



12th RNA GROUP MEETING

22-24 May, 2024

**Department of Biosciences and Bioengineering
Indian Institute of Technology Guwahati**





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Organizers

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Ananta Kesari Behera

Susmita Dey





SCHEDULE



12:00 – 13:55	Registration
12:30 – 13:45	Lunch
14:00 – 14:05	Welcome Note
14:05 – 14:15	Introduction to RNA Group Meeting
14:15 – 14:20	Inauguration of RNA India website

Session I: Regulatory RNAs

Chair: Rakesh S. Laishram and Purusharth Rajyaguru

14:20 – 14:45	IT-1	Meetali Singh
(small RNA-mediated epigenetic regulation of pathogenesis and host-parasite interaction)		
14:45 – 15:10	IT-2	Bhupendra Verma
(tRNA-derived non-coding RNAome during Dengue Virus Pathogenesis)		
15:10 – 15:35	IT-3	Veena S Patil
(Human CD4+ T cell memory subsets in infectious diseases: lessons from multi-omics analysis)		
15:35 – 16:00	IT-4	Dimple Notani
(RNA modulates phase-separation of enhancers for robust signaling response)		
16:00 – 16:25	IT-5	Zhumur Ghosh
(Regulatory Noncoding RNomics –Orchestrating the journey across pre- and post-fertilization stages)		
16:25 – 16:55	Coffee/Tea Break	

Session II: Flash Talks - I

Chair: Rakesh S. Laishram and Purusharth Rajyaguru

16:55 – 17:02	FT-1	Pratyasha Bhowal
(Unravelling the secrets of a key enzyme in a trypanosomal parasite: Insights into structural and functional attributes)		
17:02 – 17:09	FT-2	Nivedita Dutta
(Modulation of structure and thermodynamics of RNA G-quadruplexes by A to I editing: Possible route to G-quadruplex-based RNA therapeutics)		
17:09 – 17:16	FT-3	Safirul Islam
(Determining novel cytoplasmic mRNA capping enzyme-targeted, hypoxia-responsive RNAs in hypoxic osteosarcoma cellular model)		
17:16 – 17:23	FT-4	Dipanjana Banerjee
(Early life stress-induced RNA methylation regulates spatial memory)		
17:23 – 17:29	FT-5	Prerna Narwal
(Translation inhibition primes cells to face stress via activation of acute inflammation)		
17:30 – 19:30	Poster presentations	
19:30 – 21:00	Business Meeting and Dinner	

Session III: Ribonucleoprotein complexes

Chair: Sudip Kundu and Srikanta Goswami

09:00 – 09:30	IT-6	Saumitra Das
(RNA binding proteins and RNA viruses: A journey together)		
09:30 – 09:55	IT-7	Ranjit P. Bahadur
(Predicting protein-RNA recognition sites using sequence and structural features)		
09:55 – 10:20	IT-8	Jeetender Chugh
(Differential conformational dynamics in two type-A RNA-binding domains drive the double-stranded RNA recognition and binding)		
10:20 – 10:45	IT-9	Pradeepkumar P. I.
(Structural and Functional Insights into the RNA Pseudoknots of the CRISPR-Cas12a Assemblies)		
10:45 – 11:10	IT-10	Shruthi S. Vembar
(Discovery and validation of novel compact RNA-targeting CRISPR/Cas13 systems)		
11:10 – 11:40	Group Photo and Coffee/Tea Break	

Session IV: Post-transcriptional gene regulations

Chair: Pankaj V. Alone and Shovamayee Maharana

11:40 – 12:05	IT-11	Vasudevan Seshadri
(PIP4K, a kinase with RNA binding activity)		
12:05 – 12:30	IT-12	Arumugam Rajavelu
(Plasmodium falciparum YTH2 Domain Binds to m6A-Containing mRNA and Regulates Translation)		
12:30 – 12:55	IT-13	Jomon Joseph
(Autophagy-dependent regulation of processing bodies (P. bodies))		
13:00 – 14:00	Lunch Break (Mentor Mentee Lunch)	

Session V: Flash Talks - II

Chair: Pankaj V. Alone and Shovamayee Maharana

14:00 – 14:07	FT-6	Sweta Tiwari
(RGG-motif protein Scd6 affects oxidative stress response by regulating Cytosolic caTalase T1 (Ctt1))		
14:07 – 14:14	FT-7	Aditya Singha Roy
(Hidden in the Code: Exploring the Dynamic Interplay of 5'-UTR Variants, uORFs, and RNA G-Quadruplex in the regulation of human cIAPI gene expression)		
14:14 – 14:21	FT-8	Debraj Manna
(Regulation of the expression of ribosomal proteins by small downstream ORFs)		
14:21 – 14:28	FT-9	Koushick Sivakumar
(Emergence of an effective translation proofreader enabled the surge of proline-rich proteins in metazoans)		

[23-05-2024] Day 2 - Thursday

Session V: Flash Talks - II

Chair: Pankaj V. Alone and Shovamayee Maharana

14:28 – 14:35	FT-10	Payal Gupta
(Tetraphenylethene Derivatives Modulate the RNA Hairpin-G-quadruplex Conformational Equilibria in Proto-Oncogenes)		
14:35 – 14:42	FT-11	Akshay Chaudhari
(DRASTIC lncRNA may regulate DNA damage response via FUS dependent LLPS)		
14:45 – 16:45	Poster Presentations and Coffee/Tea Break	

Session VI: Ribosome Structure and Translational Control

Chair: Biswadip Das and Rajat Banerjee

16:45 – 17:10	IT-14	Jayati Sengupta
(Structural insights into the interplay of nascent chain processing factors with the 70S ribosome)		
17:10 – 17:35	IT-15	Prem S. Kaushal
(Ribosome structure of pathogenic protozoan <i>Entamoeba histolytica</i>)		
17:35 – 18:00	IT-16	Tanweer Hussain
(mRNA recruitment during protein synthesis in eukaryotes)		
18:00 – 18:30	IT-17	Umesh Varshney
(Initiator tRNA-centric mechanisms of faithful translation initiation and ribosome maturation in bacteria)		
18:30 – 19:00	Felicitation Ceremony	
19:00 – 21:00	GALA DINNER	

[24-05-2024] Day 3 - Friday

Session VII: RNA-Protein Interactions

Chair: Ansuman Lahiri and Mandar V Deshmukh

09:00 – 09:30	IT-18	Rajan Sankaranarayanan
(When Paul Berg meets Donald Crothers: An achiral connection through protein biosynthesis)		
09:30 – 09:55	IT-19	Dasaradhi Palakodeti
(Unraveling the Role of Polyadenylation Machinery in Asymmetric Stem Cell Division)		
09:55 – 10:20	IT-20	Ritu Kulshreshtha
(Decoding the role and clinical relevance of Non-coding RNAs in Glioblastoma)		
10:20 – 10:45	IT-21	Mahavir Singh
(RNA pseudoknot at the core of bacterial type III toxin-antitoxin systems)		
10:45 – 11:10	IT-22	Amanjot Singh
(Balanced interplay among the structured and disordered domains of RNA-binding protein)		
11:10 – 11:40	Coffee/Tea Break	

[24-05-2024] Day 3 - Friday

Session VIII: RNA Splicing

Chair: Sandeep M. Eswarappa and Ashish Misra

11:40 – 12:05	IT-23	Sanjeev Shukla (Unraveling the Nexus: Hypoxic Tumor Microenvironment and Alternative Splicing)
12:05 – 12:30	IT-24	Indrani Talukdar (Alternative splicing and its impact on the metabolic syndrome to stem cell differentiation)
12:30 – 12:55	IT-25	Amaresh C. Panda (Discovering Transcriptome-wide Circular RNA Interaction with mRNAs)
13:00 – 13:30	Prize Distribution and Concluding Remarks	
13:30 – 14:30	Lunch Break and Departure	

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INVITED TALK ABSTRACTS



INVITED TALKS



IT01- Meetali Singh (IISc, Bangalore)

Small RNA-mediated epigenetic regulation of pathogenesis and host-parasite interaction



IT02- Bhupendra Verma (AIIMS, New Delhi)

tRNA-derived non-coding RNAome during Dengue Virus Pathogenesis



IT03- Dr. Veena S. Patil (NII, New Delhi)

Human CD4+ T cell memory subsets in infectious diseases: lessons from multi-omics analysis



IT04- Dimple Notani (NCBS, Bangalore)

RNA modulates phase-separation of enhancers for robust signaling response



IT05- Zhumur Ghosh (Bose Institute, Kolkata)

Regulatory Noncoding RNomics-Orchestrating the journey across pre- and post- fertilization stages



IT06- Saumitra Das (IISc, Bangalore)

RNA binding proteins and RNA viruses: A journey together

INVITED TALKS

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IT07- Ranjit P. Bahadur (IIT Kharagpur)

Predicting protein-RNA recognition sites using sequence and structural features



IT08- Jeetender Chugh (IISER Pune)

Differential conformational dynamics in two type-A RNA-binding domains drive the double-stranded RNA recognition and binding



IT09- P.I. Pradeep Kumar (IIT Bombay)

Structural and Functional Insights into the RNA Pseudoknots of the CRISPR- Cas12a Assemblies



IT10- Shruthi S. Vembar (IBAB, Bengaluru)

Discovery and validation of novel compact RNA-targeting CRISPR/Cas13 systems



IT11- Vasudevan Seshadri (NCCS, Pune)

PIP4K, a kinase with RNA binding activity



IT12- Arumugam Rajavelu (IIB, Chennai)

Plasmodium falciparum YTH2 Domain Binds to m6A-Containing mRNA and Regulates Translation

INVITED TALKS

12TH RNA MEET 2024



IT13- Jomon Joseph (NCCS, Pune)

Autophagy-dependent regulation of Processing bodies (P-bodies)



IT14- Jayati Sengupta (CSIR-IICB, Kolkata)

Structural insights into the interplay of nascent chain processing factors with the 70S ribosome



IT15- Prem S. Kaushal (RCB, Faridabad)

Ribosome structure of pathogenic protozoan *Entamoeba histolytica*



IT16- Tanweer Hussain (IISc, Bangalore)

mRNA recruitment during protein synthesis in eukaryotes



IT17- Umesh Varshney (IISc, Bangalore)

Initiator tRNA-centric mechanisms of faithful translation initiation and ribosome maturation in bacteria



IT18- Rajan Shankaranarayanan (CCMB, Hyderabad)

When Paul Berg meets Donald Crothers: An achiral connection through protein biosynthesis

INVITED TALKS

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IT19- Dasaradhi Palakodeti (inSTEM, Bangalore)

Unraveling the Role of Polyadenylation Machinery in Asymmetric Stem Cell Division



IT20- Ritu Kulshreshtha (IIT Bombay)

Decoding the role and clinical relevance of Non-coding RNAs in Glioblastoma



IT21- Mahavir Singh (IISc, Bangalore)

RNA pseudoknot at the core of bacterial type III toxin-antitoxin systems



IT22- Amanjot Singh (MIRM, Bengaluru)

Balanced interplay among the structured and disordered domains of RNA-binding protein



IT23- Sanjeev Shukla (IISER Bhopal)

Unraveling the Nexus: Hypoxic Tumor Microenvironment and Alternative Splicing



IT24- Indrani Talukdar (BITS-Pilani, Goa)

Alternative splicing and its impact on the metabolic syndrome to stem cell differentiation



IT25- Amaresh C. Panda (ILS, Bhubaneswar)

Discovering Transcriptome-wide Circular RNA Interaction with mRNAs

IT01: Small RNA-mediated epigenetic regulation of pathogenesis and host-parasite interaction

Meetal Singh

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Keywords: Small RNA, Argonaute, epigenetics

Small RNAs are among the main drivers of epigenetic gene regulation. There are three main categories of small RNAs: microRNAs, piRNAs and endogenous siRNAs. These various small RNA pathways are conserved across most eukaryotes. I will talk about the lessons learnt on the biogenesis and function of small RNAs from different models. Briefly, I will talk about role of small RNAs in inheritance of memory of stress using nematode model, followed by our findings on role of microRNAs in host-pathogen interaction using SARS-CoV-2 model. We sequenced small RNAs from SARS-CoV-2 infected cells and identified a miRNA derived from a recently evolved region of the viral genome. We showed that the virus-derived miRNA locus produces two miRNA isoforms, and they were also detected in COVID-19 patients' nasopharyngeal swab samples. Moreover, predominant miRNA isoform targets the 3'UTR of interferon-stimulated genes and represses their expression, thus aiding in host immune evasion. Lastly, I will highlight our group's new ventures to understand the role of small RNAs in host- parasite interaction.

