification globally. *Pseudomonas aeruginosa*, a gram-negative bacterium, is the most common causative agent for corneal infections. Antimicrobial peptides, part of the host innate immunity, are the most potent alternative to traditional drugs. SA12, a calcium binding host-defense protein has shown to have antimicrobial effects on various microbes by acting as damage associated molecular pattern and initiate a pro-inflammatory immune response.

AIM: To elucidate the role of SA12 against *P. aeruginosa* infections.

METHODS: The increased expression of SA12 in *P. aeruginosa* infections was confirmed in corneal tissues of keratitis patient as well as *in vitro* using human corneal epithelial cells (HCEC) by immunofluorescence and by qPCR. The effect of SA12 against *P. aeruginosa* growth was checked by doing a colony forming unit assay. The immune-modulatory property of SA12 was checked by performing a cell migration assay

RESULTS: We found an increased SA12 expression in corneal tissues of patients with *P. aeruginosa* keratitis and HCEC in response to infection on both gene and protein levels. SA12 was found to inhibit the bacterial growth *in vitro* with HCECs as well as in *ex-vivo* setup. SA12 was also seen to accelerate the cell migration and wound closure in HCECs.

CONCLUSIONS: SA12 is thus likely to play a role in *P. aeruginosa* keratitis and is effective in inhibiting the growth of *P. aeruginosa.* It also has immune-modulatory property which suggests SA12 as an alternative therapeutic intervention against *P. aeruginosa*.

Poster_38

An analysis of *Candida glabrata*-epithelial cell interaction

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Candida glabrata, a non-spore-forming haploid budding yeast, resides as a commensal in the oral cavity, and gastrointestinal and genitourinary tracts in healthy individuals. This opportunistic fungal pathogen causes life-threatening deep-seated infections upon immunocompromised conditions. *C. glabrata* shares a common ancestor with the non-pathogenic yeast *Saccharomyces cerevisiae*, and is phylogenetically distant from the most prevalent *Candida* pathogen, *C. albicans*. Although antifungal drug resistance mechanisms and intracellular survival strategies of *C. glabrata* are currently being studied, the interaction of *C. glabrata* with host epithelial and endothelial cells remains largely unexplored. An interaction with tissue-resident immune cells as well as non-phagocytic epithelial and endothelial cells is essential for the colonization and invasion of diverse host niches. In the current study, we have established *in vitro* model system, using the human renal epithelial cell line A-498, to delineate *C. glabrata*-epithelial cell interaction. Besides showing that *C. glabrata* possesses an ability to adhere to A-498 cells, we have also carried out a detailed analysis of epithelial cell activation and cytokine generation response in A-498 cells in response to *C. glabrata* infection. Further, through super resolution microscopy and biochemical approaches, we have elucidated a pivotal role for a family of eleven cell surface-associated aspartyl proteases (CgYps1-11; CgYapsins) of *C. glabrata* in the modulation of cell adhesion via cell surface adhesin proteins. These results along with putative epithelial cell interactors of CgYps1 and CgYps7 proteases will be presented.

Poster_39

A rare synonymous variant in the *SELENOI* exon 2 disrupts its splicing and causes Spastic paraplegia 81

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Hereditary spastic paraplegia 81 (HSP 81) is a rare autosomal recessive disease caused by mutations in the *SELENOI* gene. HSP 81 is characterized clinically by Sensorineural deafness, Blindness, Hypotonia, Hypertonia, Cleft palate, delayed motor development, Regression of motor skills, Impaired intellectual development, Poor speech and language acquisition, Spasticity, Hyperreflexia, White matter abnormalities, and Cerebral and cerebellar atrophy. *SELENOI* codes for the enzyme ethanolaminephosphotransferase-1 (EPT1). EPT1 is one of the four enzymes involved in the Kennedy pathway of phosphatidylethanolamine synthesis. EPT1 catalyses the last step of phosphatidylethanolamine synthesis, the conversion of CDP-ethanolamine to phosphatidylethanolamine (PE). To date, five individuals from two unrelated families carrying one homozygous missense and one splicing variant in the *SELENOI* have been reported with autosomal recessive Spastic paraplegia 81. In the present study, we report two individuals affected with spastic paraplegia 81and carrying a homozygous synonymous variant in the *SELENOI* exon two NM_033505.4:c.126G>A disrupting the normal splicing and leading to complete skipping of exon two. Further, we report a novel transcript of *SELENOI* in the blood identified through cDNA analysis and RNA sequencing.

