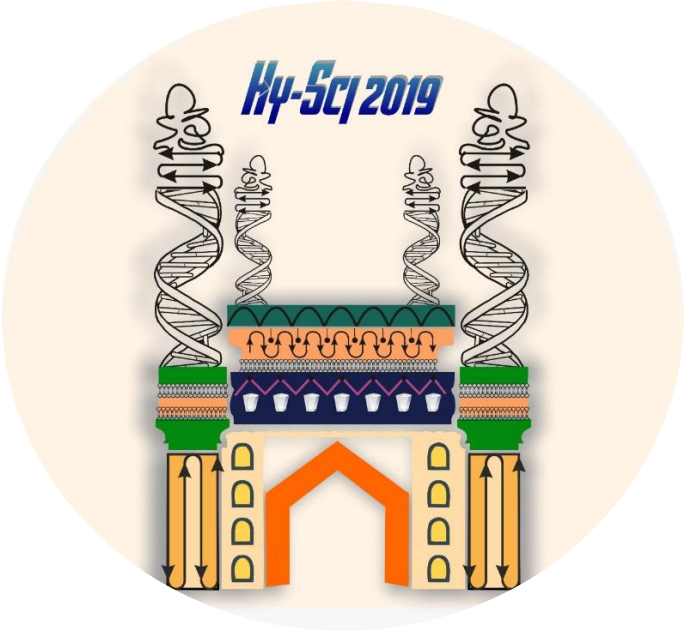

ABSTRACT BOOK

FOR

HY-SCI 2019

Host institute – CCMB

Venue for talks – IICT Auditorium

PLENARY TALK

Brenner’s elegant nonsense.

Durgadas P. Kasbekar, INSA Senior Scientist, CDFD

That UGA is a third nonsense codon was shown by Sydney Brenner, Leslie Barnett, Eugene Katz, and Francis Crick in an elegant 1967 paper. They used three “Genetics” resources: (1) the mutagens 2-aminopurine (which induces G:C to A:T, and A:T to G:C transitions) and hydroxylamine (which induces only G:C to A:T transitions); (2) *E. coli amber* and *ochre* suppressor strains, and (3) the vast collection of bacteriophage T4 rII mutants amassed by Benzer and colleagues. Around the same time, Brenner was also doing the first genetic crosses in *Caenorhabditis elegans*, and Katz, Brenner’s PhD student, and later my PhD supervisor, was the only other person in the lab the night the first results came in. *C. elegans*, a self-fertilizing hermaphrodite, makes it easy to isolate interesting recessive phenotypes in a diploid animal. Brenner’s choice of *C. elegans* influenced me to introgress *Neurospora crassa* translocation chromosomes into *N. tetrasperma*. Finally, I will discuss whether breakdown in "teacher - student transmission of values" underlies much of the all too frequent corruption of research (eg, image manipulation allegations in PubPeer) that has brought disrepute to elite Indian labs.

ABSTRACTS FOR ORAL PRESENTATION

O1

A new role for *Escherichia coli* Dam DNA methylase in prevention of aberrant chromosomal replication

Nalini Raghunathan[1,2], Sayantan Goswami[1,2,], Jakku K Leela[1], Apuratha Pandiyan[1], and Jayaraman Gowrishankar[1]

[1]Laboratory of Bacterial Genetics, Centre for DNA Fingerprinting and Diagnostics, Hyderabad 500039, India.

[2]Graduate Studies, Manipal Academy of Higher Education, Manipal, 576104, India.

The Dam DNA methylase of *Escherichia coli* is required for methyl-directed mismatch repair, regulation of chromosomal DNA replication initiation from *oriC* (which is DnaA-dependent), and regulation of gene expression. Here we show that Dam suppresses aberrant *oriC*-independent chromosomal replication (cSDR). Dam deficiency conferred cSDR and, in presence of additional mutations (Δ*tus*, *rpoB*35*) rescued the lethality of Δ*dnaA* mutants. The DinG helicase was required for rescue of Δ*dnaA* inviability during cSDR. Δ*dnaA* viability was lost upon introduction of deletions in *mutH/L/S*; thus generation of double strand ends (DSEs) by MutHLS action appears to be required for cSDR in the *dam* mutant. However another DSE-generating agent phleomycin was unable to rescue Δ*dnaA* lethality in *dam*$^+$ derivatives (*mutS*$^+$ or Δ*mutS*), but it could do so in the *dam* Δ*mutS* strain. These results point to a second role for Dam deficiency in cSDR. We propose that in Dam-deficient strains, there is an increased likelihood of reverse replication restart (towards *oriC*) following recombinational repair of DSEs on the chromosome.

O2

OsWAKL21, a putative receptor of rice cell wall damage activates alternate signaling in rice and Arabidopsis to induce immunity

Authors: Kamal Kumar Malukani[1], Ashish Ranjan[2], Hota Shiva Jyothi[1], Hitendra K. Patel[1], Ramesh V. Sonti[1,3]

[1]CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India, [2]Department of Plant Pathology, University of Wisconsin – Madison, [3]National Institute of Plant Genome Research, New Delhi, India.

Abstract:

Plant cell wall is a formidable barrier for plant pathogens which is degraded by pathogens to cause infection. Plants can perceive this cell wall damage and induce immune responses. But how do plants perceive this damage is not known in most of the plant species. My work has revealed a new rice wall associated receptor kinase *OsWAKL21* that can potentially perceive cell wall damage caused by LipA, a *Xanthomonas oryzae* secreted cell wall degrading enzyme. Overexpression of OsWAKL21 in rice mimics treatment with LipA in induction of immune responses. Ectopic expression of *OsWAKL21* in Arabidopsis also activates plant immune responses. Biochemical characterization of OsWAKL21 indicates that it is a moonlighting kinase having *in vitro* kinase and guanylate cyclase (GC) activity. Interestingly, OsWAKL21 activates rice immune responses by its kinase activity but activates Arabidopsis immune responses by its GC activity.

O3

Uip4, a novel endoplasmic reticulum protein, maintains nuclear shape and cellular homeostasis in *S. cerevisiae*

Pallavi Deolal[1], Krishnaveni Mishra[1]
[1]Department of Biochemistry, University of Hyderabad, Hyderabad, Telangana
Email-pdeolal@gmail.com

Organelles coordinate their function to maintain the cellular homeostasis. The functional status of an organelle is to a large extent dependent on its structure. Nucleus is one of the most prominent cellular organelle and changes in the structural organization of nucleus are known to be associated with aging, several laminopathies and muscular diseases. In order to understand the nuclear organization and communication with other organelles, a genome–wide screen involving deletion mutants of non-essential genes in *Saccharomyces cerevisiae* was initiated in our lab. Loss of several non-nuclear resident proteins was found to affect the distribution of inner nuclear membrane protein and nuclear pore complexes. One such uncharacterized ER resident protein, Uip4, was studied for its effect on the assembly and distribution of nuclear pore complexes. Altered levels of Uip4 protein were also found to perturb the form and function of other associated organelles and various cellular processes. Genetics and imaging-based approach was adopted to further delineate the role of Uip4. Our findings related to the effect of altered levels of Uip4p on the nuclear structure and function will be presented.

Keywords- Nuclear organization, Ulp1 Interacting Protein, Yeast, NPC, ER

Chiral Proofreader as Acetaldehyde Detoxifying Translational Apparatus

Mohd Mazeed, Raghvendra Singh, Pradeep Kumar, Shobha P. Kruparani and Rajan Sankaranarayanan

CSIR-Centre for Cellular and Molecular Biology

Reactive aldehydes such as acetaldehyde and formaldehyde create havoc in the cell by creating permanent modifications on DNA and proteins. Modifications on DNA lead to various cancers such as Fanconi anaemia and protein modifications cause a compromise in their biochemical activity, which leads to damage of organs such as liver sclerosis. In our study, we have identified a hypersensitive acetaldehyde target i.e. aminoacyl-tRNA (aa-tRNA). Acetaldehyde makes ethyl modification (adduct) on amino acid of aa-tRNA; such modified aa-tRNAs are ultra-stable and not deacylated by canonical D-aminoacyl-tRNA deacylase 1 (DTD1) and peptidyl-tRNA hydrolase. We found a chiral proofreading module DTD2, which readily removes D-aa-tRNA adducts. Interestingly, DTD2 knockout plants do not grow in the presence of acetaldehyde. DTD2 is conserved only in Archaea and land plants where acetaldehyde biosynthesis machinery is present and this suggests the strict requirement of DTD2 in physiology and evolution of Archaea and land plants.

O5

IP6K1 is essential for germ cell junction development in male mice

Aushaq Bashir Malla, Sameer Ahmed Bhat, Vineesha Oddi and Rashna Bhandari
Laboratory of Cell Signalling, Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India

Mammals possess three isoforms of inositol hexakisphosphate kinases (IP6Ks), which are enzymes that catalyse the synthesis of the inositol pyrophosphate 5-IP7 from IP6. Mice lacking the IP6K1 isoform exhibit male-specific infertility. To investigate the underlying molecular mechanisms, we performed a gene expression microarray analysis of 17 and 26-day-old *Ip6k1*+/+ and *Ip6k1*-/- testes, and observed that transcripts encoding proteins involved in cell-cell junction formation within the seminiferous epithelium are deregulated in *Ip6k1*-/- mice. Immunofluorescence and western blotting studies show that cell junction proteins including espin, claudin11, GJA2, β1-integrin, β-catenin, and E-cadherin display either deregulated expression and/or aberrant localization within the *Ip6k1*$^{-/-}$ testis. Our results show that the loss of IP6K1 in mouse testis leads to disrupted Sertoli cell-germ cell junctions and Sertoli cell-Sertoli cell junctions, including the blood-testis barrier, ultimately resulting in abnormal orientation and premature sloughing of germ cells in the *Ip6k1*-/- testis.