IT02: tRNA-derived non-coding RNAome during Dengue Virus Pathogenesis

Deeksha Madhry¹, Shivani Malvakar¹, Sushant Phadnis, Varsha Meena, Sankar Bhattacharyya² and **Bhupendra Verma**¹

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Keywords: Host-virus interaction, RNases, Angiogenin, tRNA fragments, DENV replication

DENV infection poses a major health concern globally. DENV pathophysiology relies heavily on host-cellular machinery for its replication. Currently, there is no treatment for DENV infection. There is a necessity to understand the molecular basis of DENV pathogenesis. Various small non-coding RNAs have emerged as significant elements in controlling viral replication and host responses. Transfer RNA (tRNA)-derived fragments (tsRNAs) constitute one of the novel classes of small RNAs reported to play diverse regulatory roles in various diseases. However, the role of these ncRNAs in Dengue pathogenesis remains unexplored.

tRNA fragmentation occurs under various stress conditions due to the action of various RNases. Our study suggests that the levels of several RNases are modulated during DENV infection. We demonstrate that Angiogenin is modulated during DENV infection, potentially leading to the generation of tsRNA. To define the expression patterns of tsRNA and determine the putative targets during Dengue pathogenesis, we conducted RNA sequencing of DENV infected Huh7 cell line. Our sequencing data revealed the generation of various tRNA fragments like 5'halves, 3'halves, 3'tRF, 5'tRF, and itRF. tRNA halves are mainly generated possibly by Angiogenin mediated cleavage in the anticodon loop of tRNA. Our results suggest that these non-coding RNA promotes DENV replication.

IT03: Human CD4⁺ T cell memory subsets in infectious diseases: lessons from multi-omics analysis.

Dr. Veena S. Patil

National Institute of Immunology, New Delhi

The acquisition of immunological memory to infections is the hallmark of protective immune response. The naive T cells that have not previously encountered antigen, differentiate during the primary infection into memory T cells that have specialized functions in immune defense, to a subsequent infection with the same pathogen. However, the T cell memory is highly diverse and heterogeneous and has only been categorized into few subtypes based on limited known markers. This limited knowledge has restricted the researchers in fully exploring the potential of protective role of T cells in designing vaccines. Hence, our focus has been to dissect the developmental lineage, diversity, specificity and heterogeneity of T cell memory subtypes by identifying their unique gene expression profile, epigenetic landscape and T Cell Receptor repertoire using multi-omics approaches.

IT04: RNA modulates phase-separation of enhancers for robust signaling response

Dimple Notani

National Centre for Biological Sciences, Bangalore, India

As during development, enhancers also serve as the mediators of gene expression during signaling events. However, unlike enhancers involved in development, signaling-induced enhancers need to be dynamic as upon every round of ligand stimulation, enhancers get back to their “native” state but remain poised for the next round of ligand stimulation. Such reversible enhancer dynamics during signalling is not known.

Using genomic techniques that quantify the alterations in ligand induced TF binding (Estrogen Receptor- α), nascent transcription, three-dimensional architecture during the course of estrogen signaling, our work has revealed that chromatin state under basal signaling is the key to signaling response. Certain regions in the genome are bound by estrogen receptor (ER) even before the exposure to ligand. These regions act as a seed to give rise to ER bound enhancer clusters and ER condensates to drive the signaling response. Further, these and other enhancer clusters do not function as sum-of-all but they rely on complex hierarchies that cannot be predicted *in silico*. The seed enhancers perform unique functions due to presence of eRNA (enhancer-RNA). These eRNAs, interact with ER and modulate its residence time on DNA, that potentially allows these enhancers to act as organiser of signaling response. I will discuss how TF-condensates and eRNA on enhancers regulate dynamic signaling response upon each round of ligand stimulation that is dependent on architectural framework established a priori, under basal signaling.

IT:05 Regulatory Noncoding RNomics –Orchestrating the journey across pre- and post- fertilization stages

Zhumur Ghosh

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Keywords: pre-fertilization, post-fertilization, microRNA, long noncoding RNA, zygotic genome activation

The development of an organism from single totipotent cell is a fascinating process. Following fusion of gametes, transcriptionally inactive sperm DNA is repackaged within male pronucleus and unite with female pronucleus to form 1-cell zygote. Well-coordinated cascade of events lead to transcriptional activation, epigenetic regulation which governs fertilization and embryonic development. Genome wide transcriptome mining has revealed thousands of parental noncoding RNAs (ncRNAs) which act as transcriptional regulator of gene expression in developmental events. Although the role of these ncRNAs in spermatogenesis, oogenesis and embryogenesis has been pointed out so far, precise interaction of these regulatory molecules with mRNAs and associated essential mechanisms is yet to be decrypted. We have deciphered the important set of ncRNA-mRNA interactions encompassing microRNAs and long non coding RNAs during pre- and post-fertilization events mainly prior to zygotic genome activation in mice.

IT06: RNA binding proteins and RNA viruses: A journey together**Saumitra Das**

Department of Microbiology and Cell Biology, Indian Institute of Science,
Bangalore

The interplay between viruses and host cells is extremely complex, as is the resulting disease dynamics. In the cytoplasm of host cells, (+) ss viral RNAs interact with numerous RNA-binding proteins (RBPs).

Work from our laboratory has shown that HuR, an RBP with multiple functions in RNA processing and translation, relocalizes from the nucleus to the cytoplasm upon Hepatitis C virus (HCV) infection. We have shown that two viral proteins, NS3 and NS5A, act co-ordinately to alter the equilibrium of the nucleo-cytoplasmic movement of HuR. NS3 activates protein kinase C (PKC)- δ , which in turn phosphorylates HuR on S318 residue, triggering its export to the cytoplasm. NS5A inactivates AMP-activated kinase (AMPK) resulting in diminished nuclear import of HuR through blockade of AMPK-mediated phosphorylation and acetylation of importin- α 1.

In parallel, we have shown that HuR binds to SARS-CoV-2 5'UTR. The knock-down and knock-out of HuR reduced viral RNA levels and viral titres. Using an antisense strategy, we were able to reduce the viral RNA level in wildtype cells but not in HuR-knockout cells. Interestingly, results suggest HuR supports SARS-CoV-2 life by promoting differential translational reprogramming of both genomic and subgenomic RNAs.

Taken together, we demonstrate important roles of an RNA binding protein HuR, in two RNA viruses, HCV and SARS-CoV-2, and explored different ways to target it for tackling virus infections. We are now focused to uncover the dynamic remodelling of the virus-centred ribonucleoprotein (RNP) networks upon infection and characterizing the impact of RNP remodelling on the gene expression of host cells.

IT07: Predicting protein-RNA recognition sites using sequence and structural features

Ranjit P. Bahadur

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Keywords: Protein-RNA complexes, protein-RNA interactions, RNA-binding proteins, protein-binding nucleotides, machine learning

RNA-binding proteins (RBPs) have emerged as molecules of special interest in past few decades due to their involvement in fundamental cellular processes. They interact with wide variety of RNA molecules to regulate the complex network of RNA metabolism. Several sequence and structural features of the interacting partners govern the fundamental aspects of protein-RNA recognition and aberrations caused due to misregulated interactions often results in various human pathologies. Moreover, high-throughput sequencing technologies have generated large amount of sequence data compared to available structural data of RNA-protein complexes, thereby demanding sequence-based computational investigations. A sequence-based prediction method ProRBR is designed through analysis of binding site residues in protein-RNA complexes using dipeptide composition based balanced Random forest (RF) model (DCP-BRF) to efficiently map the RNA binding residues (RBRs) in RBPs. The method developed in the study achieves an accuracy of 0.88 and AUC of 0.93. Likewise, nucleotide features in binding and non-binding sites of RNA sequences are studied and various machine learning algorithms are optimized to predict protein-binding nucleotides in RNA. Nucleotide-triplet and nucleotide-quartet (NC-triplet and NC-quartet) based RF models developed in this study achieve an overall accuracy of 0.85 and AUC of 0.93. The method is implemented in a user-friendly webserver “Nucpred”.

IT08: Differential conformational dynamics in two type-A RNA-binding domains drive the double-stranded RNA recognition and binding

Firdousi Parvez, Devika Sangpal, Harshad Paithankar, Zainab Amin, and **Jeetender Chugh**

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Keywords: Conformational dynamics, RNA-protein interactions, NMR spectroscopy, TRBP

Exploring the intricacies of RNA-protein interactions by delving into dynamics-based measurements not only adds valuable insights into the mechanics of RNA-protein interactions but also underscores the significance of conformational dynamics in dictating the functional outcome in such tightly regulated biological processes. In this study, we measure intrinsic and RNA-induced conformational dynamics in the second dsRBD, i.e., TRBP-dsRBD2, and compare the same with that carried out in the first dsRBD (TRBP-dsRBD1) of TRBP protein, a key player of the RNAi pathway. The study unveils the differential conformational space accessible to the two domains of TRBP, even though they both adopt a canonical dsRBD fold, thereby affecting how they interact with target RNAs.

IT09: Structural and Functional Insights into the RNA Pseudoknots of the CRISPR- Cas12a Assemblies

Sruthi Sudhakar¹, Masad J. Damha³, Keith T. Gagnon², **P.I. Pradeepkumar¹**

¹ Department of Chemistry, Indian Institute of Technology Bombay

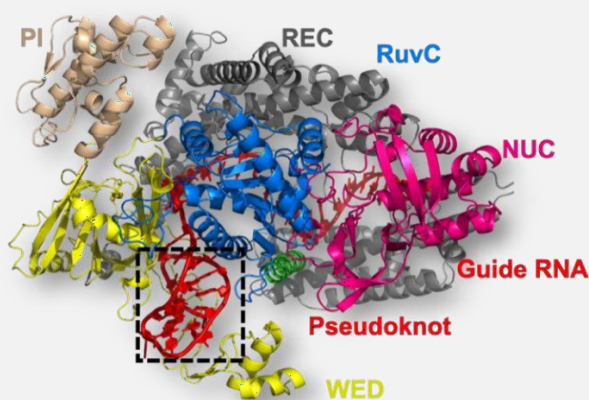
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Keywords: CRISPR-Cas12a, RNA pseudoknot, Molecular Dynamics

CRISPR-Cas12a complexes comprise the Cas12a effector protein and a single CRISPR RNA having conserved pseudoknot structure towards the 5'-handle. We have recently shown that Cas12a proteins undergo conformational rearrangements to accommodate crRNA-pseudoknot binding during RNP assembly via an induced-fit mechanism. Introducing chemical modifications in CRISPR-RNA (crRNA) is an efficient way to improve efficiency and target specificity for therapeutic gene editing. Gene editing assays revealed that the five DNA modifications towards 3' or 5' -ends in the pseudoknot RNA of Cas12a reduce the editing activity considerably. Our molecular dynamics (MD) simulations revealed that the DNA-modified complexes lost many protein contacts, critical for target DNA recognition, binding, and cleavage. Modifications also trigger large structural changes in the pseudoknot, leading to the movement of the wedge domain of the Cas12a from the pseudoknot, resulting in the loss of coordinated motion of the REC-NUC domain. This, in turn, results in alterations in the catalytic domain of the Cas12a protein, thereby reducing the editing activity. Our studies provide insights to rationally modify guide RNAs for developing CRISPR therapeutics.



IT10: Discovery and validation of novel compact RNA-targeting CRISPR/Cas13 systems

Dhanya Hegde, Varshini S, Monoswi Chakraborty, Sowmiya Ramesh, **Shruthi Sridhar Vembar**

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Keywords: CRISPR/Cas13, RNA knockdown, Transcriptome engineering, Base editing, RNA therapeutics

The ability to target RNA is integral to answering questions in basic research as well as for RNA-based diagnostics and therapeutics. Given the CRISPR/Cas boom in the past decade, single effector RNA-guided RNA-targeting Cas13 endonucleases have been touted to revolutionise this field. Cas13 contains an N-terminal region and helical domains that are involved in RNA recognition (REC lobe), and two nucleotide-binding HEPN domains that facilitate target RNA cleavage (NUC lobe). Based on domain arrangement, four Cas13 clades were initially described, Cas13a through d, with Cas13d being relatively smaller (~930 amino acids). The diversity further expanded in 2022 through the mining of metagenomic data to include clades Bt-A, Bt-B, e, f, g and i. However, the size of the smallest Cas13 reported so far is 700 amino acids, which poses a problem for efficient RNA editing and therapeutic delivery. Therefore, in this study, we used sensitive sequence-based searches implemented in PSI-BLAST and MMseqs2 to identify novel, compact Cas13 proteins (300-699 amino acids in length) in the NCBI non-redundant protein database. Based on the presence of the catalytic RX4H motif within the HEPN domains, identification of their CRISPR locus using CRISPRCasFinder, and *in silico* structural analysis, we shortlisted four compact Cas13 proteins of the a, b and d clades for experimental validation. A dual vector strategy was designed to validate RNA cleavage activity of the shortlisted systems in *Escherichia coli*, the results of which will be discussed. Additionally, the activity of the systems in human cells is being tested. If confirmed to be functional, these will be amongst the smallest known Cas13 proteins, and will prove invaluable for transcriptomic engineering and potentially for diagnostics and therapeutics.