Poster_40

Loss of backbone interaction with Tyr122 may cause loss-of-function and instability of F128L and F128I mutants of NAA10

Smita Saha

N-terminal acetylation (NTA) is one of the most ubiquitous covalent post-translational modifications among eukaryotes. This modification, occurring on ~80% of human proteins, affects half-life, folding, complex formation and localisation of proteins. NTA is catalysed by N-terminal acetyltransferase complexes. In human, the majority of the NTAs are catalysed by N-terminal acetyltransferase A complex, which comprise of a catalytic subunit NAA10, an auxiliary subunit NAA15, and an intrinsic regulator HYPK. Mutations in NAA10 have been found to be associated with X-linked disorders with a broad spectrum of clinical phenotypes, including neurodevelopmental disorder, intellectual disabilities, developmental delay etc. According to HGMD database, 26 missense mutations in NAA10 with clinical phenotypes have been reported. These mutations can be catagorised into three types on the basis of their functional consequences— mutations affecting NAA10–NAA15 association, mutation decreasing enzymatic activity and mutations causing decrease of cellular stability of NAA10. Among those deleterious mutations, F128L and F128I are present neither at the NAA10–NAA15 interaction interface nor at the active site of NAA10. According to the biochemical assays, both showed abolishment of catalytic activity and less stability compared to the wild type. To delineate the mechanistic basis for loss of stability and functional activity, we performed molecular dynamics simulation of wild type and mutant NAA10 (F128I and F128L) using GROMACS. Our MD simulation shows no fluctuation at the site of mutation in the RMSF plot, although the fluctuation occurs at the vicinity of the mutations (Tyr122). Further, we investigated the structural contribution that may cause such fluctuation. In the wild type, there is polar interaction between F128 and Y122, whereas that interaction is absent in both the mutations. Moreover, we determined the $\Delta\Delta G$ values for these mutations, which suggest that both the mutations are destabilising in nature.

Poster_41

Molecular Insights into the Role of RD3 in LCA disease pathogenesis

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RD3 encodes a 23 kDa protein that traffics retinal guanylyl cyclases from the inner to outer segments of photoreceptors. Mutations in *RD3* cause Leber Congenital Amaurosis, Type 12 (LCA12), an early-onset congenital retinal dystrophy that results in severe vision loss. To understand the role of RD3 mutations in retinal disease, an LCA12 patient-specific iPSC line was generated and differentiated into eye fields and retinal cups. Surprisingly, the control and patient iPSC lines did not show any marked differences, which suggests that the early retinal commitment remains unaffected in mutant cells. However, we hypothesized that *RD3* mutations could have significant effects during retinal maturation, gene expression, and functions. Global gene expression analysis of control and patient-specific iPSC-derived retinal cups revealed significant downregulation of several C/D box SNORDs located within the DLK1-DIO3 locus. In overexpression studies, ectopic RD3 was found localized to the cytoplasm and nucleus, as discrete punctae. The nuclear RD3 puncta were found to be in close proximity to splicing factor speckles and Cajal bodies. Also, immunopulldown studies have confirmed interactions with key nuclear sub-compartment proteins, which suggested the possible involvement of RD3 in associated RNA regulatory processes. *In silico* analysis predicted RD3 interactions with snRNAs and we further confirmed it through RNA immunoprecipitation (RIP) and qRT-PCR. These observations suggest that RD3 could be interacting with SNORDs, either directly or as a part of multi-protein RNP complexes and possibly regulate SNORD maturation, stability, trafficking, and function. Loss of RD3 may negatively impact several SNORD-dependent cellular functions, which warrants deeper investigations.

Poster_42

SWITCH1/ DYAD regulates commitment to female meiocyte differentiation

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Meiosis lies at the heart of sexual reproduction and is crucial to reduce ploidy in gamete cells. Chromosomal recombination and reduction in its number makes meiosis as a specialized cell division. The DYAD/SWI1 gene of Arabidopsis has been previously shown to be an important plant-specific regulator of meiotic chromosome organization and sister chromatid cohesion. DYAD is an early meiotic protein and expresses in both male and female meiocytes from G1 to leptotene. The *dyad* mutant shows abnormal meiosis, and converts a reductional division into equational division, which produces diploid female gametes without recombination. The central portion of DYAD contains a domain of unknown function that shows strong conservation between DYAD and its orthologs in maize (AM1), rice (OsAM1) and is also found in paralogs including MALE MEIOCYTE DEATH 1 (MMD1). The functional analysis of this conserved domain using domain swap experiments into DYAD in a *dyad* mutant background revealed that this domain is functionally conserved in maize and rice but not with the domain of Arabidopsis MMD1. These results suggest that this domain is important for DYAD function and has been conserved in DYAD orthologs in both monocots and dicots and that it has diverged from DYAD in the paralog MMD1. Expression analysis of MMD1 domain swap revealed an altered pattern of expression that suggests a role for DYAD in commitment to female meiocyte differentiation. The results suggest that DYAD play a developmental role as an identity determinant in the female germ cell lineage in addition to its known role in meiotic chromosome organization.