O6

Functional characterization of YgeR, a putative peptidoglycan hydrolase of *Escherichia coli*

Pavan Kumar Chodisetti and Manjula Reddy

CSIR-Centre for Cellular and Molecular Biology, Hyderabad India 500007

Email: pavankumarchodisetti@ccmb.res.in

Bacterial cell wall is primarily made up of peptidoglycan (PG) as a protection against internal turgor. During cytokinesis, PG synthesis happens at the cell septum to generate new poles for daughter cells. Subsequently, daughter cells separate from each other by the action of division-specific amidases. In *E. coli*, it is known that LytM-domain proteins (EnvC and NlpD) activate amidases at the division site. Here, we show another LytM-domain protein, YgeR also functions at the division site and has a role in the separation of daughter cells. We find that multiple copies of YgeR is toxic to *E. coli* cells and generate PG fragments with glycan chains lacking peptides. In addition, more YgeR leads to enhanced division defects in cells lacking amidases indicating that YgeR is either a regulator of the amidases or may possess an amidase activity by itself. However, the precise role of YgeR needs to be further investigated.

O7

Analysis of structural diversity in the kinome and grouping of kinase inhibitors based on bound conformations.

Sravani Akula*, Venkata Krishna Vanamamalai, Vishnu Prasad Nair RU, Dashavantha Reddy Vudem and Rama Krishna Kancha.

**Presenting author*

Abstract

Proteins are dynamic entities and they possess an intrinsic flexibility to adopt multiple structures essential for them to execute their functions. The ability of a protein to change conformation in response to changes in their environment and upon ligand binding provides the physical underpinning for various biological processes and important for signal transduction, protein-protein interactions and enzyme catalysis. For a given enzyme, at steady state, ensemble of structures exist in dynamic equilibrium of which only definite "reactive conformation(s)" is/are selectively bound and stabilized by a specific ligand. Therefore, depending on the chemical diversity of the ligand, several stable conformations are possible for a given enzyme thus representing the existing chemotype diversity for a target enzyme. Considering this, in this current study, we employed large scale superposition analyses for 2,107 (co-) crystal structures belonging to 149 enzymes (89 kinases and 60 non-kinases). Furthermore, we also grouped kinase inhibitors for a given enzyme based on the conformations they stabilize. Our results revealed the existence of conformational diversity among the various classes of enzymes. Of which protein kinase superfamily display extensive conformational diversity compared to non-kinases. This comprehensive study of structural diversity in the kinome is important to understand conformational features associated with kinase regulation which is crucial for selectively targeting abnormally regulated kinases in diseases.

O8

Growth Hormone Induces Notch Signaling in Podocytes

Rajkishor Nishad, Dr.P.Anil Kumar

University of Hyderabad, Hyderabad, India-500046

Growth hormone (GH) plays a significant role in normal renal function and overactive GH signaling has been implicated in proteinuria in diabetes. Previous results have shown that the glomerular podocytes, which play an essential role in kidney filtration, express the GH receptor (GHR), suggesting the direct action of GH on these cells. However, downstream pathways that are induced by the excess GH in these podocytes leading to diabetic nephropathy is not clearly established. First time using human podocytes *in vitro* and using mice model *in vivo*, we show that excess GH activates Notch1 signaling. Inhibition of Notch1 with DAPT abrogated GH-induced epithelial - mesenchymal transition (EMT) in both cultured podocytes and, in the mouse glomerular lysates and is associated with a reduction in podocyte loss. Further, DAPT prevented renal fibrosis as well as proteinuria induced by excess GH. All these results confirm that excess GH induces Notch1 signaling in podocytes, which contributes to proteinuria through EMT as well as renal fibrosis.

ABSTRACTS FOR POSTER PRESENTATION

P1

Generating LCA12 mutant models in zebrafish

Divya Pidishetty*, Praveen Joseph Susaimanickam*, Gopal Kushawah, Puja Sarkar, Rakesh Mishra, Indumathi Mariappan

Sudhakar and Sreekanth Ravi Stem Cell Laboratory, Centre for Ocular Regeneration, Hyderabad Eye Research Foundation, LV Prasad Eye Institute, Hyderabad India

Purpose:

To knockout zebrafish *rd3* linked to retinal degeneration condition in humans calledLCA12and to study its effects on retinal development.

Methods:

CRISPR-Cas9 editing tool was used to create *rd3* mutant models in zebrafish. Larvae 3-days post injection (dpi) were screened for in-dels by Sanger sequencing. The injected batch that contained in-dels,was allowed to attain juvenile stage and tail fin clip genomic DNA analysis was done to identify (F_0-*rd3*) founder fish.F_0-*rd3*was backcrossed with *wt* animals to obtain F_1-*rd3*heterozygotes (F_1-*rd3*$^{+/-}$). Interbreeding of F_1-*rd3*$^{+/-}$resulted in F_2-*wt*, F_2-*rd3*heterozygotes *(*F_2-*rd3*$^{+/-}$) and F_2-*rd3*null homozygotes (F_2-*rd3*$^{-/-}$), whose retinal morphology was compared by immunohistological analysis.

Results:

The edit efficiency was found to be 16.66%. Immunostaining for cone arrestin at different time points showed gross reduction in the cell number and lamination defects in the retina, indicative of retinal degeneration.

Conclusion:

Zebrafish *rd3* null mutants with retinal degeneration were created.

P2

GREEN SYNTHESIS OF SILVER NANOPARTICLES OF ONION DNA AND SCREENING FOR *IN VITRO* ANTITYROSINASE ACTIVITY

SNEHA THAKUR[1*], Dr. G. KRISHNA MOHAN[2]

[1]Department Of Pharmacognosy, Bojjam Narasimhulu Pharmacy College For Women, Saidabad, Hyderabad-500059, Telangana, India.

[2] Department Of Pharmacognosy, CPS, IST, JNTUH, Kukatpally, Hyderabad-500059, Telangana, India.

Abstract

The main objective of the research work was to evaluate the anticancer potential of onion DNA silver nanoparticles The onions were procured from the local market and DNA was extracted by standard method. The isolated DNA acts as reducing agent for synthesis of silver nanoparticles by green method. Further they were characterized by UV-Vis, FTIR, SEM, XRD and DLS studies. The results revealed that the particles were uniform in shape, 153±0.5 nm in size with face centered cubic structure. The DNA silver nanoparticles were further investigated for its antityrosinase against the standard kojic acid and were to have anticancer potential nearer to the standard.

P3

Enumeration of frequency of ERBB2 kinase mutations in solid cancers based on clinical data sets

Archana Katta, Rama Krishna Kancha

Molecular Medicine and Therapeutics Laboratory, Centre for Plant Molecular Biology, Osmania University, Hyderabad, India

Abstract:

ERBB2/HER-2 is a receptor tyrosine kinase which plays a crucial role in cell survival and proliferation pathways. ERBB2 mutations were shown to be oncogenic and a proven therapeutic target in multiple solid cancers. In the present study, we collected the data regarding the incidence of ERBB2 mutations in various solid cancers, analyzed their expression patterns

P4

Natural Products as drug candid ates against inflammatory bowel disease (IBD)

Kotha Anilkumar, Gangappa Dharmapuri, and Pallu Reddanna
Department of Animal Biology, School of Life Sciences,
University of Hyderabad, Hyderabad 500046, India

IBD is a chronic relapsing inflammation afflicting any part of the gastrointestinal tract. It is the result of a deregulated, aberrant and even inappropriate mucosal response in the intestinal wall due to the defects of barrier function of the intestinal epithelium and mucosal immune system. The two distinct subtypes of IBD, classified basing on the location and duration of inflammation, include Ulcerative Colitis (UC) and Crohn's Disease (CD). As there is no complete cure for IBD the goal is to decrease the relapse episodes and increase the patient quality of life. Anti-inflammatory drugs are often the first step in the treatment of IBD. They include: anti-inflammatory drugs, immune system suppressors, antibiotics and other medications. However, these have a number of side effects, including digestive distress and headache. (Talaeti et al., 2013; Hassan and Soliman, 2010). A number of studies have revealed an association between the elevation of eicosanoids, the metabolites of arachidonic acid formed principally via the lipoxygenase (LOX) and cyclooxygenase (COX) pathways, and the pathophysiology of IBD (Sklyarov et al., 2011). However, the treatments with non-steroidal anti-inflammatory drugs (NSAIDs) are associated with variable clinical response and frequent deterioration in response. The selective COX-2 inhibitors (COXIBs), on the other hand, have cardiac side effects. In the light of the above it is proposed to develop COX-2 and 5-LOX dual inhibitors (CLOXIBs), from natural source as potential candidates for the treatment of inflammatory Bowel Diseases.