IT11: PIP4K, a kinase with RNA binding activity

Sourav Halder, Ayushi Kumari and **Vasudevan Seshadri**

National Centre for Cell Science, Pune

Phosphatidyl inositol 5-phosphate 4-kinase (PIP4K) is a key regulator of phosphoinositide metabolism. We showed that PIP4K2A is imported into Plasmodium from the host erythrocytes and interact with specific transcripts. It interacts with UUGU motif of plasmodium Rad51 3' UTR. We hypothesized that PIP4K can also act as a RNA binding protein in host cells and regulate gene expression. PIP4K knockout mouse shows attenuated glutamate uptake through the glutamate receptor and defects in neuromuscular communication. Surprisingly the neuromuscular defects are also exhibited by drosophila PIP4K mutants. We explored if PIP4K can regulate the expression of GluRIIA. We found that Drosophila PIP4K (dPIP4K) can interact with the 3'UTR of GluRIIA mRNA and the interaction may be UUGU motif dependent. We also show that neuronal knock down (KD) of PIP4K shows drastic increase of GluRIIA expression in drosophila. The molecular mechanism of the regulation of GluRIIA expression by PIP4K and its interacting partners will be discussed.

IT12: *Plasmodium falciparum* YTH2 Domain Binds to m6A-Containing mRNA and Regulates Translation

Gayathri Govindaraju¹, Jabeena CA¹, Devadathan VS¹, Sreenivas Chavali²,
Arumugam Rajavelu¹

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²Department of Biology, Indian Institute of Science Education and Research (IISER) Tirupati., Andhra Pradesh, India.

The malaria parasite carries unique epigenetic signatures, which are required for optimal gene expression in the parasite during its development in red blood cells (RBCs). *Plasmodium falciparum* exhibits high translational plasticity during its development in RBCs, yet the regulation at the post-transcriptional level is not well understood. The N6-methyl adenosine (m6A) is an important epigenetic modification primarily present in mRNA that controls the levels of transcripts and efficiency of translation in eukaryotes. Recently, the dynamics of m6A on mRNAs at all three developmental stages of *P. falciparum* in RBCs have been profiled; however, the proteins that regulate the m6A-containing mRNAs in the parasites are unknown. Using sequence analysis, we computationally identified that the *P. falciparum* genome encodes two putative YTH (YT521-B Homology) domain-containing proteins, which could potentially bind to m6A-containing mRNA. We developed a modified methylated RNA immunoprecipitation (MeRIP) assay using PfYTH2 and found that it binds selectively to m6A-containing transcripts. The PfYTH2 has a conserved aromatic amino acid cage that forms the methyl-binding pocket. We show that F98 residue is essential for m6A binding on mRNA through site-directed mutagenesis experiments and molecular dynamics simulations. Fluorescence depolarization assay confirmed that PfYTH2 binds to methylated RNA oligos with high affinity. Further, MeRIP sequencing data revealed that PfYTH2 has more permissive sequence specificity on target m6A containing mRNA than other known eukaryotic YTH proteins. We identify and characterize PfYTH2 as the primary protein that could regulate m6A-containing transcripts in *P. falciparum*.

IT13: Autophagy-dependent regulation of Processing bodies (P-bodies)

Nikhil More and **Jomon Joseph**

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Key Words: P-bodies, Autophagy, CaMKK2, AMPK, Cytosolic Ca^{2+}

P bodies (PBs) are conserved, non-membranous cytoplasmic condensates of RNA-protein complexes. PBs are implicated in post-transcriptional regulation of gene expression through messenger RNA (mRNA) decay, translational repression and/or storage. While much is known about the de novo formation of PBs involving liquid-liquid phase separation through multiple protein-protein and protein-RNA interactions, subcellular localization and turnover mechanisms of PBs are less understood. Here we report the presence of a sub-population of PBs in proximity to ER-mitochondria contact sites (ERMCSs) at the steady state condition. Disruption of ERMCSs, achieved through depletion of ER-mitochondria tethering proteins, leads to disappearance of PBs. This effect can be reversed by inhibiting autophagy through both genetic and pharmacological means. Additionally, we find that the disruption of ERMCSs leads to cytosolic calcium (Ca^{2+})-induced activation of CaMKK2 and AMPK, ultimately resulting in autophagy-dependent decrease in PB abundance. Collectively, our findings unveil a mechanism wherein disturbances in ERMCSs induce autophagy-dependent loss of PBs via activation of the Ca^{2+} /CaMKK2/AMPK pathway, thus potentially linking ERMCS dynamics with post-transcriptional gene regulation.

IT14: Structural insights into the interplay of nascent chain processing factors with the 70S ribosome

Jayati Sengupta

CSIR-Indian Institute of Chemical Biology, Kolkata

Bacterial co-translational N-terminal methionine excision (NME), an early event of nascent polypeptide chain processing, is mediated by two enzymes - peptide deformylase (PDF) and methionine aminopeptidase (MetAP). Nascent peptide deformylation by PDF is an essential event in prokaryotes. Excision of the deformylated methionine is catalyzed by co-translationally acting enzyme MetAP. Trigger factor (TF), the only ribosome-associated bacterial chaperone, offers co-translational chaperoning assistance.

Cryo-EM structures of ribosome in complex with the nascent chain processing factors, published by our laboratory, provided crucial information on dynamic interplay of protein biogenesis factors at the ribosomal tunnel exit site to accomplish processing of nascent polypeptide chain.

Moreover, while *E. coli* expresses a single subclass of type1, MetAP1a, Mycobacteria contains two subclasses i.e. MetAP1a and MetAP1c differentiated by the presence of a 40 amino acid N-terminal extension in the later. We are trying to explore the exact reason for maintaining the two subclasses in mycobacteria.

IT15: Ribosome structure of pathogenic protozoan *Entamoeba histolytica***Prem S. Kaushal**

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Keywords: Ribosome, *Entamoeba histolytica*, protozoa, Cryo- EM and single particle reconstruction.

Entamoeba histolytica (Eh), a parasite protozoan, is responsible for amebiasis, an intestinal infection that results in bloody diarrhea and liver abscesses. Amoebiasis is more predominant in tropical areas with poor sanitation conditions, including India. Therefore, amoebiasis puts a huge economic burden on our country. Although ~40% of antibiotics in clinical use target functional centers for protein synthesis machinery, the ribosomes, the current treatment involves only one such drug (paromomycin) for clearing the asymptomatic Eh infection. The currently used drugs have their own side effects, and the drug resistance strains of Eh are also emerging. Moreover, there is no vaccine available to prevent amoebiasis. We determine Eh ribosome's high-resolution cryo-EM structure using cryo-EM single particle reconstruction of the 60S and 80S at 2.8 Å and 3.4 Å resolution, respectively. The structural analysis revealed several unique features such as triple helix structure in 28S rRNA, co-evolution of rRNA segment and r-protein, several Eh specific expansion segments in rRNA, and extension in r- protein. The unique features of Eh ribosome architecture will be presented.

IT16: mRNA recruitment during protein synthesis in eukaryotes

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Keywords: mRNA, ribosomes, protein synthesis, eIF4, translation initiation

The initial steps of protein synthesis in eukaryotic translation is highly complex requiring a myriad of eukaryotic initiation factors (eIFs). Various eIFs namely, eIF1, eIF1A, eIF3, eIF5 and the eIF2- GTP-initiator tRNA ternary complex bind to the 40S small ribosomal subunit to form the 43S pre- initiation complex (PIC). The mRNA is activated for initiation by the eIF4 group of factors, which help in recruiting mRNA-eIF4 complex to the 43S PIC resulting in the formation of the 48S PIC. eIF4B belongs to the eIF4 group of factors that help in mRNA recruitment to the 43S PIC in all eukaryotic organisms. However, there is no clear understanding of the location of eIF4B on the small ribosomal subunit and how eIF4B helps in the recruitment of mRNAs. Using cryo-electron microscopy we show that yeast eIF4B binds near the entry site of the mRNA channel of the small ribosomal subunit, thereby remodelling the entry site. The structural analysis of yeast eIF4B-bound ribosomal complex provide insights into possible events during mRNA recruitment.

IT17: Initiator tRNA-centric mechanisms of faithful translation initiation and ribosome maturation in bacteria

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The step of translation initiation comprises multiple stages. In bacteria it uses a special tRNA (tRNA^{fMet}). The tRNA^{fMet} is aminoacylated with methionine and then formylated with N10-formyl- tetrahydrofolate (N10-fTHF). Both methionine and N10-fTHF are produced via one-carbon metabolism, which makes the process of translation dependent on energy sufficiency in cell. The fidelity of tRNA^{fMet} binding to the ribosomal peptidyl-site (P-site) is attributed to the structural features in its acceptor stem, and the highly conserved three consecutive G-C base pairs (3GC pairs) in its anticodon stem. The acceptor stem region is important in formylation of the aa-tRNA^{fMet} and in its initial binding to the P-site. The 3GC pairs are crucial in transiting the i-tRNA through various stages of initiation. Interestingly, the use of the same tRNA^{fMet} in initiation and elongation steps (for example in mammalian mitochondria) is regulated by the extent of its formylation. Our studies provide renewed ways of use of trimethoprim and sulfa drugs to control bacterial growth. Further, we show that initiation complex formation, in the pioneering round of initiation, triggers the final stages of ribosome maturation. Interestingly, use of an anticonvulsant drug, lamotrigine, has provided important insights into the novel roles of IF2 in ribosome maturation.

IT18: When Paul Berg meets Donald Crothers: An achiral connection through protein biosynthesis

Rajan Sankaranarayanan

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Despite the presence and biological role of D-amino acids, the fundamental issue of how proteins are made only with the L-chiral entities was largely ignored. Over the last two decades, it has become clearer as to how multiple ‘Chiral Checkpoints’ work in concert to avoid D-amino acids from getting incorporated into proteins. Our recent work has shed light on how chiral proofreading systems have played critical roles in important evolutionary transitions. In this talk, I will introduce the work that came out of the laboratories of two of the pioneers of nucleic acid research in the area of protein biosynthesis, Paul Berg and Donald Crothers, more than half a century back. Their work on the identification of D- aminoacyl-tRNA deacylase (DTD) and ‘Discriminator hypothesis’, respectively, were hugely ahead of their time and were partly against the general paradigm at that time. In both of the above works, the smallest and the only achiral amino acid turned out to be an outlier as DTD can act weakly on glycine charged tRNAs with a unique discriminator base of ‘Uracil’. This peculiar nature of glycine remained an enigma for nearly half a century. With a load of available information on the subject by the turn of the century, our work on ‘chiral proofreading’ mechanisms during protein biosynthesis serendipitously led us to revisit these findings. Our analysis has uncovered an unexpected connection between them that has implications for evolution of different eukaryotic life forms.

IT19: Unraveling the Role of Polyadenylation Machinery in Asymmetric Stem Cell Division

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Asymmetric division is pivotal for stem cell maintenance and progenitor generation, facilitated by the uneven distribution of organelles and mRNA. Our research focuses on understanding the mechanisms governing mRNA partitioning in asymmetric division using Planarian models. Neoblasts are the pluripotent stem cells that are distributed widely throughout the body and essential regeneration, offer an ideal system for studying this process. In my presentation, I will discuss how polyadenylation machinery and polyA tails mediate mRNA partitioning in neoblasts during asymmetric division. We have found that disrupting polyA tail length, by targeting a critical PolyA polymerase isoform, hampers mRNA partitioning and inhibits asymmetric division. These findings highlight the crucial role of polyadenylation machinery in orchestrating mRNA localization during asymmetric stem cell division.

IT20: Decoding the role and clinical relevance of Non-coding RNAs in Glioblastoma

Ritu Kulshreshtha

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GBM is the most aggressive and fatal form of malignancy among the central nervous system (CNS) tumors. Patients suffering from this insidious disease have a median overall survival (OS) of 15 months, and only 5% of patients survive beyond five years of diagnosis. The standard care of treatment is maximum safe surgical resection, followed by concomitant chemo- and radiotherapy. However, the tumor almost invariably recurs leading to dismal prognosis. There is a unanimous consensus among researchers that an in-depth understanding of the molecular pathogenesis of GBM progression is required to design more effective therapy. Our work is focused on a miRNA, miR-210 and its host gene lncRNA- miR- 210HG both of which are shown to be hypoxia regulated and play oncogenic role in GBM. miR-210HG/miR-210 are induced by transcription factor- HIF1A under hypoxia. miR- 210HG/miR-210 are also significantly correlated with poor prognosis in GBM. Functional analyses of miR-210HG/miR-210 show that they play oncogenic role by promoting cellular proliferation, migration and inhibit apoptosis. miR-210 is also shown to promote glycolysis and inhibit mitochondrial oxidative phosphorylation in GBM. A detailed mechanistic study identifies novel target genes (NeuroD2, ALDH5A1) of miR-210 in GBM and identify their clinical relevance. miR-210 thus emerges as a potential therapeutic target in GBM. MiRNA therapy, although sounds promising, however lacks efficient delivery agents. To target the highly oncogenic miR-210, we have developed a novel, transglutaminase- based nanoflowers (TGNFs) functionalized with PEI that efficiently delivers anti-miR-210 to GBM cells, resulting in decreased proliferation, migration, and increased apoptosis. Overall, this study highlights the importance of miR-210 in GBM progression and offers an interesting therapeutic approach of delivering anti-miR-210 either alone or in combination using a novel miRNA nanocarriers.