Poster_43

Influence of Magnetite and Zinc Oxide Nanomaterial on the Fermentative Process towards Medium Chain Fatty Acids Production

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The biological production of fuel and chemicals offers a sustainable way to meet the increasing demand for value-added products. Nanotechnology has provided several possibilities for enhancing the rate of product formation through bacterial metabolic upregulation. The current study focuses on the application of nanomaterials towards the production of medium chain fatty acids (MCFA) along with green H_2. To increase the operational stability, microbial metabolism and enhanced fermentative by-products, nanomaterial additives such as Magnetite (M) and Zinc oxide (ZnO) were used in the current demonstration. The experiment was carried out in fed-batch mode where short chain fatty acids (SCFA: acetic, propionic, and butyric acids) were transformed into medium chain fatty acids (MCFA: caproic acid) through chain elongation process using glucose as the substrate (50 g/L) and ethanol (15 g/L) as an electron donor along with designed synthetic waste media. Caproic acid was produced in higher quantity in the reactor with M (7.32 g/L) followed by the ZnO reactor (3.46 g/L) after a period of 17 days with 52% substrate utilization. Meanwhile, total biogas (16.02 L) and green H_2 (6.5 L) was observed higher with M compared to the reactor with ZnO (10.8 L, 3.3 L). Overall, nanomaterials changed the product spectrum and steered to caproate formation with upregulated bacterial metabolism.

Poster_44

Impact of vitamin B12 supplementation on streptozotocin-induced diabetic rat brain

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Background & Objectives: Diabetes mellitus is associated with increased risk for neurodegenerative disorders. Vitamin B12 is an essential micronutrient, whose deficiency has also been linked to neurodegenerative conditions including cognitive impairment. Accumulation of misfolded proteins, altered neurotrophic support, astrogliosis, synaptic dysfunction, and defects in autophagy are associated with neurodegenerative diseases. Hence, in this study, we investigated the neuroprotective role of vitamin B12 and its molecular basis in diabetic rat brain.

Methods: Diabetes was induced in two-month-old Sprague-Dawley rats using streptozotocin and animals were divided into 3 groups: control (CN), diabetes (D), and diabetes with B12 supplementation (DBS). DBS group was fed double the amounts of B12 (50 µg/kg diet) in comparison to the CN and D groups. At the end of four months, the cerebral cortex (CC) tissue was collected. The histopathology was performed using H&E staining and nissil body staining, and cell death by TUNEL assay. Furthermore, markers of neurotrophic support, synaptic density, ER stress, the ubiquitin-proteasome system (UPS), and autophagy were analyzed by immunoblotting and immunofluorescence methods.

Results: Vitamin B12 supplementation to diabetic rats showed a reduction in cellular degeneracy with fewer halos around the cells, increased neuronal cell density, and decreased chromatolysis. Additionally, B12 supplementation restored the neurotrophic factors, improved the synaptic density-related markers, and attenuated the astrogliosis. Furthermore, B12 supplementation reduced the ER stress and alpha-synuclein expression. In tune with these changes, B12 supplementation decreased neuronal cell death.

Conclusion: This study demonstrates a neuroprotective role of B12 in ameliorating diabetes-induced neurodegeneration by modulating ER stress, astrogliosis, neurotrophic support, and abating cell death. This study is likely to help in developing B12 mediated therapeutic strategies for diabetes.

Poster_45

The Host F-box protein 22 enhances the uptake of *Brucella* by macrophages and drives a sustained release of pro-inflammatory cytokines through degradation of the anti-inflammatory effector proteins of *Brucella*.