P5

Estimation of variability in reported activities of chemotherapeutics on commonly used cancer cell lines

Shravanthi Ravula, Dedeepya Chermala, Sravani Akula, Dashavantha Reddy Vudem and Rama Krishna Kancha

Molecular Medicine & Therapeutics Laboratory, CPMB, Osmania University, Hyderabad

ABSTRACT

Cancer cell lines such as HeLa, A549, MCF7 and K562 are commonly employed for determining the bio-activity of newly synthesized chemical entities. A reference chemotherapeutic is used as a reference compound to compare the activity of novel compounds. However, considerable variation exists in published literature with regards to IC50 values for these reference compounds towards cancer cell lines leading to erroneous interpretation of anti-cancer activity. In the present study we have estimated the variability in anti-cancer activity and derived a consensus IC50 value and acceptable range for each drug-cell line pair which may serve as a reference for medicinal chemists to test the activity of new molecules. Further,based on the consensus IC50 values, the cell lines are classified as either sensitive or resistant and the factors that influence the variability were determined.

P6

In vitro & *In vivo* Toxicological Studies of Synthesized Chromium (III) Oxide Nanoparticles: β-cyclodextrane coated Nanoparticles in Medicinal usage

SANKARARAO GANTA, Dr. Mohd. Mahboob

CSIR-INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY

Nanoparticles has, potential to revolutionize our lives as they have unique physico-chemical features encompassing beneficial properties surpassing those of traditional substances. Such features; Chromium (III) Oxide NPs possess enhanced electrical and thermal conductivity, more efficient catalysts, high tensile strength or improved drug delivery vehicles. Consequently NPs have potential applications in a wide range of products with applications in diagnosis, drug delivery, food industry, paints, electronics, sports, environmental cleanup, cosmetics, and sunscreens. Smart ways of delivering drugs to the site of interest is the goal of the study; mitigates drug leakage in the system; *inter alia* decrease side of effects of potential cancer drugs.

P7

MAP kinases as new players in translation regulation

Haripriya Parthasarathy, Sana Parveen, Dhiviya Vedagiri, Krishnan H. Harshan

Centre for Cellular and Molecular Biology (CSIR-CCMB), Hyderabad

Protein translation is a highly energy-expensive process whose regulation is essential for cellular homeostasis. mTOR complexes are considered master regulators of translation by mediating phosphorylation of molecules such as 4EBP1 that sequesters cap-binding eIF4E. MAP kinases respond to various stress stimuli, influencing translation via eIF4E. To understand their role, we used polysome profiling to compare effects of mTOR and MAPKs inhibition. Interestingly, dual MAPK inhibition of p38 and ERK1/2 causes a more pronounced impact on translation. Western blotting results indicate that two major players of stress response, eIF2α and AMPK may mediate this. Their concomitant activation in relation to MAPKs is yet to be reported. Simultaneous eIF2α activation-p38 inhibition shows a large impact, indicating p38 MAPK may have a major role in translation regulation and will help in further understanding the intricacies of translation regulation.

P8

HUMAN PAX6 PROMOTER A AND ITS REGULATION IN LIMBAL STEM CELLS

Vinay Kumar Pulimamidi, Savitri Maddileti, Indumathi Mariappan

Sudhakar and Sreekanth Ravi Stem cell Laboratory, Center for Ocular Regeneration, Hyderabad Eye Research Foundation, LV Prasad Eye Institute, Hyderabad, India

Purpose: To characterize the human *PAX6* P_A promoter and to assess its activity in limbal stem cells

Methodology:OSE enhancer and different promoter regions of human *PAX6, p63, K3/K12*and PAX6 variants (wt and 5a) were cloned into pGL3-Basic and lentiviral constructs.

Results: *PAX6*-$P_A$promoter is active in HCE and ARPE19 and moderately active in 661W and HLE-3B cells. The OSE enhancer repressed $P_A$activity in HCE cells and induced $P_A$activity in lens and retinal cells. BIO treatmentactivated the wt-$P_A$but not the mutant promoter in HCE andHDAC inhibition by valproic acid treatment activatedboth the wt and mutant $P_A$in HCE. Thissuggests that $P_A$is regulated by wnt signals and HDACs in HCE.All PAX6 variants repressed the activities of *K3, K12, TAp63* and *dNp63* promoters

Conclusion:P_A is the dominant promoter in corneal cells and tightly regulated by wnt dependent and independent mechanisms.

P9

Human primary retinal cells as an in-vitro cell culture model for investigating defective signalling caused by Optineurin mutants associated with glaucoma

Zuberwasim Sayyad[1], Sushma Vishwakarma[2], Tarjani Vivek Dave[2], Inderjeet Kaur[2], Vegesna Radha[1], Ghanshyam Swarup[1,*]

[1]CSIR- Centre for Cellular and Molecular Biology, Hyderabad, India 500007
[2]Prof Brien Holden Eye Research Centre, L.V. Prasad Eye Institute, Hyderabad, India

The E50K and M98K mutants of Optineurin (OPTN) associated with glaucoma induce cell death specifically in mouse Retinal Ganglion Cells (RGCs) but not in other cell types. Studies carried out on the pathogenesis of glaucoma using murine cell lines and animal models require to be validated in human cells, but authentic model systems are lacking. We have characterised adult Human Primary Retinal Cells (hPRCs) in culture that attain RGC-like properties suggestive of dedifferentiation. Glaucoma associated mutants of OPTN selectively induce cell death in these cells. M98K-OPTN induced cell death signalling is dependent on activity of TBK1, CaMKKβ and AMPK. Amlexanox, a clinically approved drug, which is a TBK1 inhibitor, also inhibited cell death induced by both M98K and E50K suggesting its therapeutic potential for glaucoma therapy/ management. These cells can serve as in-vitro model system that be used to explore the molecular mechanisms of glaucoma pathogenesis and for drug testing.

P10

Mitochondrial dysfunction on cellular homeostasis: Importance of integrated stress response pathway

K Rama Gopal Reddy , Naresh B Sepuri, Ramaiah V Kolluru.

Department of Biochemistry, UoH.

Corresponding Author: ramaiah.kolluru@gmail.com

CCCP that affects mitochondrial function promotes phosphorylation eIF2α independent of PERK or GCN2 but dependent of HRI activation. CCCP induced eIF2α phosphorylation promotes translation of ATF4 and CHOP and plays a role in promoting autophagy or cell death. In addition, CCCP reduces expression of BiP, an ER chaperone, XBP1 splicing, PARP cleavage and caspase activity in Thapsigargin treated cells suggesting that it reduces ER stress induced UPR. Further, CCCP induced eIF2α phosphorylation is also correlated in our studies to AMPK activation, reduction in S6 kinase and eIF4EBP phosphorylation. ISRIB, a small molecule inhibitor of ISR pathway reduces CCCP induced ATF4 &CHOP expression and autophagy but fails to reducing the activation of AMPK. Further ISRIB reduces CCCP induced cell death which is independent of caspase activation and ER stress mediated apoptosis in HepG2 cells.

P11

Cell Cycle Dependent Modulation of Membrane Dipole Potential: Correlation with Membrane Cholesterol Content

Bhagyashree D. Rao, Parijat Sarkar and Amitabha Chattopadhyay*

CSIR-Centre for Cellular and Molecular Biology , Uppal Road, Hyderabad 500 007, India

E-mail: bhagyashree@ccmb.res.in

Cell cycle is a well-organized sequence of events which occurs in living cells of multicellular organisms and leads to cell multiplication for growth and tissue repair. Since cholesterol is modulated in different cell cycle stages, this could be accompanied by alterations in membrane physical properties. An important physical property of biological membranes is dipole potential which could be crucial for protein function. In this context, we explored membrane dipole potential in different stages of cell cycle. Our results show that dipole potential is highest in the G1 phase relative to S and G2/M phases. This is accompanied by variation in membrane cholesterol content in a cell cycle specific manner. Importantly, we show that cell cycle dependent modulation in dipole potential correlates closely with membrane cholesterol content. Such cell cycle dependent modulation of membrane dipole potential could affect physiological events related to the cell cycle.

P12

IP6K1 participates in the removal of RAD51 from sites of DNA damage

Shubhra Ganguli and Rashna Bhandari
Laboratory of Cell Signalling, Centre for DNA Fingerprinting and Diagnostics, Hyderabad

The inositol pyrophosphate 5-IP_7 is an energy-rich small signalling molecule. Mechanistically, 5-IP_7 modulates cellular functions by either directly binding to proteins or by bringing about protein pyrophosphorylation, during which it transfers its β-phosphate to a pre-phosphorylated serine residue in a protein. We observed that upon treatment with genotoxic stressors, DNA damage foci marked by γH2AX and RAD51 persisted longer after drug removal in U-2 OS cells with reduced IP6K1 levels, indicating that 5-IP_7 is essential to complete homologous recombination (HR) mediated DNA repair. Surprisingly, we did not observe any defect in an HR reporter assay, suggesting that the defect lies in the removal of repair proteins. In parallel, we identified that the C-terminal domain of BRCA2, required for RAD51 removal, undergoes phosphorylation by CK2, which is prerequisite for pyrophosphorylation. We are currently investigating whether pyrophosphorylation of BRCA2 regulates its interaction with RAD51 and its consequent removal from DNA damage sites.