IT21: RNA pseudoknot at the core of bacterial type III toxin-antitoxin systems**Mahavir Singh**

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Key words: toxin-antitoxin system; ToxIN, RNP, pseudoknot, structure

The nucleic acids can organize in various noncanonical structures such as G-quadruplexes, triplexes, and pseudoknots. These higher order structures are recognized by proteins resulting in specific nucleo-protein complexes, involved in various cellular events. Toxin- antitoxin (TA) systems are genetic modules in bacteria that play roles in plasmid maintenance, persister cell formation, drug tolerance, and phage resistance. Type III TA systems consist of a protein toxin and an RNA antitoxin (resulting in an TA RNP complex). In this talk, I will present our results on understanding the assembly, structure, and activation of bacterial type III toxin-antitoxin systems. Structures of type III TA complexes have revealed unique pseudoknot structure in the core of antitoxin RNA that is essential for the toxin inhibition by formation of a closed TA assembly. Understanding the TA complex assembly is important, as specific disruption of TA complexes, thereby releasing free toxins in pathogenic bacteria, has been envisioned as a potential antibacterial strategy.

IT22: Balanced interplay among the structured and disordered domains of RNA-binding protein

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Most proteins possess regions which are highly structured as well as regions which do not show any structure that are labeled as “disordered”. Ataxin2 is one such RNA-binding protein which has been implicated in the progression of hereditary neurodegenerative diseases as Amyotrophic Lateral Sclerosis (ALS) or Spinocerebellar Ataxia type 2 (SCA2). Disease progression depends upon the length of the polyQ repeats that are present in the protein. At the cellular level, Ataxin2 is important for cell viability and cellular responses to various types of stresses as well as itself is part of stress granules (SGs); at an organismal level, Ataxin2 regulates circadian rhythms, metabolism, and consolidation of long-term memory formation in *Drosophila*. To identify RNA targets of Ataxin2, we used TRIBE (Targets of RNA binding proteins Identified By Editing) technology which uses the property of adenosine deaminase (ADAR) to edit mRNAs. Ataxin2 seems to act as a stabilizer for its target mRNAs in the RNP granules, where it preferentially binds AU-rich elements (AREs) in the 3'UTRs. Detailed analyses also revealed the way the different structured and disordered domains of Ataxin2 regulate its interaction with RNAs and its ability to form condensates in the cells. I will also elaborate on our efforts to understand the assembly and clearance of such condensates.

IT23: Unraveling the Nexus: Hypoxic Tumor Microenvironment and Alternative Splicing

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Keywords: Hypoxia, Alternative splicing, CTCF, DNA methylation, Cancer

In this talk, I will delve into the fascinating world of hypoxia-mediated regulation of gene expression and alternative splicing in cancer. Hypoxia, a state of low oxygen, plays a crucial role in tumor progression and metastasis. Our laboratory investigates the molecular mechanisms by which hypoxia alters gene expression and splicing patterns, leading to significant changes in cellular behavior and phenotype. In one of our studies, we have demonstrated that the non-canonical pyruvate kinase M2 (PKM2) regulates the hypoxia- responsive element (HRE) mediated upregulation of PFKFB3 in hypoxic cancer cells by facilitating HIF-1 α and p300 enrichment, while the absence of PKM2 leads to poised chromatin states that restrict HIF-2 α -induced PFKFB3 expression, and the inhibition of PKM2 nuclear translocation by Shikonin demonstrates potential therapeutic implications for breast cancer. In another story, we have discovered an intricate interplay between the splicing factor SRSF2, DNA methylation, CTCF recruitment, and RNA polymerase II occupancy to regulate the angiogenesis-related isoforms of VEGFA-165 under normoxic and hypoxic conditions. Additionally, we have also discovered an intricate interplay between CTCF-mediated promoter-upstream looping and CTCF-mediated RNA polymerase II pause at exon, regulating alternative splicing outcome of EMT-associated gene. My talk will give you an overview of the intricate connections between hypoxia and the dynamic landscape of gene regulation and alternative splicing in cancer.

IT24: Alternative splicing and its impact on the metabolic syndrome to stem cell differentiation

Indrani Talukdar

BITS-Pilani, K K Birla Goa campus.

Alternative splicing is a post-transcriptional gene regulatory mechanism that controls various aspects of cellular function and causes a diverse array of proteins from the same genetic material. More than 95 % of human genes are alternatively spliced sometimes in tissue and developmental stage-specific manner. The aberrant splicing phenomenon has been attributed to various pathological conditions in humans.

Our group is interested in understanding the role of AS and its regulatory players in metabolic syndromes as well as in stem cell differentiation. Metabolic syndromes (MetS) are a cluster of conditions that may occur together, including but not limited to obesity, insulin resistance, and Type II diabetes. We have created insulin-resistant mouse skeletal muscle cells (C2C12) by treating the differentiated myotubes with palmitic acid or TNF- α and analyzed the expression pattern of various AS-regulatory proteins (mostly known as RNA binding proteins, RBPs). Our data suggest an overall downregulation of RBPs, especially those belonging to the heteronuclear ribonucleoprotein family (HnRNPs) of proteins under insulin-resistant conditions. We have observed a similar trend in the liver in the high-fat diet-induced pre-diabetic animals. Consequently, we also have observed that the splicing pattern of some of the target genes of these RBPs, which play contributory roles in MetS, has also altered, establishing the correlation between MetS and AS.

Other than trying to understand the role of AS and associated RBPs in MetS, we are also interested in their roles in the maintenance and differentiation of pluripotent stem cells. Given the potency status of the mouse embryonic and human iPSC stem cells, we have identified that the expression profile of a group of HnRNPs is highly regulated. Lineage-specific differentiation drives drastic downregulation of these hnRNPs, whereas their expression is vastly upregulated in the pluripotent state. We have shortlisted two such hnRNPs, hnRNP U and D, to investigate their roles further. The siRNA-based analysis confirmed the role of hnRNP U in the upregulation of differentiation markers in mESCs, whereas the CRISPR-based gene editing in human iPSC followed by a transcriptome-wide RNA seq analysis confirmed the role of hnRNP U mostly in definitive endodermal differentiation and hnRNP D in muscle cells (especially cardiac muscle) fate commitment and maintenance of their structure and function. Taken together our studies show the crucial roles played by AS and its regulators in MetS and stem cell differentiation.

IT25: Discovering Transcriptome-wide Circular RNA Interaction with mRNAs

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Keywords: Circular RNA, cross-linking, RNA hybrid, RNA-sequencing, mRNA expression

Circular RNAs (circRNAs) represent a family of ubiquitously expressed, abundant, single-stranded RNA molecules without free ends. Due to their high stability and resistance to cellular exonucleases, they have been shown great potential as cellular regulators of gene expression by interacting with various molecules. CircRNAs regulate gene expression by interacting with microRNAs and RNA-binding proteins. However, the interaction of circRNAs with the protein-coding mRNAs is mostly unexplored. Here, we developed an AMT-mediated cross-linking of RNA-RNA duplexes followed by selection of mRNAs, enrichment of circRNAs, and high-throughput sequencing to identify mRNA-associated circRNAs in intact cells. We invented a novel Cross-Linking, Poly(A) Pulldown, RNase R, Sequencing (CLiPPR-seq) method that identified hundreds of circRNAs interacting with protein-coding mRNAs in diverse cell types. These findings suggest that the novel circRNA-mRNA interactions may alter mRNA translation, localization, or stability. Silencing of mRNA-interacting circRNAs altered the expression of target mRNAs in β TC6 and C2C12 cells, indicating the discovery of novel circRNA-mediated gene regulation by direct interaction with mRNAs. Together, the discovery of transcriptome-wide mRNA-circRNA interactions by CLiPPR-seq indicates the necessity to characterize these novel interactions thoroughly.



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DRASTIC lncRNA may regulate DNA damage response via FUS dependent LLPS

**FT01: Unravelling the secrets of a key enzyme in a trypanosomal parasite:
Insights into structural and functional attributes.**

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Keywords: *Trypanosoma cruzi*, arginyl-tRNA synthetase, drug target, Transfer RNA, Fluorescence.

Aminoacyl-tRNA synthetases (aaRSs) are essential and omnipresent “housekeeping” enzymes responsible for charging amino acids onto their corresponding tRNAs. These enzymes provide the substrates necessary for global protein synthesis. Recent research suggests additional roles for multiple aaRSs in pathological processes, highlighting their potential as pharmacological targets and valuable therapeutic agents. Chagas disease, a disease that echoes through the lush rainforests of Latin America, is a life-threatening illness caused by the *parasite Trypanosoma cruzi*. While treatments such as Nifurtimox and Benznidazole exist, they have undesirable side effects. Clearly, new solutions are urgently needed to combat this complex disease. We identified a unique feature of the arginyl-tRNA synthetase (TcArgRS) of this parasite: a 100- amino acid segment missing in its human counterpart. Intrigued, we performed a preliminary characterization of the enzyme using biophysical, biochemical, and bioinformatics tools. We expressed this protein in *E. coli* and validated its enzymatic activity in vitro. Additionally, analysis of DTNB kinetics, circular dichroism (CD) spectra, and ligand-binding studies using intrinsic tryptophan fluorescence measurements helped us gain insights into its structure despite the lack of a 3D crystal structure. Our findings suggest that TcArgRS can distinguish between the natural amino acid (L-arginine) and its analogues. This study paves the way for the development of targeted therapies by designing small molecules that specifically interact with TcArgRS's unique features to potentially disrupt the parasite's protein production, offering a novel approach to combat Chagas disease.

FT02: Modulation of structure and thermodynamics of RNA G-quadruplexes by A to I editing: Possible route to G-quadruplex-based RNA therapeutics

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Keywords: G-quadruplex, RNA modifications, A to I editing, RNA therapeutics, Structure and dynamics

Inosine (I) is one of the most abundant RNA modifications and it plays a significant role in several essential biological processes. The effect of inosine has been extensively studied within RNA duplexes but its effect on RNA quadruplex structures is required to be better explored. The occurrence of RNA G-quadruplex (rG4) structures in non-coding RNAs (ncRNAs), e.g. miRNAs, lncRNAs etc. and non-coding regions (UTRs and introns) of mRNA¹, suggests their potential role in the regulation of ncRNA function and ncRNA-protein interactions. The inosine (A to I editing) modification, frequently occurring within ncRNAs², has been reported to activate latent rG4 structures by forming GI-quadruplexes³. In the present study, we have analyzed the structure, dynamics, energetics and hydration patterns of I-, G-, A-tetrads within similar rG4 structures using extensive molecular dynamics simulations to understand the effect of inosine substitutions on the stability of such structural motifs. Our results reveal increased stabilization of I-tetrad compared to A-tetrad indicating the possibility of formation of stable G-quadruplexes with I-tetrad/s as a result of A to I RNA editing. Additionally, we also report the effect of 2'-O-methylation of the A, G and I residues, on the conformations of the tetrads and the quadruplexes. The properties of the quadruplexes and tetrads reported in this work will help in elucidating the possibility of utilizing modified RNA G-quadruplex motifs as therapeutic targets and might also aid the design of potential G-quadruplex-based RNA therapeutics incorporating RNA modifications.

FT03: Determining novel cytoplasmic mRNA capping enzyme-targeted, hypoxia-responsive RNAs in hypoxic osteosarcoma cellular model

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Keywords: Hypoxia, cytoplasmic capping enzyme (cCE), posttranscriptional modification.

Hypoxia is the hallmark of solid tumors and is one of the main reasons for cancer-related mortality. Hypoxia-inducible factors (HIFs) are the key molecules that elicit a hypoxic response by changing cellular transcriptomic output. Most of the RNA pol II transcript has a 5' cap. Cap protects the RNA transcripts from exonucleolytic cleavages and helps their export to the cytoplasm. The previous notion that capping takes place in the nucleus was changed by the identification of a cytoplasmic pool of capping enzyme (cCE) that can add a cap onto decapped transcripts. Cellular recovery from a brief arsenite-induced oxidative stress requires cCE to recap its target transcripts that were decapped during stress. However, cCE-mediated posttranscriptional regulation of hypoxia-responsive RNAs has not been studied yet. This study focuses on identifying novel hypoxia-responsive cCE-targeted mRNAs. Using an RNA Seq approach we analyzed the cytoplasmic transcriptome of U2OS cells treated with CoCl₂, a hypoxia mimetic reagent. Differential expression of genes and pathway enrichment analyses a few transcripts were selected that were associated with hypoxic stress response for their further validation in osteosarcoma cells. qPCR and immunoblotting analysis showed upregulation of RORA and KCTD16 transcripts upon hypoxic stress. Pharmacological inhibition of HIF1 α downregulated the expressions of RORA and KCTD16 which suggests that their expressions are mediated by HIF1 α during hypoxia. Cap analysis of gene expression (CAGE) revealed internal CAGE clusters on RORA and KCTD16, thus implying that they could be recapped by cCE. In cytoplasmic capping inhibited cells (K294A) the expressions of the selected transcripts were attenuated as confirmed by qPCR. Upon inhibition of cCE, these transcripts were found to be decapped as revealed by the in vitro XRN1 susceptibility assay that was used to measure the cap status of the transcripts. Hence, this study highlights the novel role of cCE in targeting the hypoxia-responsive RNAs post transcriptionally.