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Brucella species are intracellular bacterial pathogens, causing the world-wide zoonotic disease, brucellosis. *Brucella* invade professional and non-professional phagocytic cells, followed by resisting intracellular killing and establishing a replication permissive niche. *Brucella* also modulate the innate and adaptive immune responses of the host for their chronic persistence. The complex intracellular cycle of *Brucella* majorly depends on multiple host factors but limited information is available on host and bacterial proteins that play essential role in the invasion, intracellular replication and modulation of host immune responses. By employing an siRNA screening, we identified a role for the host protein, FBXO22 in *Brucella*-macrophage interaction. FBXO22 is the key element in the SCF E3 ubiquitination complex where it determines the substrate specificity for ubiquitination and degradation of various host proteins. Downregulation of FBXO22 by siRNA or CRISPR-Cas9 system, resulted diminished uptake of *Brucella* into macrophages, which was dependent on NF-κB-mediated regulation of phagocytic receptors. FBXO22 expression was upregulated in *Brucella*-infected macrophages that resulted induction of phagocytic receptors and enhanced production of pro-inflammatory cytokines through NF-κB. Furthermore, we found that FBXO22 recruits the effector proteins of *Brucella*, including the anti-inflammatory proteins, TcpB and OMP25 for degradation through the SCF complex. We did not observe any role for another F-box containing protein of SCF complex, β-TrCP in *Brucella*-macrophage interaction. Our findings unravel novel functions of FBXO22 in host-pathogen interaction and its contribution to pathogenesis of infectious diseases.

Poster_46

Antibiotic resistance surveillance in urban water environment

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Antimicrobial resistance (AMR) means the development of resistance in a microbe against antimicrobials to which they are previously sensitive. Over millennia, environmental microorganisms developed diverse resistance mechanisms. The presence of pollutants (drugs, disinfectants, and heavy metals) in the environment as a result of anthropogenic activity causes significant selection pressure and accelerates the lateral transfer of associated antibiotic resistance (ARGs (emerging pollutants)) genes among bacterial populations (non pathogenic to pathogenic). Therefore, understanding the origins, evolution, and mechanisms of transfer of resistance elements is vital to address AMR. Surveillance is one of such approach where pollutant behavior, dissemination and fate can be studied in a real time. As a result, the current study aimed to examine ARGs occurrence, dissemination, and assembly mechanisms in urban water environments as they are interface between humans and ecosystem. Surveillance was carried for a period of three months to better understand the pollutant behavior in seasonal dynamics. Water characteristic parameters and other pollutant components (antibiotics) that contribute to cause a selection pressure for AGR were also examined. A total of 136 ARGs were screened, where aminoglycoside beta-lactamase and multidrug are the most abundant. The average absolute abundance of ARGs and mobile genetic elements (MGEs) are in higher magnitude. Horizontal Gene Transfer (HGT) processes enabled by MGEs play a significant role in the lakes ARG community. These findings suggest that urban lakes are ARG hotspots and underline their importance in ARG propagation. These findings give comprehensive information on the contamination level of ARGs on a local scale, and they will be effective in managing and preventing ARG pollution.

Poster_47

Evidence that dephospho-PtsN inhibits the activation of the K^+/H^+ antiporter YcgO by its cytoplasmic C-terminal region in *Escherichia coli.*

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Potassium (K^+) is a major intracellular cation that plays an important role in various cellular processes. Earlier studies from this laboratory have identified a role for PtsP-PtsO-PtsN phosphotransferase system in potassium metabolism in *Escherichia coli.* A strain lacking PtsN displays a K^+ limited growth (K^L) in minimal media with K^+ concentrations greater than 5 mM that is alleviated by the absence of YcgO encoding a putative K^+/H^+ antiporter. Absence of dephospho-PtsN was shown to lead to K^L and the YcgO as the mediation of K^L. Studies reported herein, have shown that YcgO possesses an "$N\text{-}_{out}\ C\text{-}_{in}$" configuration in the membrane with a transmembrane domain (TMD) comprising 382 amino acids and a 196 amino acid long C-terminal region (CTR) that is cytoplasmic. Deletion of CTR abolished YcgO function. Two amino acid substitutions in the TMD and four in the CTR led to constitutive activation of YcgO, that is, expression of these mutant proteins led to K^L, even in a wild type strain. Furthermore, constitutively active YcgO versions bearing mutation in the TMD displayed a CTR independent K^L. Furthermore, we have identified amino acid substitutions in CTR that impair YcgO function. Genetic studies also indicate that dephospho-PtsN interacts with YcgO. Our studies implicate CTR as an allosteric activator of YcgO and as a site with which dephospho-PtsN interacts to inhibit YcgO activation.