P13

P S Kesavan

Monitoring global changes in chromatin compaction states upon localized DNA damage with tools of fluorescence anisotropy

DNA in cells is subject to continuous damage from endogenous and exogenous sources. Failure to repair such damage can cause mutations that give rise to diseases like cancers and neurodegenerative diseases. DNA is compactly packed in the form of chromatin inside the nucleus. The compact chromatin structure acts as a barrier for efficient repair of DNA, but also can make DNA more refractory to damage. Chromatin has to be remodeled transiently for DNA damage responses (DDR) to work efficiently. We use fluorescence anisotropy imaging of histone H2B-EGFP to image the compaction state of the chromatin. H2B-EGFP anisotropy maps can be used to map out the euchromatin or heterochromatin like regions in live and fixed cells. We induce local double strand breaks with laser microirradiation and observe the dynamics of chromatin compaction states in live cells for 2 h after damage, and then later correlate it to markers of damage using immunofluorescence. Compact nodes of chromatin nodes were formed, and overall there was a global compaction of chromatin upon local irradiation. We also observed the dynamics of endogenous repair factors using chromobodies for PARP1 and PCNA, which were immediately recruited to the site of damage. PARP1 showed a more transient recruitment than PCNA. Together these studies are yielding insight into changes in chromatin compaction states and associated responses upon local induction of clustered double strand breaks.

P14

Membrane-cytoskeleton interaction shapes GPCR dynamics and function

Parijat Sarkar* and Amitabha Chattopadhyay

CSIR-Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India

*E-mail: parijat@ccmb.res.in

The function of actin cytoskeleton in cellular motility and trafficking has been widely studied. However, reorganization of actin cytoskeleton upon modulation of membrane cholesterol is addressed only rarely. In this work, we have explored the reorganization of actin cytoskeleton and its implications in membrane protein dynamics and function, upon modulation of membrane cholesterol by statins. Our results showed that F-actin content significantly increases in response to membrane cholesterol depletion, which could be due to a synergistic effect of multiple pathways. In this context, we explored the role of actin cytoskeleton in regulating the dynamics of the serotonin$_{1A}$ receptor, a crucial neurotransmitter G protein-coupled receptor (GPCR) that plays a major role in the generation and modulation of cognitive and behavioral functions. On a broader perspective, these results assume significance in understanding the modulatory role of the membrane environment on the organization and function of GPCRs.

P15

RAPGEF1, interacts with and inhibits GSK3β activity to promote C2C12 differentiation.

Divya Sriram[1], Kunal Dayma[1], A Sharada Devi[1], Vegesna Radha[1]
[1]Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India.

GSK3β, a ubiquitously expressed Ser/Thr kinase, regulates cell metabolism, proliferation and differentiation. In this study, we identify RAPGEF1(C3G), a protein essential for mammalian embryonic development, as an interacting partner and substrate of GSK3β. Modelling and mutational analysis, identify a GSK3β interaction domain in RAPGEF1. In vivo and in vitro interaction assays demonstrate that GSK3β and Akt are present in a complex with RAPGEF1. GSK3β phosphorylates RAPGEF1 on primed and unprimed sites. Inhibition of cellular GSK3β activity enhances RAPGEF1 localization to nucleus. Over-expression of RAPGEF1 results in activation of Akt and inactivation of GSK3β, dependent on PI3K activity. RapGEF1 knockdown C2C12 cells (CRISPR/Cas9 mediated) fail to differentiate and exhibit reduced Akt activation and high GSK3β activity. RAPGEF1 induced C1C12 differentiation is dependent on its ability to inactivate cellular GSK3β activity. Our results identify reciprocal regulation between GSK3β and RAPGEF1, and elucidates mechanism of RAPGEF1 induced myogenic differentiation.

P16

IP6K1 regulates P-body formation by modulating mRNA degradation

Akruti Shah[1,2] and Rashna Bhandari[1]

[1]Laboratory of Cell Signalling, Centre for DNA Fingerprinting and Diagnostics, Hyderabad
[2]Manipal Academy of Higher Education, Manipal

IP_6 kinases (IP6K1/K2/K3 in mammals) form the inositol pyrophosphate 5-IP_7 from the inositol phosphate IP_6. In mice, IP6K1 regulates the assembly of male germ-cell-specific chromatoid bodies. We report the effect of IP6K1 on P-bodies, functional analogs of chromatoid bodies in somatic cells involved in mRNA storage and degradation. Loss of IP6K1 causes diminished P-bodies, and downregulation of P-body marker proteins- EDC4, DCP1A/B and DCP2. IP6K1 interacts with these proteins on ribosomes, suggesting an IP6K1-mediated decapping complex formation on ribosomes. P-bodies are formed when the decapping complex replaces the translation initiation complex on the mRNA cap. We demonstrate that IP6K1 also interacts with the mRNA cap recognizing translation initiation proteins, eIF4G1 and eIF4E. We are currently investigating whether IP6K1 promotes the replacement of the translation initiation complex with the mRNA degradation complex at the mRNA cap. In summary, we propose that IP6K1 regulates P-body formation by modulating mRNA metabolism on ribosomes.

P17

Cholesterol-induced Switch in GPCR Endocytosis and Trafficking: Excitements and Opportunities

G. Aditya Kumar and Amitabha Chattopadhyay*

CSIR-Centre for Cellular and Molecular Biology, Hyderabad 500 007, India

Endocytosis is a key regulatory mechanism adopted by G protein-coupled receptors (GPCRs) to modulate receptor-mediated downstream signaling responses. The function, organization and dynamics of GPCRs are intimately associated with their surrounding lipid environment. Previous work from our group has delineated the role of membrane cholesterol in the organization, dynamics and function of the serotonin$_{1A}$ receptor, a representative GPCR. However, the role of cholesterol in GPCR endocytosis and intracellular trafficking remains largely unexplored. In the present study, we show that statin-induced chronic cholesterol depletion switches the endocytic pathway of the serotonin$_{1A}$ receptor from clathrin- to caveolin-mediated endocytosis. Importantly, upon chronic cholesterol depletion, a significant proportion of endocytosed receptors is rerouted toward lysosomal degradation, with a considerable reduction in their fraction undergoing membrane recycling. Our work assume significance in the backdrop of emerging literature suggesting that the combination of antidepressants and statins exhibit superior antidepressant effects relative to antidepressant treatment alone.

P18

Nuclear Matrix has a nonrandomly arranged protein core

Ashish Bihani, Rakesh K. Mishra

Nuclear regulation of gene function is controlled and executed by a myriad of processes, *e.g.*, transcription, transcript processing and splicing, chromatin condensation and decondensation, chromatin translocation, *etc*. The factors involved in all of these processes have been observed to be anchored to Nuclear Matrix (NuMat), an architectural element biochemically isolated from the nucleus. Using a charged chaotrope, Guanidine Hydrochloride, we attempt to find the elements of NuMat that potentially constitute a nucleoskeleton, characterize their structural dynamics during cell cycle and their role in gene regulation. We suspect that proteins contributing to the bulk of NuMat proteome are arranged in a definite spatial arrangement.

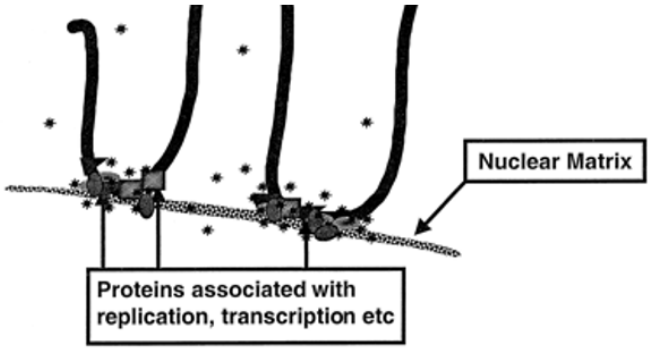

Figure modified from
Brown, 1999, Cancer Research

1. Differentiate between loosely bound proteins and tight core proteins
2. Check for a hierarchy amongst filamentous proteins and globular proteins

Extraction with 1M, 2M, 3M, 4M Guanidinium Hydrochloride

P19

Repurposing the biased visibility in Hi-C datasets to mark dynamically regulated condensed and decondensed chromatin states genome-wide

Prashanth Kumar Guthikonda[$,1],Keerthivasan Raanin Chandradoss[$,2] , Srinivas Kethavath[1], Monika[2], Harpreet Singh[2], Rakhee Nayak[1], Kuljeet Singh Sandhu[*,2], Sreenivasulu Kurukuti[*,1]

[1]Department of Animal Biology, School of Life Sciences, University of Hyderabad (UoH), Professor CR Rao Rd, P O, Central University, Gachibowli, Hyderabad, Telangana 500046, India

[2] Department of Biological Sciences, Indian Institute of Science Education and Research (IISER) – Mohali ,Knowledge City, Sector 81, SAS Nagar 140306, India

[$]Equal contribution

[*]Co-corresponding authors (*skurukuti@uohyd.ac.in*, *sandhuks@iisermohali.ac.in*)

Proximity ligation based techniques, like HiC, involve restriction digestion followed by ligation of formaldehyde cross-linked chromatin. Through analysis of lamina-associated domains (LADs) and polytene bands in fly, we first established that the DNA in condensed chromatin had lesser accessibility to restriction endonucleases used in HiC as compared to that in decondensed chromatin. The observed bias was independent of known systematic biases, was not appropriately corrected by existing computational methods, and needed an additional optimization step. We then repurposed this bias to identify novel condensed domains outside LADs, which were bordered by insulators and were dynamically associated with the developmentally regulated epigenetic and transcriptional states. Our observations suggested that the corrected one-dimensional read counts of existing HiC datasets can be reliably repurposed to study the gene-regulatory dynamics associated with chromatin condensation and decondensation

P20

3-Dimensional organisation of inter chromosomal regions during cellular differentiation

Dharmapal Burne[1], Srinivas Kethavath[1], G. Prashanth Kumar[1], Sreenivasulu Kurukuti[1,]*

[1]Department of Animal Biology, School of Life Sciences, University of Hyderabad – 500046.