FT04: Early life stress-induced RNA methylation regulates spatial memory

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Keywords: Early-life stress, Maternal separation, RNA methylation, Long non-coding RNA, Spatial memory.

Early-life stress (ES) significantly impacts diverse cognitive functions including memory. The ES-induced behavioural deficits rely on transcriptomic changes. However, our understanding of post-transcriptional regulation following ES remains elusive. Emerging studies have implicated RNA methylation, particularly N6-methyladenosine (m6A) modification, as a new regulatory switch that governs the function of developing as well as adult brains. The reversibility of m6A tagging pivotally positions a subset of transcripts to tune the synaptic activity upon external stimuli. Prompted by these observations, we hypothesized that ES could influence transcript-specific RNA methylation in the brain and reversal of this modification could rescue ES-induced cognitive function, such as spatial memory and anxiety. We have used the maternal separation paradigm to model ES in mice and evaluated the impact of ES on anxiety and memory. The maternal separation paradigm involves separating mouse pups from their mothers for three hours daily from post-natal day 2 (P2) to P14. m6A tag in transcripts was immunoprecipitated from the hippocampus isolated from P21 mice and the identity of these transcripts was assessed by transcriptomic analysis (RNA-IP Seq). The RNA-IP Seq analysis identified a selection of coding as well as non-coding transcripts including long non-coding RNAs. The bioinformatic analysis revealed that m6A tagging predominantly occurs in the untranslated region of the coding transcripts.

The ES-induced compartmentalization of transcripts via the addition of the m6A mark is accompanied by deficits in memory and enhanced anxiety. We observed that this hypermethylation of non-coding transcripts occurs due to a reduced level of brain-enriched demethylase FTO (Fat mass and Obesity-associated). The overexpression of FTO in hippocampal neurons in vivo could rescue memory deficits but not anxiety. Taken together, our study provided a novel therapeutic approach to rescue memory deficits upon ES.

FT05: Translation inhibition primes cells to face stress via activation of acute inflammation

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Keywords- Sterile inflammation, self-immunogenic RNA, translation inhibition, phase-separated condensates, stress granules

Organisms face a variety of stresses, including oxidative stress, hypoxia, heat shock, pathogenesis, and nutrient deprivation. Cells often respond to these stresses by reprogramming transcription and translation to activate acute inflammation. Inflammation is also associated with normal cellular functioning, like cell proliferation, differentiation, and wound healing, but prolonged inflammation can lead to cell death, aging, and various diseases like type I interferonopathies. These diseases are associated with a state of low-grade systemic chronic inflammation appearing even in the absence of external infection and, therefore, called sterile inflammation. Hence, it is important to understand the source and the buffering mechanism of endogenous sterile inflammation.

A common factor between various stresses is inflammation and inhibition of translation. Hence, we hypothesize that translation inhibition can cause inflammation directly. We found that a short pulse of translation inhibition by puromycin or cycloheximide was enough to cause translocation of immune response-related transcription factor- IRF3 into the nucleus and transcription upregulation of inflammatory RNAs. This triggering of the immune response coincided with the increase in concentration of dsRNA in the cells.

dsRNA can be produced in the cells from various sources, including sense-antisense RNA, nuclear transcripts, or mitochondrial transcripts. The RNA-based sterile infection was shown to cause inflammation in cells transfected with RNAs purified from ribosomes. Interestingly, our experiments with translation inhibition drugs also show that mRNA released from the ribosome is not the only source of inflammation. Recent literature suggests that the free mRNA and endogenous immunogenic dsRNA are known to be sequestered in RNA-RBP condensates called stress granules, which buffer inflammation. In the future, we will identify the dsRNA and probe the role of stress granule proteins in buffering the inflammation caused by the acute increase of dsRNA during translation inhibition.

FT06: RGG-motif protein Scd6 affects oxidative stress response by regulating Cytosolic caTalase T1 (Ctt1)

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Keywords: Catalase, Translation, RGG-motif proteins, oxidative stress, post-transcriptional regulation.

Upon stress, cells undergo intricate gene expression changes to combat external challenges. As a coping mechanism, cells employ a conserved strategy known as global downregulation of translation since it is an energy expensive process, which involves sequestering mRNA molecules in RNA granules or RNP condensates. In oxidative stress, genes responsible for combating oxidative stress, such as catalases and glutathione peroxidase, are strongly induced. The post-transcriptional regulatory events affecting these genes during H₂O₂ stress are not well- explored. RNA binding proteins, particularly those containing RGG motifs, play crucial roles in mediating translation regulation. Scd6, an RGG motif-containing protein in yeast, is known to act as a translational repressor by interacting with eIF4G. While its general function as a translational regulator is established, its impact on specific mRNA targets remains elusive. This study sheds light on the involvement of Scd6 in the oxidative stress response by elucidating its role in regulating cytoplasmic catalaseT1 (CTT1). Upon H₂O₂ treatment, Scd6 forms dynamic puncta that contain RNA. Interestingly, alteration of Scd6 levels affects Ctt1 protein leading to changes in ROS levels and physiological behaviour of the cell in response to oxidative stress. Notably, the overexpression of a Scd6 mutant lacking the RGG motif fails to affect Ctt1 protein levels, emphasizing the importance of this domain in regulating Ctt1 expression. This study also demonstrates the interaction between CTT1 mRNA and Scd6, showing that increased interaction correlates with reduced Ctt1 protein levels upon oxidative stress. Furthermore, we show the increased localization of LSM14A (human homologue of Scd6) to granules hinting toward the conserved role of Scd6 family proteins in oxidative stress response. Overall, this research unveils a novel role for a conserved RNA-binding protein in response to H₂O₂ treatment, providing valuable insights into the mechanisms governing cellular adaptation to external stressors.

FT07: Hidden in the Code: Exploring the Dynamic Interplay of 5'-UTR Variants, uORFs, and RNA G-Quadruplex in the Regulation of Human cIAP1 Gene Expression

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Keywords: Alternative 5'-UTRs, uORFs, RNA G-Quadruplex, Translation

We have explored the post-transcriptional regulation of the anti-apoptotic human cIAP1 gene, which is frequently overexpressed in various cancer cells, emphasizing its alternative 5'-UTR variants. Our research focused on two primary alternative transcript variants of the cIAP1 gene - 213 and 201 - that differ mainly in their 5'-UTR compositions. Detailed investigations through luciferase reporter assays suggested distinct translation efficiencies among these alternative 5'-UTR variants in cells. Interestingly, transcript variant 201 includes exon 1A along with exon 2 in its 5'-UTR, whereas the 5'-UTR of transcript variant 213 is composed of exon 1B and exon 2. We observed that the selection of exon 1A or exon 1B significantly affected the translation output of each 5'-UTR. In particular, we identified an evolutionarily conserved sequence motif in exon 1A known to fold into a stable RNA G-quadruplex structure that promotes translation¹. Upon further investigation of exon 2, which is conserved in both transcript variants, an evolutionarily conserved upstream Open Reading Frame (uORF) was revealed to repress luciferase translation. These findings deepen our understanding of the complex mechanisms behind post-transcriptional regulation and highlight the important roles of alternative 5'-UTRs, uORFs, and RNA G-quadruplex structure in modulating human cIAP1 gene expression. Future work will further explore these regulatory elements and their potential as therapeutic targets in diseases where cIAP1 overexpression is implicated.

FT08: Regulation of the expression of ribosomal proteins by small downstream ORFs

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Keywords: Translation initiation, ribosomes, downstream ORFs.

Translation in the 3'UTR of mRNAs can occur due to stop codon readthrough or reinitiation at a downstream ORF (dORF). While stop codon readthrough results in a C-terminally extended protein isoform, translation reinitiation results in more than one protein from a single transcript. Both these phenomena are observed across different kingdoms of life. Ribosomes are large multimolecular ribonucleoprotein particles that decode genetic information from mRNA to form proteins through translation. We looked for evidence of translation events in the 3'UTRs of ribosomal protein transcripts. Analyses of ribosome profiling data and the evolutionary conservation of mRNAs that encode ribosomal proteins revealed that 3'UTRs of human RPS27A (eS31) and RPL36A (eL42) mRNAs undergo translation. Further experimental analyses using luminescence-based assays, qRT-PCRs, fluorescence microscopy, and western blots validated that S27A and L36A encode a translatable short ORF in their 3'UTRs that generate microproteins. The presence of the dORFs increases transcript levels, leading to increased S27a and L36a protein expression, as observed using qRT-PCR and western blot. CRISPR-based deletion of the dORFs of either S27A or L36A resulted in decreased mRNA levels of the transcript. The deletion clones also demonstrated slow proliferation and reduced global translation compared to parental wild-type cells. This study demonstrates a unique mechanism of regulation of ribosome formation and translation in mammalian cells.

FT09: Emergence of an effective translation proofreader enabled the surge of proline-rich proteins in metazoans

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Keywords: Animalia-specific tRNA deacylase (ATD), tRNA, evolution, proline-rich proteins.

Gene duplication and neofunctionalization of multiple genes is one of many events that increased the complexity of the early metazoans and ultimately dictated the origin of metazoans. Animalia-specific tRNA deacylase (ATD), a translational proofreader, is one such protein that originated in choanozoa to remove alanine mischarged on tRNAs with G4•U69 by alanyl-tRNA synthetase (AlaRS). Previous studies indicated that the presence of ATD and tRNAThr (G4•U69) is well correlated and ATD's activity on Ala-tRNAThr becomes essential under oxidative stress. Thorough bioinformatics analysis showed that the genomic fixation of tRNAThr (G4•U69) occurred during chordate evolution (500 Mya) and the emergence of ATD in choanoflagellate (900 Mya). The inception of a proofreader around 400 million years before the proposed substrate suggests that the origin of ATD is not directly linked with Ala-tRNAThr mischarging but that the activity is acquired later. On the other hand, biochemical analysis by mutating tRNA elements showed that ATD prefers pyrimidine at the discriminator base position (U/C73). These two independent observations led to the proposition that ATD is a multi-substrate translational proofreader that can remove more than one specific mischarged tRNA. In accordance, we noted that ATD can effectively deacylate Ala-tRNAPro, a mischarged product of prolyl-tRNA synthetase (ProRS). Further analysis of 280 proteomes for individual amino acid content shows that the emergence and presence of ATD are in correlation with the sudden expansion of proline-rich proteins (PRPs) seen in the metazoan lineage. Overall, the study points out a stringent tRNA error correction strategy in metazoans that allowed evolution to shape the complex proteome.

FT10: Tetraphenylethene Derivatives Modulate the RNA Hairpin-G-quadruplex Conformational Equilibria in Proto-Oncogenes

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Keywords: Cancer, Conformational equilibria, G- quadruplex, Ligand design, Proto-oncogenes, TPE

RNA G-quadruplexes (GQs) sequences in 5' UTRs of certain proto-oncogenes colocalize with hairpin (Hp) forming sequence resulting in intramolecular Hp-GQ conformational equilibria, which is suggested to regulate cancer development and progression. Thus, regulating Hp-GQ equilibria with small molecules is an attractive but less explored therapeutic approach. Herein, two tetraphenylethenes (TPE) derivatives, TPE-Py and TPE-MePy, were synthesized, and their effect on Hp-GQ equilibrium was explored. The FRET, CD, and molecular docking experiments suggested that cationic TPE-MePy shifts the Hp-GQ equilibrium significantly towards the GQ conformer mainly through π - π stacking and Van der Waals interaction. In the presence of TPE-MePy, the observed rate constant values for the first and second folding steps increased by 14.6 and 2.6-fold, respectively. The FRET melting assay showed a solid stabilizing ability of TPE-MePy ($\Delta T_m = 4.36$ °C). Notably, the unmethylated derivative TPE-Py did not alter the Hp-GQ equilibrium. Subsequently, the luciferase assay demonstrated that the TPE-MePy derivatives suppressed the translation efficiency by 5.7-fold by shifting the Hp-GQ equilibrium toward GQ conformers in 5' UTR of TRF2. Furthermore, Western Blot suggests a decrease in NRAS proto-oncogene expression by 1.8-fold in MCF-7 cells at 24 hours. TPE-MePy also suppresses the expression of NRAS by 2.1-fold at 72 hours in MD-MB-231 cells, which are highly proliferative triple-negative breast cells and are resistant to many drugs. In conclusion, our data suggest that HpGQ equilibria could be selectively targeted with small molecules to modulate translation for therapy.

FT11: DRASTIC lncRNA may regulate DNA damage response via FUS dependent LLPS**Akshaykumar Chaudhari**, Juhi Srivastava and Vidisha Tripathi

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akki.com26@gmail.com**Keywords:** lncRNA; DNA damage response; DRASTIC; LLPS; FUS;

DNA is constantly exposed to various types of extrinsic (chemicals or UV radiation) or intrinsic (replication errors and cellular metabolism) challenges. Prolonged or repetitive exposure to these factors may lead to the formation of DNA double-stranded breaks (DSBs), resulting in DNA damage response. DSB is the most lethal form of DNA damage in cells and is repaired by the NHEJ, HR, or MMEJ pathways. Although the molecular players of these repair pathways have been well characterized; recently there has been much discussion about the precise roles and molecular mechanisms by which lncRNAs regulate DNA damage response (DDR). In our laboratory, we have discovered a nuclear-retained lncRNA DRASTIC [DNA Repair Associated Transcript In Cell] that plays important role in cellular proliferation. Depletion of DRASTIC leads to arrest of cells at G1/S and S phase with a concomitant decrease in G1 phase of the cell cycle. Furthermore, DRASTIC depleted cells showed defective loading of pre-RC components onto chromatin and stalled replication forks in addition to DNA damage during S phase leading to intra-S checkpoint activation. Several components of the major repair pathways were underrepresented in DRASTIC depleted cells suggestive of severely defective DNA repair program, implicating its role in regulating DNA repair in cells. We further confirmed that DRASTIC RNA interacts with several proteins involved in the DNA repair pathway including FUS, RBMX, NONO, SFPQ, PARP1. Interestingly, FUS is one of the upstream regulators of the DDR pathway as it recruits DDR factors onto DNA damage sites by promoting LLPS thereby physically contributing to the efficient recruitment of key DDR factors. DRASTIC depletion led to increased levels of FUS suggesting that FUS is stabilized upon DRASTIC depletion. Additionally, FUS protein was homogenously redistributed in the nucleus in DRASTIC depleted cells suggesting that interaction of DRASTIC with FUS helps it to form aggregates. To summarize, our results indicate that DRASTIC lncRNA interacts with FUS protein and promotes LLPS thereby might play a central role in guiding FUS to recruit at damage sites and initiate repair.



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P01: Investigating the molecular mechanism of non-AUG codon-based translation initiation in yeast

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Keywords: Non-AUG codon, Trans-acting factor, Ribosome heterogeneity, Start codon selection, mTRIP

Establishing an Open Reading Frame (ORF) is crucial for decoding the genetic code and subsequent translation into protein. While most mRNAs utilise the AUG codon as the start codon for translation, there are instances where non-AUG codons, such as CUG or GUG, can also initiate translation. In yeast, one such example is the α -ketoglutarate dehydrogenase (KGD4) gene, which is translated into two isoforms: one using the conventional AUG start codon and the other utilising an upstream UUG codon, both from a single mRNA. The translation initiation process is typically stringent in its selection of start codons. However, non-AUG codons like UUG can still be chosen as the start codon, raising questions about the underlying molecular mechanisms. We hypothesised that this UUG codon-based initiation might involve trans-acting factors associated with the ribosome during non-AUG codon selection. To explore further, we conducted mRNA-Tethered Ribosome Interactome Profiling (mTRIP) to identify trans-acting factors on the 48S ribosomal complex associated with KGD4 mRNA. Remarkably, we identified a non-canonical translation initiation factor associated with the 40S ribosomal complex on KGD4 mRNA, absent in control mRNA. This finding suggests that the selection of a non-AUG codon as the translation start site may involve an alternative translation initiation mechanism distinct from the canonical one. The 48S complex facilitating non-AUG codon selection may differ from the canonical 48S complex, resulting in ribosome heterogeneity within the cell. Further investigation aims to uncover other mRNAs where this mechanism governs translation initiation and elucidate how these ribosomes are recruited to the mRNA and, particularly, to the non-AUG start site.

P02: Understanding the role of altered RNA metabolism upon inflammation

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Keywords: RNA metabolism, inflammation, phase separation, nuclear speckles, splicing

Many cellular condensates consist of RNA and RNA Binding proteins (RBPs) and cellular concentration of RNAs has been shown to affect the condensate formation and integrity. A high concentration of RNA dissolves condensates and a low concentration of RNA can nucleate condensates. The presence of RNA can also affect the transition of liquid-like condensates into solid-like aggregates. In inflammatory conditions, selective degradation of cellular RNAs via activation of RNase L leads to a widescale rewiring of cellular transcriptome. However, how the changes in cellular RNA concentrations affect the phase behaviour and function of condensates is unclear. Previous literature showed that dsRNA-mediated inflammation activates cellular RNaseL leading to lower levels of cellular RNA and translational shutdown within 2hrs of induction of inflammation. We measured the effect of inflammation on the nuclear splicing speckles. Nuclear splicing speckles or speckles are RNA-RBP condensates which consist of splicing machinery and are known to influence splicing. Speckles are irregularly shaped, liquid-like condensates present in euchromatin regions. Their formation is driven by intrinsically disordered domain-rich scaffold RBPs like SON & SRRM2, and it also consists of RNAs like polyA-tailed RNAs, and long non-coding RNAs- MALAT1 & Meg3.

In our study, we found a global decrease in cellular RNA concentration by inflammation induced by lipopolysaccharides or nucleic acids like dsRNA and dsDNA. We also found a concomitant change in the speckle shape and organisation of its scaffold RNA and RBPs. SRRM2 forms a dense spherical structure at the speckle centre whereas SON and polyA RNA form a more irregularly organized peripheral ring in the speckles. Apart from changes in speckle organization, mislocalisation of the speckle proteins to the cytoplasm was observed under dsDNA-mediated inflammation. In future, we will be testing our hypothesis of the loss of transcription-mediated contact of the speckles with the euchromatin as the underlying reason for changes in the phase behaviour of speckles during inflammation-mediated rewiring of global RNA metabolism.

P03: Exploring RNA Structure-to-Function role through Chemical Probing and Mutational profiling

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Keywords: RNA, Chemical probing, Mutational profiling, RNA frameshift element, RNA therapeutics

Although RNA is being considered as a central molecule in molecular and cellular biology, it is experimentally challenging to detail it because of its fragility and dynamicity. However, advances in RNA technology, like chemical structure probing, mutational profiling, next-generation sequencing and RNA-targeted therapeutics, have made RNA biology considerably more accessible experimentally. These advances provide opportunities to establish specific functional and regulatory aspects of RNA. With my expertise in RNA biology I intended to develop a unique program to investigate RNA structure, modifications and functions in disease models. Using combination of above methodologies, I have discovered alternative conformations of Telomerase RNA from *T. brucei* causing Trypanosomiasis. This alternative conformation is required to regulate the function of *T. brucei* telomerase RNA during its dual life cycle in humans and insects. In other study I have also determined the conformational ensembles of RNA frameshift element (FSE) from SARS-CoV 2 virus enabling the pathogen to translate viral proteins from two alternative open reading frames. This translational regulation is critical for the virus to express multiple proteins from its compact genome. Recently, I have also identified structural modulations in pre-miR-675 upon its interaction with H19 lncRNA. This mechanistic insight provides the basis of regulatory feedback mechanism of H19 lncRNA on miR-675 in cancer. These studies illustrate the local and global arrangements that an RNA can undergo to regulate gene expression and underline the conformational dynamicity of an RNA molecule which is required by the cells to regulate its normal physiological processes.

P04: Oncogenic role of miR-X in regulating Epithelial-to-Mesenchymal transition (EMT) and stemness in breast cancer

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Keywords: Breast cancer; EMT; microRNAs; stemness

Breast cancer is the most frequent cancer diagnosed in females worldwide. The onset of metastasis via activation of the epithelial-to-mesenchymal transition (EMT), and the occurrence of cancer stem cells render cancer therapy ineffective, thereby increasing mortality rates. Non-coding RNAs are currently emerging as major mediators of cancer pathways. In this study, we have worked on one such oncogenic microRNA, miR-X, which is highly expressed in breast cancer. We used the antisense inhibitor of miR-X, and miR-X-overexpressing clone to assess effects of miR-X on breast cancer cell lines with different subtypes: MCF-7 and MDA-MB-231. Cells with modulated expression of miR-X were subjected to wound healing assays, flow cytometry based CD44/CD24 analysis, western blot analysis for EMT and stemness proteins, qRT-PCRs for measuring transcript levels, and treated with commonly used chemotherapeutic drugs. Potential targets of miR-X were explored through bioinformatic tools and further validated with Luciferase reporter assays. The suppression of miR-X through anti- miR-X was found to inhibit the migration of breast cancer cells; with a decrease in the EMT- transcription factor ZEB1, and other mesenchymal markers. We also observed a decrease in the transcript and protein of stemness-associated transcription factor OCT4, KLF4, Nanog and marker CD133. Induced overexpression of miR-X was found to display opposite effects. The percentage of CD44^{high}/CD24^{low} breast cancer stem cells was significantly decreased after treatment with anti-miR-X, indicating the suppression of stem cell population. Additionally, we obtained novel targets of miR-X, that were further validated through 3' UTR Luciferase assays, that might be key players in mediating miR-X mediated oncogenesis. In conclusion, our study highlights the potential of anti-miR-X treatment to suppress the EMT and stemness phenotype in breast cancer cells, paving the way for development of a novel therapeutic agent to curb breast cancer aggressiveness.

P05: Physiological significance of the two isoforms of initiator tRNAs in *Escherichia coli* K strain

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Keywords: i-tRNA^{fMet1}, i-tRNA^{fMet2}, tRNA stability, co-culture experiment

E. coli genome harbours four copies of initiator tRNA (i-tRNA) genes. Three of these (*metZ WV*) reside at 63.5' locus as and the fourth one (*metY*) resides at 71.5'. In *E. coli* B, all four i-tRNA genes (*metZ WV* and *metY*) encode identical i-tRNAs (tRNA^{fMet1}) and possess G46 (as m⁷G46). In *E. coli* K, *metZ WV* encodes for tRNA^{fMet1}, whereas *metY* encodes a variant, tRNA^{fMet2} possessing A46 in place of m⁷G. To check the physiological relevance of the allelic variations at 46th position, we generated *E. coli* strains, which are sustained either on *metYA46* (*metY* of *E. coli* K origin encoding i-tRNA^{fMet2}) or its derivative *metYG46* (encoding i-tRNA^{fMet1}) in single (chromosomal) or plasmid borne copies. Co-culture experiments followed by northern blotting for i-tRNA show that strains sustained on i-tRNA^{fMet1} have growth fitness advantage over those sustained on i-tRNA^{fMet2} in LB media. However, in nutrient poor media (M9 media containing 0.2% glycerol), strains supported on i-tRNA^{fMet2} show somewhat better fitness. We also show that in LB media i-tRNA^{fMet1} is more stable than i-tRNA^{fMet2} by rifampicin assay as well as molecular simulation studies. Interestingly, i-tRNA^{fMet2} stability is comparable with i-tRNA^{fMet1} in nutrient limiting condition. Thus, these experiments suggest that these allelic variation in i-tRNA (G46 or A46) might have been selected to offer optimal growth/fitness under varying nutrient conditions in the environment.

P06: Investigating the crosstalk among canonical and non-canonical initiation factors in recruitment of viral IRES to the ribosome

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Keywords: translation initiation, IRES, PTB1, initiation factors, ribosome

The trajectory of protein synthesis or translation follows initiation, elongation, termination, and recycling. Among these, initiation serves as the major rate regulatory step at the level of mRNA recruitment or amino-acylated-tRNA delivery. This recruitment of mRNA on ribosomes could be cap-dependent or independent. Cap-independently, it could be brought about by Internal Ribosomal Entry Sites or IRESs, present in the 5' untranslated region of mRNA and originally discovered in viruses. The viral IRESs have been divided into 4 major groups based on the requirement of initiation factors. Where the mechanism of initiation on Type 3 and Type 4 IRESs is well known, we lack knowledge in understanding the Type 1 and Type 2 IRESs.

Our study aims to understand the mechanism of recruitment of Type 2 IRES on the ribosomal complex and understand its regulation at the 48S preinitiation stage. The formation of 48S PIC on Type 2 IRES requires the 40S ribosomal subunit and a subset of eukaryotic initiation factors (eIFs) and majorly the most common IRES trans acting factor (ITAF)- Polypyrimidine tract binding protein1 (PTB1). Therefore, to understand how the canonical and non-canonical factors interact with one another and facilitate the loading of IRES on the ribosome, we have applied pull-down/reconstitution approaches and Cryo-EM. The preliminary low resolution Cryo-EM map of the complex shows a tentative position of the IRES on the ribosome. Thereby suggesting a possible recruitment mechanism of RNA on the ribosome.