3D organization of the genome has been shown to play a major role in orchestrating cell type specific gene regulatory networks. Though chromosomes were shown to occupy discrete locations in the nucleus termed Chromosome Territories, there exist multiple functional interactions between the chromosomes. Many recent studies have focused on understanding the principles behind chromosomal architecture at the level of topological associated domains (TADs) and sub-TADs. However, very few studies focused on understanding the principles of inter chromosomal domains and interactions clusters. We chose Lactogenic differentiation of mouse Mammary Epithelial Stem like Cells (HC11) under the influence of Glucocorticoids and Prolactin as a model system to understand the 3D interactions between the chromosomes and their functional relevances. We analysed in-house generated *in-situ* Hi-C data and studied the 3D clusters of interactions between functional elements in the context of lactogenic differentiation. Our results provide valuable insights into principles governing 3D intermingling chromosomes regions and clustering during cellular differentiation in general.

Keywords: Chromosomal architecture, TADs, inter chromosomal domains.

P21

Kinetic Model of the Sodium Glucose Co-transport, A Kinetic Simulation

Adithya Pallepati[1], Ravi Kumar Gutti[1], Chanchal K Mitra[1]
[1] Department of Biochemistry, School of Life Sciences, University of Hyderabad.

***Abstract*—** We describe the kinetic simulation of a protein assisted (transporters) transport of ions (univalent cations like Na^+ and K^+) as well as neutral molecules (glucose for example) across the biological membrane (phospholipid bilayer) using conventional kinetic model (Michealis-Menten equation) and an open-source software to simulate the dynamics. We assume constant electric field within the lipid layer and hence a constant force on the ionic species being transported. We also consider the free energy contribution from the concentration gradient (and this applies to both charged and neutral species). We ignore the contribution from the protein due to conformational changes (because little details are available) although this may possibly be significant. The simulation is carried out using Octave (https://www.gnu.org/software/octave/) that follows a matlab like syntax. Most of our results are presented graphically.

Key words: Transporters, simulation, dynamics, octave

P22

Developing tumor model using zebrafish embryos for rapid screening of potential anti-tumor candidates

Sulagna Mukherjee

Abstract

Currently preclinical studies depend on mouse xenografts to assess the efficacy of potential anti-tumor drug molecules and compounds. These studies on humanized murine models are technically challenging with toxicity and reproducibility issues and are time consuming and expensive. We propose to develop an alternative tumor model using zebrafish embryos, which will complement the existing methodology with more rapid identification of compounds entering pre-clinical and clinical trials. This could also be used to rapidly screen patient tumor sample for the personalized medicine initiative. The alternative tumor model using zebrafish embryos is based on the fact that the oncogenic signaling and gene expression signatures are highly conserved between human, mice and zebrafish tumors. Zebrafish can spontaneously develop a range of benign and malignant tumors in many organs - liver, GIT, pancreas, muscle, and skin in a manner akin to tumor development in humans. Here, we use the tumor transplant approach in early stages of zebrafish embryos to study early events of tumor development, neovascularization and invasion. This short-term assay will be useful to identify candidate tumorigenic inhibitors.

P23

Differential role of EGFR pathway members in *Drosophila* eye development revealed by single molecule FISH

Nikhita Pasnuri[1] and Aprotim Mazumder[1]
TIFR Centre for Interdisciplinary Sciences, Tata Institute of Fundamental Research Hyderabad, 500107, Telangana, India.

The eye imaginal discs from the 3rd instar larva of the fruit-fly *Drosophila melanogaster* serves as an interesting system to understand pattern formation as the ommatidial numbers and arrangement in the adult eye are highly reproducible.

The EGFR signaling pathway plays important roles during eye development. Multiple activating ligands and feedback loops mediated by positive and negative regulators of the pathway fine-tune the signaling spatiotemporally, which can act cell autonomously or non-cell autonomously depending on their diffusion properties and mode of action.

Through single molecule RNA Fluorescent *in situ* Hybridization (smRNA FISH), we track the cell-types which transcribe *spitz*, the principle EGF and *argos*, a transcriptional target of EGFR signaling which attenuates the signaling by sequestering Spitz. Both the proteins act through diffusion across several cell diameters. We observe that *spitz*, which is expressed by the photoreceptors, acts non-cell autonomously as the *argos* expression is seen exclusively in neighbouring non-photoreceptor cells.

I will present how we aim to correlate mRNA numbers of downstream targets (eg. *argos*) to EGFR pathway strength and in turn to cell cycle indices to finally elucidate cell autonomous vs non-cell autonomous relations between different members of EGFR pathway.

P24

Reordering the bithorax complex: Understanding spatial collinearity of *cis*-regulatory elements

Nikhil Hajirnis, Rakesh K. Mishra

CSIR-Center for Cellular and Molecular Biology, Hyderabad 500007, India

Homeotic genes (Hox) encode transcription factors that set up anterior-posterior body axis of a developing embryo and are conserved across all bilaterians. Despite having the same number of Hox genes in arthropods, their mere expression pattern provides the diversity that we see in the phylum. The differential expression is attributed to *cis*-regulatory elements (CREs) of these genes. The significance of spatially colinear positioning of CREs in genome with respect to their pattern of expression in developing anterior posterior body axis is still debatable. We are trying to reorganize CREs of one such Hox gene, *Abdominal-B* that is responsible for the specification of A5 through A8 abdominal segments in developing embryo of *Drosophila melanogaster.* Specific deletion mutations have been characterised but there has been no attempt to redesign the *cis*-regulatory landscape by adding more regulators or inverting the positions of existing ones. We observe as yet unreported phenotypes of fly indicating their positional significance in driving different types of body axes across different phyla upon reorganizing the cis-regulatory landscape.

P25

IP6K1 plays an essential role in mammalian gastric physiology

Jayraj Sen and Rashna Bhandari
Laboratory of Cell Signalling, Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India

Inositol hexakisphosphate kinases (IP6Ks) catalyse the synthesis of the inositol pyrophosphate 5-IP7 (5-diphosphoinositol pentakisphosphate) from IP6 (inositol hexakisphosphate).Mice lacking the IP6K1 enzyme show different physiological defects including male infertility, delay in blood clotting, reduced insulin levels,and lower body weight compared to wild type mice. The expression of IP6K1 is conserved throughout the gastrointestinal tract epithelium, including stomach, ileum, colon, and rectum. We have observed that IP6K1 is highly expressed in isolated mouse gastric glands, which secrete enzymes including pepsin that are involved in dietary protein digestion.Immunofluorescence studies show that IP6K1 is localized to chief cellsin the gastric gland. IP6K1 knockout mice have a reduced number of chief cells and lower secretary granules in these cells. We propose that IP6K1 expression in gastric chief cells plays an important role in the regulation of secretory granule formation and digestion physiology in mammals.

P26

Reconstitution of the mouse germ cell development *in vitro* from embryonic stem cells

Umesh Kumar, Nithyapriya Kumar, P Chandra Shekar, K Thangaraj

Centre for Cellular and Molecular Biology, Hyderabad, India

Embryonic stem cells (ESCs) are pluripotent cells have the potential to differentiate into all different somatic cell types and germ cells. Primordial germ cells, the precursors of germ cells arise at post implantation stage of the embryo. PGC differentiates from a subset of the cells of the epiblast stem cells (EpiSC) in response of signaling molecule from the vicinity cells. Recently different regimes for the in vitro germ cell differentiation from ESCs and induce pluripotent cells (iPSCs) have been identified. In the study, we created a double reporter ESC line for two different stage germ cell markers, DPPA3 and DDX4. DPPA3 expresses exclusively in primordial germ cells. DDX4 is also a germ cell specific marker express into pre meiotic germ cells. We tagged DPPA3 with mCherry and DDX4 with GFP, endogenously using RISPR-CAS9 assisted recombination, By using this cell line, we develop an efficient and simplest protocol to differentiate ESC to PGC and then spermatogonia. We achieve up to 50% PGC differentiation these PGC further differentiate into spermatogonia. By using the developed protocol we demonstrated the role of a novel gene Tex13 which we identified as a target for male infertility. The study will be helpful to explore the regulatory mechanisms for germ cell development and useful for developing regenerative medicines to rescue the infertility.

P27

Deepanwita Purohit

Captive breeding programs are used as valuable tool for the conservation and restoration of threatened species by supplying population stock to various reintroduction programs. Notwithstanding, captive bred individuals often have reduced fitness when introduced into the wild. Several hypotheses explain the cause of reduced fitness like, inbreeding, relaxed natural selection and adaptation to captivity.The present work aims to investigate the occurrence of inbreeding depression in a captive population of critically endangered pygmy hog comprising individuals from six generations. In addition, the study also estimates the presence of major histocompatibility complex variants in each individual which will inform its vulnerability/resistance to infectious diseases. Furthermore, associations between inbreeding, MHC variability and fitness will be tested for each individual. The study will inform the mechanism behind the reduced fitness in captive population and will help in genetic management of breeding population.