P07: Gene and methylation signature under hypoxia in meningioma identifies novel biomarkers and therapeutic targets

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Keywords: Meningioma, Hypoxia, RNA transcriptome, DNA-methylation

Meningioma, the most common benign brain tumor, accounts for 20% of patients with significant morbidity and mortality. The link between hypoxia and tumor aggression, including high recurrence rates and poor prognosis, is well established. However, the underlying mechanisms remain largely unexplored. Our study presents the first detailed analysis of RNA transcriptome and genome-wide DNA methylation in meningioma cell line under hypoxic conditions. Differential expression analysis revealed that 1863 mRNAs and 540 lncRNAs were upregulated, whereas 2121 mRNAs and 653 lncRNAs were downregulated under hypoxia. The top 200 differentially expressed genes (DEGs) were analyzed for their association with meningioma prognosis. Of these, 33 had prognostic significance; wherein high levels of 13 genes, including SLITRK2, KRT17, ALDOA, NR1D1 and low levels of 6 genes including LRP1B, CALCRL, AK7 were associated with poor prognosis. Furthermore, KEGG pathway analyses revealed that upregulated genes were enriched in the HIF-1, MAPK, renal cell carcinoma, and p53 signaling pathways. Downregulated genes were enriched in systemic lupus erythematosus, neutrophil extracellular trap formation, DNA replication, arginine and proline metabolism. Among the top 100 DEGs, 13 upregulated such as SLC38A5, NDRG1, SLAMF9, CNTFR, ENO2, and 7 downregulated genes such as RAPGEF5, RNASEH1, AK7 were found to have hypoxia-response elements (HREs). Furthermore, analysis of the methylation data identified 165 hypermethylated and 416 hypomethylated CpG probes along with the associated genes, revealing differentially methylated genes (DMGs). Upon intersection of the DEGs and DMGs, 23 methylation-regulated DEGs (MeDEGs) were identified. Overall, our study revealed the gene/lncRNA and methylation signature of hypoxia in meningioma with the broad aim of identifying novel biomarkers and therapeutic targets for the treatment of aggressive meningioma.

P08: Identification and characterization of LCS containing modulators of RNA granule dynamics

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Keywords: RNA granules, RGG motif proteins, phase separation, Low complexity sequences

RNA granules are conserved, phase-separated, membrane-less and dynamic condensates formed by multivalent RNA-RNA, RNA-protein and protein-protein interactions. Aberrant RNA granule dynamics have been linked to neuro-degeneration, cancer and aging. Persistent RNA granules that arise as a result of aging and chronic stresses act as a focal point for the aggregation of disease-associated RNA binding proteins. Several mechanisms of RNA granule assembly have been reported. However, it is still unclear how RNA granules disassemble, allowing the repressed mRNAs to enter translation. Low-complexity sequences (LCS) are important for the phase separation and assembly of RNA granules, owing to their ability to promote self-assembly and multivalent weak interactions with RNA and other proteins. Disease-causing mutations in the low-complexity sequences of RNA binding proteins like FUS, TDP43, EWSR1, hnRNPA1, and hnRNPA2 lead to protein mislocalization, aberrant phase separation and formation of amyloid inclusions along with RNA granule proteins. LCS containing proteins might play a crucial role in regulating the dynamics and disassembly of RNA granules. In this project, we first aim to screen and identify LCS-containing modulators of RNA granule dynamics and elucidate their mechanisms. So far, we have screened the deletions of twenty LCS-containing RNA binding proteins for their Processing body and stress granule phenotypes and identified some potential modulators of RNA granule assembly. Secondly, we plan to test the potential of the identified RNA granule disassembly factors in the dissolution of diseases-relevant condensates like FUS and TDP43. To this end, we have tested the effect of the P body disassembly factor Sbp1 on FUS condensate formation. Our in vitro sedimentation assay suggests that the RGG motif containing translation repressor protein Sbp1 can suppress the in vitro phase separation of FUS in a concentration-dependent manner.

P09: CTCF and BORIS-mediated Autophagy regulation via Alternative splicing of BNIP3L in Breast Cancer

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Keywords: BNIP3L-F, BNIP3L-Δ1, DNA methylation, Hypoxia, SRSF6

Autophagy is a pivotal regulatory and catabolic process, induced under various stressful conditions, including hypoxia. However, little is known about alternative splicing of autophagy genes in the hypoxic landscape in breast cancer. Our research unravels the hitherto unreported alternative splicing of BNIP3L, a crucial hypoxia induced autophagic gene. We showed that BNIP3L under hypoxic condition forms two isoforms, a full-length isoform (BNIP3L-F) and a shorter isoform lacking exon 1 (BNIP3L-Δ1). The hypoxia induced BNIP3L-F promotes autophagy, while under normoxia the BNIP3L-Δ1 inhibits autophagy. We discovered a novel dimension of hypoxia mediated epigenetic modification that regulates the alternative splicing of BNIP3L. Here, we showed hypoxia mediated changes in methylation of BNIP3L intron 1 cause reciprocal binding of CTCF and its paralogue BORIS. Additionally, we also highlighted the role of CTCF and BORIS impacting autophagy in breast cancer. The differential binding of epigenetic factor CTCF and BORIS results in alternative splicing of BNIP3L forming BNIP3L-F and BNIP3L-Δ1, respectively. The binding of CTCF on unmethylated BNIP3L intron 1 under hypoxia results in RNA Pol II pause and inclusion of exon 1, promoting BNIP3L-F and autophagy. Interestingly, the binding of BORIS on methylated BNIP3L intron 1 under normoxia also results in RNA Pol II pause, but leads to the exclusion of exon 1 from BNIP3L mRNA. Finally, we reported the critical role of BORIS-mediated RNA Pol II pause, which subsequently recruits SRSF6, redirecting the proximal splice-site selection, promoting BNIP3L-Δ1, and inhibiting autophagy. Our study provides novel insights into the potential avenues for breast cancer therapy by targeting autophagy regulation, specifically under hypoxic conditions.

P10: Integrated analysis of transcriptome and small RNA sequencing data provides miRNA candidates for grain quality traits aligning with different developmental stages in *Triticum aestivum* (L.)

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Keywords: miRNA, RNA-Seq, Wheat, Grain quality

Wheat is the second major staple crop worldwide constituting 18% of total calorie intake and 19.5 % of daily per capita protein. Breeding efforts in wheat are primarily directed toward enhancing seed-related traits, but these are often influenced by complex genetic mechanisms. MicroRNAs play a crucial role in regulating plant seed development by modulating gene expression at the post-transcriptional or translational level. Due to their regulatory function, miRNAs hold significant potential for targeted improvement of grain quality traits. However, the specific roles of miRNAs in *T. aestivum* seed development remain largely unexplored in comparison to other model organisms. Here we report transcriptome analysis along with small RNA profiling and miRNA identification from two developmental stages of the seeds of cultivar C306, distinguished for its best chapati-making qualities and robust adaptability to varying environmental conditions. We found 20,422 DEGs from the transcriptome analysis. We identified 416 microRNAs including 369 known and 47 novel miRNAs. 95 miRNAs were found to be differentially expressed including 14 novel miRNAs. Integrating miRNAome and transcriptome data identified 5,453 putative miRNA-target modules. The categorization of differentially expressed genes and miRNAs into distinct groups predicated upon variety- specific upregulation, alongside comprehensive functional analysis of targets using pathway mapping, gene ontology, transcription factor mapping, and candidate gene analysis has unveiled potential candidates implicated in grain quality traits. Our study furnishes a comprehensive report of miRNAome expression dynamics of different developmental stages of wheat seeds elucidating prospective candidate genes and their corresponding regulatory miRNAs relevant to the genetic enhancement of grain quality traits.

P11: Regulation of the human beta-globin gene expression

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Keywords: Translation regulation, stop codon readthrough, HBB, protein degradation

In eukaryotes, the regulation of gene expression is controlled at two levels: the transcriptional level, where the mRNA transcribed from a gene gets regulated, or the post-transcriptional level, where the stability of the mRNA and its translation is controlled. Human mature erythrocytes are transcriptionally inactive as they lack the nucleus. So, post-transcriptional control is the only means of gene expression regulation in these cells. Alpha and beta-globin chains form the functional tetrameric hemoglobin molecule, which makes up most of the human mature erythrocyte proteome. So, alpha (HBA) and beta (HBB) globin gene expression must be tightly regulated for the erythrocytes to function properly. Stop codon readthrough (SCR) is a mechanism of translation regulation in eukaryotic cells. In this process, the translation is continued beyond the canonical stop codon of an mRNA to give rise to a C-terminally extended protein, which may have a different function, localization, or stability than its canonical counterpart. Here, we studied the 3'untranslated region-mediated translation control of the human beta-globin (HBB) gene. We demonstrated SCR across the canonical stop codon of HBB mRNA using luminescence-based assays in ex vivo and in vitro translation systems. This phenomenon produces two proteins from the single HBB mRNA, the canonical beta-globin protein and the beta-globin readthrough protein with 20 amino acids extension at the C- terminus. Our experiments indicated that a pyrimidine-rich sequence patch near the canonical stop codon is essential for the SCR. The mutation of the pyrimidine-rich tract abrogated the SCR to the basal level. Stability assays revealed that beta-globin readthrough peptide confers instability to green fluorescence protein (GFP) when tagged downstream to it. The GFP expression is rescued post-treatment with proteasome inhibitors, affirming that the readthrough peptide is prone to proteasomal degradation. The results reveal that the HBB SCR provides a mechanism for HBB expression regulation in humans.

P12: Characterization of eukaryotic initiation factor 4G binding proteins involved in repression of Translational Initiation

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The eukaryotic initiation factor 4F (eIF4F) complex comprises of the 5' mRNA cap-binding protein eIF4E, the scaffold protein eIF4G, and DEAD-box RNA helicase eIF4A. eIF4F complex plays a pivotal role in the initiation of translation by facilitating the recruitment of mRNA to the 43S ribosomal preinitiation complex (PIC). Despite its fundamental importance, the intricate structural details of the eIF4F complex with and without mRNA remain elusive. Further, eIF4G-binding proteins (eIF4G-BPs) act as translational repressors and impede mRNA recruitment to the ribosome by binding directly to eIF4G. Herein, we propose to determine the structure the eIF4F complex both in its apo state and in complex with mRNA to unravel the mechanistic intricacies underlying mRNA activation. Moreover, we aim to investigate the inhibitory mechanisms orchestrated by eIF4G-BPs. Notably, it is established that Npl3, an eIF4G-BP, interact with eIF4G via RGG motif, leading to the repression of translation initiation. We outline a methodological approach involving the optimization of NPL3 purification and the subsequent reconstitution of the NPL3-eIF4F complex. Initial attempts failed to form a stable NPL3-eIF4F complex. In future, we aim to reconstitute a stable complex and optimize conditions conducive for cryo-electron microscopy (cryo-EM) studies for high-resolution structural analysis of the eIF4F complex in complex with mRNA and repressor proteins. Through this comprehensive structural investigation, we anticipate gaining crucial insights into the molecular mechanisms underlying mRNA activation by the eIF4F complex and the regulatory roles of translational repressor proteins in modulating translation initiation.

P13: Characterizing eIF2A-mediated Non-Canonical Translation Initiation in Eukaryotes

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Keywords: eIF2A (Eukaryotic initiation factor 2A), tRNA_i (Initiator transfer RNA), Ribosome, β -propeller and CTT (C-terminal tail).

In eukaryotes, translation initiation is a highly regulated and complex process involving various factors. The canonical translation initiation process relies on eIF2 complex, which delivers the initiator tRNA to the 40S ribosomal subunit. However, during cellular stress the alpha subunit of eIF2 undergoes phosphorylation, inhibiting its ability to deliver tRNA_i through the canonical pathway. Apart from eIF2 complex, a few proteins can also deliver tRNA to 40S ribosome and can initiate translation. eIF2A is one of non-canonical factor that is unrelated to eIF2 complex and can perform tRNA-delivery activity. However, the detailed mechanism of tRNA delivery by eIF2A needs to be understood. Our goal is to uncover the details of the tRNA delivery process by eIF2A for translation initiation.

eIF2A has a β -propeller domain and an unstructured C-terminal tail (CTT). We characterized the binding regions of yeast eIF2A with tRNA_i and the ribosome by making deletions and site-directed mutagenesis in yeast eIF2A. Regions in CTT show interactions with tRNA and ribosomes. Further, we created an mutant eIF2A construct of unstructured CTT only by deleting the β -propeller and examined its interaction with tRNA_i and the ribosome. The CTT- only construct also showed binding with both tRNA and ribosomes. Interestingly, the nine-bladed β -propeller domain of eIF2A is highly conserved; however, its role is not clearly understood. So, we disrupted the nine-bladed structure of the β -propeller and assessed its interaction with tRNA_i. Further, we plan to figure out the factors involved in eIF2A-mediated translation initiation using pull-down assays.

P14: Deciphering the role of LNC707 RNA during DNA replication

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Keywords: Cell cycle, Replication stress response, DNA damage.

The explicit DNA replication process during cellular division is critical for cell survival. Unlikely, numerous internal and external stresses challenge the cell for a faithful DNA replication process. This interference leads to a stalled or interrupted replication process, resulting in replication stress. The counter-response of cells is replication stress response that is activated via ATR halting the replication process and preventing new origin firing. Studies have showed that inability of cells to overcome the replication stress leads to DNA damage response pathway that results in DNA repair. Additionally, long noncoding RNAs have been reported to play significant role in DNA damage response and repair. In our laboratory, we have identified a nuclear-retained lncRNA [LNC707] that is enriched during S phase of the cell cycle. Interestingly, depletion of LNC707 results in cell cycle arrest with a significant increase in the S phase population of cells. Further examination of the cells revealed that the majority of cells are in early S phase, indicating important function of LNC707 during S phase progression. Additionally, prolonged depletion of LNC707 resulted in significant DNA damage as observed by increased γ -H2AX foci and comet assay results. Differential proteomic analysis of control and LNC707 depleted cells revealed aberrant levels of genes associated with DNA damage response, DNA repair, replication and cell cycle upon LNC707 depletion. Furthermore, in vitro RNA pull down assay followed by mass spectrometry confirmed interaction of LNC707 with various proteins involved in DDR pathway including NONO, SFPQ and MATR3. Our preliminary results indicate that LNC707 associates with specific DDR factors and regulates their function during cell cycle. Additionally, LNC707 may potentially have important function during DNA replication and may act as a replication stress response RNA.