P28

Molecular and functional venom variation in geographically distinct populations of Saw-scaled viper *Echis carinatus*

Siddharth Bhatia, Karthikeyan Vasudevan
Centre for cellular and molecular biology (CCMB), Hyderabad, India

Snakebite in India claims around 50000 lives annually and several victims are left with physical disability. This alarming mortality can be attributed to lack of infrastructure in PHCs, people resorting to faith healer, Antivenom ineffectiveness, etc. There are several reports showing inability of Indian polyvalent antivenom (ASV) to neutralize the snake venom. We hypothesize that the ineffectiveness of ASV could be due to the geographical venom variation. Since ASV is made from venom sourced majorly from Irula coop based in Chennai, ASV might not be effective in neutralizing snake venom from other regions. To test the hypothesis, we collected saw-scaled viper venom from Tamil Nadu, Goa and Rajasthan and perform detailed compositional analysis using RP-HPLC, SDS-PAGE and Mass Spectrometry. Our study found toxins belonging to 10-12 toxin families which differ in its composition among venom collected from these locations. We analyzed the antivenom-venom immune complex formation for different venoms using size-exclusion chromatography. The dose dependent parameters estimated showed variation in binding of ASV to venoms from different locations. We conclude that there is geographical venom variation in saw-scaled viper, causing differential binding of ASV. This makes the commercially available polyvalent ASV inefficient for treatment of snakebite victims.

Characterization of sex pheromones in endangered Indian Mouse deer (*Moschiola Indica*)

Vinod Kumar and G Umapathy

Laboratory for the Conservation of Endangered Species (LaCONES), CSIR-Centre for Cellular and Molecular Biology (CCMB)

Pheromones are airborne chemical signals that are released by an individual which affect the physiology or behavior of other members of the same species. We discovered androstenone and androstenol pheromones in mouse deer, which are supposed to be involved in reproduction. The present study aimed to characterize the androstenone at molecular level in Mouse deer. We used 5' and 3' RACE PCR procedures to isolate, clone and obtained the full-length sequence of cDNA encoding P450c17.A full-length cDNA and open reading frame encoding P450c17 from the testicular tissue of mouse deer was of 1539 bp in length and encoding a putative enzyme of 512 amino acids. We found 6 extra amino acids in the coding frame of P450c17 in mouse deer as compared to porcine and human in which pheromone was already reported. Further, a detailed study is required to examine the enzymatic activity of P459c17 for conversion of pregnenolone to 5,16 androstadien -3beta-ol.

P30

Uncoupling translation from transcription as a contributor to R-loop formation during sense transcription in *Escherichia coli.*

Apuratha Pandiyan and J. Gowrishankar, Laboratory of Bacterial Genetics
Centre for DNA Fingerprinting and Diagnostics, Uppal, Hyderabad

Abstract:

R-loops are three-stranded structures comprised of an RNA:DNA hybrid and a displaced single-stranded (ss)DNA. Bisulphite-treatment (which selectively deaminates Cytosines in ssDNA to Uracils) followed by whole-genome-sequencing was done earlier in the lab to identify regions prone to form R-loops which were termed as bisulphite-reactive-regions (BRRs). In this study, we have compared the occurrence of R-loops as defined by BRR percentiles during sense transcription with translation-efficiency, nascent transcriptome (NET-Seq), and RNA abundance (RNA-Seq), for the genes in *E. coli*. We found low translation-efficiency for genes with low (< 20 %ile) BRRs and high (> 80 %ile) BRRs. Surprisingly, the NET-Seq and RNA-Seq of Rho-inhibited cells show greater expression of low BRRs but not high BRRs. UvsW (an R-loop helicase) expression in Δ*rho* mutant shows a higher expression of high BRRs. Therefore, our data implies that low translation-efficiency leads to R-loop formation in high BRRs in absence Rho-dependent transcription termination.

P31

Analysis of Telomere Length and Mitochondrial DNA Copy Number Variation in Apparently Healthy Adults: A Cross Sectional Study in South Indian Population

Guruvaiah Praveen[1], PhD; Tattari Shalini[1], MSc; Mudili Sivaprasad[1], PhD; Geereddy Bhanuprakash Reddy[1*,] Ph.D.
Affiliation: [1]Biochemistry, National Institute of Nutrition, Hyderabad, India.

Background: The main aim of this study is to analyze leukocyte telomere length (TL) and mitochondrial DNA copy number (mtCN) in aged individuals.

Methods: The present community-based cross-sectional study involves 428 subjects which were categorized into <60 (n=242) and ≥60 (n=186) years age groups. Relative TL, mtCN variation measured by q-PCR and plasma folate, vitamin B12 levels are analyzed by solid phase radioimmunoassay.

Results: The subjects with ≥60 age group has significantly shorter telomeres ($p<0.001$) and lower mtCN ($p=0.002$) than the <60 years age group. A significant positive correlation observed between the relative TL and mtCN ($p<0.001$). Plasma folate and vitamin B12 levels were positively correlated with the relative TL and mtCN.

Conclusion: We report for the first time, decline of TL and mtCN with age in Indian population and their association suggests that they may co-regulate each other with age.

P32

CD40 signal modulates cell fate determinants for prospective memory B cell differentiation

Satyajeet Salunkhe, Dr Tushar Vaidya

CSIR-Centre for Cellular and Molecular Biology,Hyderabad

CD40 signal serve as minimum essential factor for commitment of B cell to memory compartment.in Mice lacking T cell, administered with agonistic anti-CD40along with antigen, B cells commits to memory phenotype. Nature of intrinsic changes brought by CD40 signal that helps cell fate choice to memory compartment are unclear. We decided to explore the molecular events under control of CD40 signal that possibly help to explain B cell fate choices as memory or plasma cell.DICER(miRNA biogenesis component) knock out under Activation induced cytidine deaminase promoter, shown to reduce memory B cell formation, which highlighted importance of unkown set of miRNA contributing in cellular fate decision. Therefore, we used candidate miRNA approach and selected miRNA, which get affected in B cell development or differentiation. We observed upregulation of miR 155,292 post 24 hr stimulation and downregulation of miR150,17,146,26 at 48 hr time point in CD40 signalled B cell. We further decided to explore the miRNA-targets that participate in B cell differentiation and found differential regulation of CCND2,BTLA,c-MYB and TRAF6 in CD40 signalled B cell. Furthermore, we performed gene set enrichment analysis for miRNA-targets of differentially regulated miRNA and found significant enrichment of miRNA-targets at 48 hr time point but not at 24 hr post stimulation. Therefore, we conclude that CD40 confers intrinsic changes upon B cell fate via modulating key cell fate determinants that helps in commitment of B cell to memory phenotype.

Keywords:Plasma cell, Memory B cell, miRNA

P33

PPE2: a blessing in disguise

Ravi Pal[1,2], Madhu babu batu[1], Sangita Mukhopadhyay[1]

[1]Center for DNA fingerprinting and diagnostics, Hyderabad, Telangana

[2]Gaduate studies, Manipal academy of higher education, Manipal, Karnataka

PPE2 is a member of the PPE family of proteins in *Mycobacterium tuberculosis*. Previous studies have shown that PPE2 inhibits nitric oxide production in macrophages by specifically binding to TATA box of *iNOS* promoter. We observed that, during infection in mice, *Mycobacterium smegmatis* expressing PPE2, reduced tissue resident mast cells. Since, mast cells are major players in inflammation, we tested recombinantly purified PPE2 (rPPE2) as an anti-inflammatory agent. In formalin-induced paw inflammation, mice treated with rPPE2 showed reduction in edema and mast cell population. Presence of a "TAT protein" like motif in PPE2 enables its energy-independent uptake in tissue fibroblasts. Further, KitL; a fibroblast cytokine that regulates tissue mast cells, was found to be transcriptionally downregulated in rPPE2 treated mice. Also, unlike a majority of the NSAIDs, rPPE2 did not cause any hepatotoxicity. Therefore, rPPE2 emerges as a novel therapeutic for the treatment of inflammation, with no apparent side effects.

P34

CD40 signalling-mediated delay in terminal differentiation of B cells enables alternate fate choices at early divisions

Devi Prasad V[1], Tushar Vaidya[1]

[1]CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India

B cells constitute a major arm of adaptive immunity. Post-activation, they can become quiescent memory cells or differentiate into antibody-secreting plasma cells. The role of T cell help in this decision-making process is of prime importance but the underlying mechanisms are unclear. In our study, we have used a CD40 agonist, to substitute for T cell help, and studied its effect on lipopolysaccharide-induced differentiation. Earlier reports have suggested that CD40 signalling arrests terminal differentiation. However, we show that although CD40 signalling initially diverted cells towards memory- and germinal centre-like fates, it eventually facilitated the generation long-lived plasma cells. Dissecting the signalling components revealed that the regulation of differentiation happened at two independent levels−CD40 signalling inhibited the commitment to differentiation, while the proliferative potential of cells controlled the extent of differentiation. Elucidating such mechanisms will lead to better design of vaccines and management of immune disorders.

P35

Deciphering the role of the unusual *PPE50 (Rv3135) – PPE51 (Rv3136)* gene cluster in Mycobacterium tuberculosis physiology

Ravi Prasad Mukku[1], Sangya Yadav[2] and Raghunand R. Tirumalai[*]

CSIR - Centre for Cellular and Molecular Biology (CCMB), Uppal Road, Hyderabad - 500007, India

*Correspondence: raghu@ccmb.res.in

The extraordinary success of *Mycobacterium tuberculosis* (*M.tb*) has been attributed to its ability to modulate host immune responses. The genome of *M.tb* encodes multiple immunomodulatory proteins, including several genes of the multigenic PE_PPE (named after the conserved Proline-Glutamate and Proline-Proline-Glutamate residues at their N-termini) family, which comprise about 10% of its coding potential. The presence of these proteins in pathogenic mycobacteria strongly suggests that they play a role in disease pathogenesis. Several of the PE_PPE genes are organised in clusters that include *PE-PPE, PE-PE* and *PPE-PPE* genes arranged in tandem. While the functional properties of individual PE/PPE proteins have been examined, little information exists on the integrated functions of such gene clusters. To understand its role in *M.tb* physiology we have begun to characterise the *PPE50 (Rv3135)-PPE51 (Rv3136)* gene cluster, one of four *PPE-PPE* clusters in the *M.tb* genome. Using RT-PCR we demonstrated that this cluster encodes a co-transcriptional unit and then hypothesised that this locus encodes interacting proteins, a common feature of operons. Using Mycobacterial Protein Fragment Complementation we showed that PPE50 and PPE51 interact *in vivo*, and validated this finding with *in vitro* pull-downs using purified preparations of these proteins. To examine the role of these proteins in host-pathogen interaction we expressed *PPE50* and *PPE51* as c-myc fusions in the surrogate saprophytic host *Mycobacterium smegmatis* (*M. smegmatis*) and observed that they localise to the cell surface. CFU counts of THP-1 macrophages infected with recombinant *M. smegmatis* strains expressing *PPE50* and *PPE51* individually and in combination suggested that PPE50 and PPE51 play a role in intracellular bacillary survival. In pull-down experiments, PPE50 was observed to interact with TLR1, consistent with its probable role in immune signaling. We are now performing cytokine profiling experiments to study the possible immunomodulatory functions of these proteins during infection.

P36

Characterization of unusual Atg12-Atg5 system of *Plasmodium*

Divya Das, Renu Sudhakar, Rajesh Prasad, Puran Singh Sijwali*

Autophagy is a highly conserved pathway, known to maintain cellular homeostasis in eukaryotes, but little has been explored about *Plasmodium* autophagy. Here, we are investigating *Plasmodium* Atg12 and Atg5 which are known to play important role in lipidation of Atg8, in-spite of the speculated absence of Atg10, Atg16 homologues. Bioinformatics analysis revealed unusual features of PfAtg12 and PfAtg5.PfAtg12 lacks the canonical c-terminal glycine residue which is required for conjugation, while PfAtg5 is a much larger protein than its other eukaryotic counterparts.Atg12 showed diffused localization and Atg5 has a punctate localization throughout the parasite cytoplasm. Knock-out of Atg12 could not be achieved while knock down study of Atg5 inferred they are essential for the parasite survival.

P37

Lyse-Reseal Erythrocytes for Transfection of *Plasmodium falciparum*

Gokulapriya Govindarajalu, **Zeba Rizvi**, Deepak Kumar and Puran Singh Sijwali*

CCMB, Hyderabad

Plasmodium falciparum causes malaria and yet to be controlled. *In vitro* culture and genetic manipulation are necessary to know its biology. Among the available transfection techniques for *P. falciparum*, electroporation-based methods are routinely used. Nonetheless, transfection is still considered a laborious and resource-intensive procedure. Here, we report a simple and economic transfection method for *P. falciparum*, which is termed as the lyse-reseal erythrocytes for transfection (LyRET). It involves lysis of erythrocytes with a buffer containing the plasmid DNA, followed by resealing. These DNA-encapsulated erythrocytes, were infected with *P. falciparum* subjected to drug selection to obtain resistant parasites. This method was successful for transfection of two strains with three different plasmids. The efficiency and cost effectiveness of LyRET method give it an edge over the existing transfection methods particularly in resource-limited settings.

P38

Identification and validation of protein neddylation in malaria causing parasites

Manish Bhattacharjee, Navin Adhikari, Puran Singh Sijwali*

Centre for Cellular and Molecular Biology, Hyderabad – 500007

The Ubiquitin Proteasome System (UPS) is known to be critical for proteostasis in *Plasmodium*. It was predicted to have a neddylation pathway which is an important regulator of UPS in most eukaryotes. Further, *P. falciparum* growth was inhibited in culture, upon treatment with the universal neddylation inhibitor,MLN4924. Unlike other organisms, *P. falciparum* NEDD8 (PfNEDD8)lacks a c-terminal tail beyond Gly_{76} and is constitutively available for activation upon translation. Immuno-blotting revealed the presence of multiple higher molecular weight bands of HA tagged-PfNEDD8, indicating the covalent conjugation of NEDD8 onto substrate proteins. Immunoprecipitation of HA-PfNEDD8 expressed in *P. falciparum* led to enrichment of PfNEDD8 and higher molecular weight bands,predicted to be neddylated cullin-1 and cullin-2 in immuno-blotting. PfNEDD8 was also shown to biochemically complement the neddylation pathway in *Saccharomyces cerevisiae* NEDD8 knock-out line. This system is currently in use to identify the true E2 of the *Plasmodium* neddylation pathway among the multiple predicted candidates.

P39

Immune response induction in rice due to co-expression of the *Xanthomonas oryzae* pv. *oryzae* type III effectors XopQ and XopX

Sohini Deb[1], Hitendra K. Patel[1], Ramesh V. Sonti[1,2]

[1]Centre for Cellular and Molecular Biology (CSIR-CCMB), Hyderabad, India,
[2]National Institute of Plant Genome Research, New Delhi, India

Keywords: 14-3-3 protein, resistance, rice, *Xanthomonas oryzae* pv. *oryzae*, effector

Xanthomonas oryzae pv. *oryzae* (Xoo) causes bacterial blight, a serious disease of rice. Xoo uses the type III secretion system (T3SS) to suppress rice immune responses. The T3SS secreted effectors XopQ and XopX suppress rice immune responses by interaction with different rice 14-3-3 proteins. Sub-cellular localisation of XopQ and XopX mutants that are defective in 14-3-3 binding and suppression of immune responses indicates that, for suppression, XopQ and XopX are spatially segregated. Hence, both XopQ as well as XopX individually act as suppressors of rice immune responses. However, we find that when XopQ is delivered through *Agrobacterium* along with XopX, it becomes an inducer of immune responses. We also find that XopQ and XopX can interact with each other. This raises the possibility that besides being a suppressor of immune responses, XopQ can under certain circumstances also function as an inducer of immune responses.

P40

SHUKR and SHUKR-Like Molecular Characterization with respect to Sporophytic to Gametophytic transition in *Arabidopsis thaliana*

Sivakumar Prakash, Veeraputhiran Subbiah, Aparna Singh, Chandan Kumar, Saurabh Pandey, Ramesha A, and Imran Siddiqi*

Centre for Cellular and Molecular Biology

The sporophytic (diploid) stage to the gametophytic (haploid) stage transition is important for successful plant gamete development. Meiosis acts as a bridge in this transition. Molecular players mediating this transformation are not well understood. We had isolated a novel sporophytic mutant having gamete development defects leading to male sterility in *Arabidopsis*. The gene responsible for this phenotype was named SHUKR (SKR) upon confirmation via genetic complementation. SKR is expressed from mid-prophase to the early microspore stage and shows a dynamic nucleo-cytoplasmic localization, it also associates with chromatin. We find that SKR interacts with H3K9me2 (a repressive histone mark) *in vitro*. During this study, we found that *skr* null mutants are rescued by keeping the plants at lower temperatures. By exploiting this phenomenon we screened genetic suppressors of *skr*. We are further studying SHUKR-Like (SKL) the only paralogue of SKR - having similar phenotypes as that of *skr* when mutated. By genetic and biochemical interaction studies, we are studying the mechanism of sporophytic to gametophytic transition in plants.

P41

Microstructure and Dynamics of Water at the Membrane Interface: Relevance in Membrane Biology

Sreetama Pal[a,b,c], Nirnay Samanta[d], Debashis Das Mahanta[d], Rajib Kumar Mitra[d,*] and Amitabha Chattopadhyay[a,c,*]

[a]CSIR-Centre for Cellular and Molecular Biology, Hyderabad 500 007, India
[b]CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, India
[c]Academy of Scientific and Innovative Research, New Delhi, India
[d]S. N. Bose National Centre for Basic Sciences, Kolkata 700098, India

Water in biology is universally recognized as the matrix that holds life together. However, the functional relevance of water in complex cellular processes has largely been overlooked, particularly in membrane biology. This is especially paradoxical since the self-assembly of lipids into a membrane can be attributed to unfavorable interaction of lipids with their aqueous microenvironment. Despite recent reports correlating membrane interfacial hydration to membrane protein structure and function, these evidences are largely based on indirect readouts due to technical challenges in direct measurements of membrane hydration. In this work, we have explored the microstructure and collective dynamics of membrane interfacial water using terahertz time-domain spectroscopy (THz-TDS). Our results implicate membrane electrostatics and steric crowding as key players in dictating the organization and dynamics of water at the membrane interface. We envision this lipid-stringent membrane hydration to expand the mechanistic framework of lipid-protein interactions.

P42

MOLECULAR INSIGHTS INTO THE ROLE OF RD3 IN LCA12 DISEASE PATHOGENESIS

Sudipta Mahato,Savitri Maddileti, Vinay Kumar, Praveen Joseph,Indumathi Mariappan

Sudhakar and Sreekanth Ravi Stem Cell Laboratory, Center for Ocular Regeneration,Hyderabad Eye Research Foundation, L V Prasad Eye Institute, Hyderabad, India

Purpose: To dissect out the unknown functions of *RD3* in the nucleus and to understand the effects of pathogenic mutations on retinal cell functions.

Methods: RD3-wt and RD3-mut coding regions were cloned into retroviral and mammalian expression vectors. The sub-cellular and nuclear localization of RD3 and other organelle markers were examined by immunocytochemistry. Immunopull-down was performed to identify the known and unknown interacting protein partners of RD3.

Results: Ectopically expressed RD3-wt protein was localized within the nucleus and the cytosol as discrete punctas. In dual staining, the nuclear RD3 punctas partially co-localized with sub-nuclear compartment markers such as PML and coilin bodies. Immunopulldown assay followed by mass spectrometry identified many interacting proteins that are involved in non-coding RNA processing, ribonucleoprotein assembly, coiled body and nucleolar functions.

Conclusion: The localization of RD3 in nucleus and its association with the nuclear sub-compartment proteins suggests its possible involvement in an unknown gene regulatory function.

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The epigenetic regulator PRDM2 controls the Notch pathway and restricts differentiation in quiescent myoblast.

Sujoy Deb[1],Amena Saleh[1,2,3], and Jyotsna Dhawan[1,2]

[1] Center for Cellular and Molecular Biology-CSIR, Hyderabad, India

[2]Institute for Stem Cell Biology and Regenerative Medicine, Bangalore, India,

[3]Manipal University, Manipal, India

Satellite cells, the stem cells present in adult skeletal muscles, are essential for muscle repair. They are maintained in a poised undifferentiated state, ready to re-enter the cell cycle partly through the action of the epigenetic regulator PRDM2, an H3K9 methyl transferase. Earlier, we showed that PRDM2 associates with and represses muscle-specific promoters in quiescence, performing a dominant role in suppressing differentiation. Notch signaling, a key repressor of muscle differentiation, directy inhibits MyoD and Myogenin in quiescence. We find that PRDRM2 associates with the promoters of multiple Notch pathway genes including Notch 1, 3 and Notch targets Hes-1, Hey-1, Hey-L, but not the key transcriptional effector RBPJ-k. Knockdown of PRDM2 results in reduced expression of the Notch pathway genes, with a concomitant increase in the muscle differentiation markers, while over-expression of PRDM2 results in induction of Notch target genes, and suppression of differentiation. Our results indicate that PRDM2 directly regulates expression of Notch downstream targets in an RBPJk independent mechanism. We propose a model where inhibition of differentiation in quiescence is controlled by PRDM2 via a checkpoint involving Notch target genes.

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Structure-function analysis of *Xanthomonas oryzae* pv. *oryzae* virulence factor CbsA

Rajkanwar Nathawat[1], Roshan V. Maku[1], Ramesh V. Sonti[1, 2]and Rajan Sankaranarayanan[1]

1. Centre for Cellular and Molecular Biology, Hyderabad – 500007
2. National Institute of Plant Genome Research, New Delhi, Delhi 110067

ABSTRACT

Xanthomonas oryzae pv. *oryzae* (*Xoo*) causes bacterial blight disease of rice.*Xoo* uses several cell wall degrading enzymes as virulence factors.Cellobiosidase (CbsA) is an important enzyme, which is required for the complete virulence of *Xoo*.It has an N-terminal catalytic domain (exoglucanase), which belongs to the GH6 family,and a C-terminal Fibronectin type 3 (FN3) domain. Based on the crystal structure of CbsA catalytic domain and available structural and functional studies on other members of GH6 family, putative catalytic residues were identified for CbsA. Mutation of these residues does not abrogate the activity of CbsA but changes its activity, which is closer to endoglucanase activity. Interestingly, a deletion mutant of FN3 domain is virulence deficient and shows almost 95% reduction in secreted CbsA level. Western analysis of different cellular fractions suggests role of FN3 domain in efficient transport and stability of the CbsA protein. In the presence of FN3 domain, CbsA catalytic domain does not act upon carboxymethylcellulose, a substrate on which the catalytic domain is otherwise active. Currently, we are trying to understand the molecular mechanism of this inhibition using SAXS.

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Animalia-specific tRNA deacylase (ATD): A new player in translation quality control

Kuncha S.K.[*], Dahate P., Venkadasamy V.L., Amudhan G., Pottabathini S., Shobha P. Kruparani S.P.,and Sankaranarayanan R.

CSIR–Centre for Cellular and Molecular Biology, Hyderabad, India

[*]*Academy of Scientific and Innovative Research (AcSIR), CSIR-CCMB campus, India*

The ever growing genome data has shed light on the explosion of tRNA genes in higher eukaryotes. Recent studies have shown the regulatory roles of tRNA and tRNA-derived fragments in different cellular processes such as apoptosis, intergenerational inheritance, spermatogenesis, translation and RNAi [1]. This versatility of tRNA is possible due to the emergence of tRNA isodecoders (tRNAs with identical anticodon but different body). However, this introduces a peculiar problem of tRNA mis-selection in Animalia, wherein alanyl-tRNA synthetase (AlaRS) mischarges $tRNA^{Thr}$ containing G4•U69 (tRNA determinant for AlaRS is G3•U70) and generates L-Ala-$tRNA^{Thr}$. Recently, using bioinformatic, structural and biochemical studies, we have identified a novel proofreading factor named Animalia-specific tRNA deacylase (ATD) which proofreads L-Ala-$tRNA^{Thr}$ and thereby avoids mistranslation of Thr codons [2]. The structural understanding and the physiological importance of ATD will be presented in detail.

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Biochemical and dynamic basis for combinatorial recognition of H3R2K9me2 by dual domains of UHRF1

Suman Abhishek[1,#],M. Angel Nivya[1,#], Naveen Kumar Nakarakanti[1,#], Waghela Deeksha[1], Sanjeev Khosla[2,*] and Eerappa Rajakumara[1,*]

[1]Department of Biotechnology, Indian Institute of Technology Hyderabad, Kandi, Sangareddy, 502285, Telangana, India

[2]Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad 500001, India.

Abstract:

UHRF1 is a multi-domain protein comprising of a tandem tudor (UHRF1 TTD), a SET and a RING-associated domain. It is required for the maintenance of CG methylation, heterochromatin formation and DNA repair. Isothermal titration calorimetry binding studies of unmodified and methylated lysine histone peptides establish that the UHRF1 TTD binds dimethylated Lys9 on histone H3 (H3K9me2). Further, MD simulation and binding studies reveal that TTD-PHD of UHRF1 (UHRF1 TTD-PHD) preferentially recognizes dimethyl-lysine status. Importantly, we show that Asp145 in the binding pocket determines the preferential recognition of the dimethyl-ammonium group of H3K9me2. Interestingly, PHD finger of the UHRF1 TTD-PHD has a negligible contribution to the binding affinity for recognition of K9me2 by the UHRF1 TTD. Surprisingly, Lys4 methylation on H3 peptide has an insignificant effect on combinatorial recognition of R2 and K9me2 on H3 by the UHRF1 TTD-PHD.

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Dynamic basis for Auranofin drug recognition by thiol-reductases of human pathogens, and intermediate coordinated adduct formation with catalytic cysteine residues

Nitin Kulhar, **Suman Abhishek**, Eerappa Rajakumara

Department of Biotechnology, Indian Institute of Technology Hyderabad, Kandi, Sangareddy, Telangana 502285, India

ABSTRACT

Auranofin (AF), an FDA approved gold-conjugated drug, is known to selectively target thiol-reductases, key enzymes involved in ROS metabolism. Several studies have shown the inhibitory activity of AF against a diverse range of pathogens. Co-crystal structures of thiol-reductases complexed with AF revealed that Au(I) was coordinately linked to catalytic cysteines, but the mechanism of transfer of Au(I) from AF to catalytic cysteines still remains unknown. In this study, we have employed computational approaches to understand the interaction of AF with thiol-reductases of selected human pathogens. Also, we have shown that tailor-made analogs of AF can be designed against selective thiol-reductases for targeted inhibition. MD studies show that the AF-intermediates, linked to one of catalytic cysteines remains stable in the binding pocket of thiol-reductases for *L. infantum* and *P. falciparum*. This suggests that the organic moieties of AF may be sequentially eliminated during transfer of Au(I) to catalytic cysteines of receptor.

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Diels Alderase – A thriller story of nature

Authors: Rajnandani Kashyap and Dr. Anthony Addlagatta *

Applied Biology, CSIR-Indian Institute of Chemical Technology, Hyderabad-500007, India.

Abstract: Diels Alderase is an enzyme that catalyzes a [4+2] cycloaddition reaction between a conjugated diene and dienophile molecules. Diels Alderase have been remained an interesting topic for organic chemists for last several decades. However, there are no naturally occurring enzymes that have been shown to catalyze bi-molecular Diels-Alder reactions. In the facade of 21st Century, it poses an intriguing question on the existence of natural Diels Alderase catalyzing intermolecular reactions. Hence this curiosity led us to dive in finding out a natural enzyme, which can catalyze an intermolecular reaction. Here we have performed a three-component aza-Diels-Alder reaction between aromatic aldehyde, aromatic amine and 2-cyclohexen1-one using four putative natural Diels Alderase enzymes.

Keywords: Diels-Alder reaction, Diels Alderase, Inter-molecular reaction, Nature, Structural biology

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