P15: Synapse-enriched long non-coding RNA regulates inhibitory synapse development

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Keywords: lncRNA, Synapse development, Inhibitory synapses, Hippocampus, In-utero electroporation

Synapse formation in hippocampus has been shown to be regulated by various transcriptional control. Although, RNA-based mechanism of glutamatergic synapse development gained an attention, our understanding of inhibitory synapse development involving regulatory RNA remains elusive. More recently, our transcriptomic analysis identified a set of synapse-enriched long non-coding RNAs (lncRNAs). An expression profile during the window of synapse development identified a subset of lncRNAs that showed an exponential increase in expression during this period. Prompted by these observations, we hypothesized that lncRNAs could regulate synapse development. We have analyzed the excitatory and inhibitory synapse density in hippocampus in vivo following knockdown of two key lncRNAs based on their peak expression during synapse formation. We observed that the knockdown of NO1Rik, but not Zfas1, in embryonic CA1 neurons reduces inhibitory synapse density without influencing excitatory synapse formation. The knockdown of NO1Rik in CA1 neurons showed a significant reduction of both perisomatic and dendritic inhibitory synapse density. We have measured synaptic activity using whole-cell patch clamp recordings from hippocampal neurons following knockdown of NO1Rik. Our patch clamp data showed a significant reduction in amplitude, but not frequency, of miniature inhibitory post-synaptic current (mIPSC). Consistent with our observations from synapse density analysis, we observed that the knockdown of No1Rik has no effect on miniature excitatory post-synaptic current (mEPSC). Experiments are in progress to define the molecular frame work that defines NO1Rik -mediated inhibitory synapse development. Taken together, our study provides a lncRNA-mediated novel layer of gene regulatory control that drives inhibitory synapse development.

P16: miRNA-mediated regulation of p53 isoforms and its consequences**Pritam Kumar Ghosh**, Apala Pal, Saumitra Das

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Keywords: p53 isoform, $\Delta 40p53$, miRNA

p53 is a well-known nuclear transcription factor and tumor suppressor which is commonly known as the ‘Guardian of the genome’. p53 is mutated in more than 50% of human cancers and consists twelve isoforms. The only translational isoform of p53 is $\Delta 40p53$, which can affect cancer progression. Our laboratory has previously shown that, translation initiation from two different Internal Ribosomal Entry Sites (IRES) can generate the full-length p53 protein and the $\Delta 40p53$ isoform from the same p53 mRNA. Different RNA binding proteins and miRNAs can regulate the synthesis of these isoforms. There are not many studies of miRNAs binding to 5’UTR of p53 RNA. Bioinformatics analysis has predicted miR-34a, a tumor suppressor miRNA, to interact with 5’UTR of p53. Incidentally, the MIRX34 (miR-34a mimic) is reported as a potential cancer therapeutic. The current study revealed the interactive outcome of miR-34a and p53. The interaction of miR-34a with p53 5’UTR results a decrease in p53 mRNA level and reduces canonical translation. In contrast, during IRES mediated translation, protein level of $\Delta 40p53$ was significantly downregulated by miR-34a but p53 mRNA level remained unchanged. Interestingly chemotherapeutic drug, Doxorubicin-induced stress can rescue the p53 protein and mRNA levels due to increased abundance of the ITAF PTB.

As p53 functions as a tetramer and levels of FL-p53 and $\Delta 40p53$ are differentially regulated by miR-34a, it led us to investigate FL-p53 and $\Delta 40p53$ mediated regulation of miRNA levels. For this purpose, after transfection of FL-p53 and $\Delta 40p53$ in H1299 cell line (p53 null), small RNA sequencing was performed. miR-4671 has been identified as one of the differentially regulated miRNA. The level of miR-4671 was found to be down-regulated by $\Delta 40p53$. Finally, the levels of the putative mRNA target SGSH showed reciprocal correlation with miR-4671 and positive correlation with $\Delta 40p53$. Importantly, overexpression of miR-4671-5p triggered intra-S-phase cell cycle arrest. Thus, $\Delta 40p53$ -miR-4671-5p-SGSH axis emerges as a novel axis capable of regulating cell cycle progression. Collectively, these data enhance understanding of $\Delta 40p53$ functions mediated by miRNAs that help to maintain metabolic and cellular homeostasis independently of FLp53.

P17: PIWI-RNA sensitizes tongue cancer cells to cisplatin

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Keywords: PIWI-interacting RNA, piR-hsa-30937, GAB2, cisplatin, tongue cancer

The rise of tumor recurrence and incomplete remission in cancer treatment can primarily be attributed to gradual loss of drug sensitivity and/or the increase in chemoresistance. Consequently, this presents a serious threat to public health, potentially triggering a global crisis. Fortunately, the advent of advanced molecular techniques has unravelled small non- coding RNAs which are known regulators of oncogenic events to also prompt sensitivity towards chemo drugs. As such, our lab has deciphered piRNAs in different solid tumors and established their mechanisms of action in tumorigenesis including its role in chemoresistance. In the present study, we reported that piR-hsa-30937 sensitizes tongue cancer to cisplatin by regulating cell proliferation, apoptosis, and ROS production. Further, GAB2 is predicted as the target of piR-hsa-30937 whose expression drastically reduced upon transient overexpression of this piRNA. Moreover, cisplatin treatment increased the expression of piR- hsa-30937 several folds, the rationale of which is being investigated. Altogether, this study might pave way towards piRNA-target based therapeutics to wrestle chemoresistance in cancer.

P18: Dynamic interplay between miR-133a and RBMX during dengue virus infection

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Keywords: Dengue Virus, Host-virus interaction, miRNA, miR133a, RNA-binding proteins, RBMX.

The incidence of viral infection is spreading rapidly, posing a dramatic threat to public health due to their severe fatality and high transmission rate. Dengue is the arboviral disease with varying degree of therapeutic outcomes ranging from mild to fatal life-threatening conditions. Viruses are obligate intracellular parasites with limited genome capacity, relying entirely on host factors and cellular machinery for sustainable infection. Among the various host factors, RNA-binding proteins are the key host players. They are indulged in regulating the various molecular mechanisms related to RNA metabolism due to which they become equally important for the viral life cycle. Previously, many RBPs have been characterized where their expression and localization landscape has been changed during infection but studies are still lacking to highlight the regulation of these RBPs during viral infection.

Here, we demonstrate that Dengue infection modulates the expression of RBMX (an RNA-binding protein) and miR-133a. Viral infection elevates the expression of the RBMX gene while down-regulates the level of miR-133a. Additionally, miR-133a possesses a potential binding site in the 3'UTR region of the RBMX gene and can regulate RBMX expression in the absence of dengue virus. Intriguingly, our time point study in Huh7 cells over-expressing the synthetic form of miR-133a indicates the convoluted interaction between miR-133a and RBMX regulation during DENV infection. After 24 hours post infection, miR-133a significantly suppresses both the RBMX expression and viral RNA levels, acting as an anti-viral agent by targeting the expression of the RBMX gene. Additionally, we highlight the central role of DENV 3'UTR in regulating the expression of both RBMX and miR-133a. Furthermore, RBMX over-expression study illuminates the vital function of RBMX protein in the DENV life cycle. Over-expression of the RBMX gene in ivermectin-pretreated cells rescues viral replication. This comprehensive study explicates the dynamic miRNA/RBPs regulatory axis during DENV pathogenesis.

P19: The interplay between Host and Viral miRNAs in Dengue virus infection and pathogenesis

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Keywords: Dengue virus, 3'UTR, Host miRNAs, Virus miRNAs

Dengue virus is a positive sense single stranded RNA virus belonging to the genus Flavivirus, which causes Dengue Hemorrhagic Fever. Virus-induced pathogenesis mainly depends on the translation and replication efficiency of the virus inside the host. Host factors such as RNA binding proteins (RBPs) and miRNAs, bind to untranslated regions (5' and 3'UTRs) of the viral RNA and control virus translation and replication. We have performed in-silico analysis to get a pool of miRNAs, which can bind directly with Dengue virus 3'UTR. Subsequently, we have performed tagged RNA affinity pull-down assay (TRAP assay) and demonstrated that miR-183-5p, miR-184, and miR-296-5p interact with DENV-3'UTR. Overexpression of miR-183-5p, miR-184 decreased viral RNA and protein expression however miR-296-5p did not show any effect. Reporter-based assays showed that these miRNAs bind to DENV-3'UTR and decrease viral RNA translation. Thus, these host miRNAs are negatively regulating virus lifecycle in DENV-2 and we predicted that these miRNAs will differentially regulate lifecycle of other serotypes as binding sites are not conserved. Interestingly, Dengue virus (serotype 2) 3' UTR generates viral miRNAs (vmiRs) and we observed that vmiR-5 and vmiR-6 are generated upon Dengue virus infection and our in-silico analysis suggests that these vmiRs can target host mRNAs related to innate immunity genes. Currently, we are trying to understand whether the host miRNAs binding with virus 3'UTR interfere with the production of viral miRNAs. Also, exploring the possible role of the interplay between viral and host miRNAs in regulation of cellular pathogenesis.

P20: Uncovering the role of ribosome heterogeneity in hepatocellular carcinoma
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Translational regulation of mRNAs acts as a nexus for multiple oncogenic signals, allowing cancer cells to modulate protein expression in response to resource constraints while sustaining cellular growth and viability. Amidst the integrated stress response (ISR) triggered by various factors which normally shut down global protein synthesis cancer cells manage translation of a subset of mRNAs essential for survival and proliferation. Numerous studies demonstrate the significance of eIF2 α phosphorylation as a key element in how cells manage their survival and proteostasis allowing cells to reconfigure translation for adapting to stress. Ribosomes catalyze peptide bond formation with highly conserved core units, however their complexity increases from bacteria to humans. Recent studies link ribosome composition variability, like alternative rRNAs and their post-transcriptional modifications, and ribosome associated factors with significant effects on global protein translation. However, the regulatory role of these variants in protein synthesis or specific mRNA translation remains largely unexplored. Furthermore, upstream regulators of ribosome composition are not fully understood.

In mammals, both translation and ribosome biogenesis are regulated by a highly conserved serine-threonine kinase mTOR. Deregulation of mTOR is associated with various disorders such as diabetes and cancers. In Liver cancers, the majority of which is comprised of hepatocellular carcinoma (HCC), among 50% of cases, mTOR pathway upregulation is noted, regulated by molecules like PIK3CA, RAS, RAF, and others. It acts as a vital sensor for physiological stresses, governing HCC growth via control of eIF4E-BP1 and S6K expression. We propose to investigate the role of ribosome-associated factors under stress conditions and their impact on mRNA translation in HCC. In addition, we will also investigate how the mTOR signaling in tumors is manipulating the composition of ribosome-associated factors. Using polysome profiling and mass spectrometry, we'll identify mRNA characteristics and associated ribosomal molecules, aiming to understand reprogramming of protein synthesis in HCC and identify therapeutic targets for HCC.

P21: Short code from a long message: cytoplasmic capping targeted long non-coding RNAs translate into micropeptides

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Keywords: cytoplasmic capping; lncRNA; uncapped RNA ends; ncRNA translation; micropeptide

The N7methyl guanosine (m7G) cap is added to RNA polymerase II (Pol II) transcribed RNAs by RNA guanylyltransferase, commonly called mRNA Capping Enzyme (CE). Cytoplasmic pool of this enzyme (cCE) returns a cap to specific uncapped RNA targets, thus protecting those uncapped RNAs from decay. Recently our study shows cytoplasmic capping activity is not only restricted to mRNAs, but also several long noncoding RNAs (lncRNAs) are regulated by cCE. Current research advancement in ribosome profiling explored that lncRNAs are not only regulatory non-coding elements, but also serve as templates for peptide translation which contributes to the complexity of cellular proteome. Several lncRNAs harbor short open reading frames (sORFs) that give them coding potentiality. Although not all sORFs are translatable within the cell, some of them indeed encode cryptic peptides localized to various cellular locations. Many of these peptides are reported to play biological functions similar to or differently from the parent lncRNA. Here we have reported that cCE targeted lncRNAs harbor functional Kozak sequence and translatable open reading frames (ORFs) which generate micropeptides of unknown functions.

P22: Revealing Target of Rapamycin (TOR) signaling network with the help of auxin inducible degron (AID) system

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Keywords: TORC1, TORC2, AID, phospho-proteome

The target of rapamycin, TOR, is a eukaryotic serine-threonine protein kinase conserved from yeast to mammals (mammalian TOR, mTOR). It is a member of the phosphatidylinositol 3-kinase-related kinase family of protein kinases. TOR is present in two different protein complexes, TORC1 and TORC2 with distinct composition and function. TORC1 is sensitive to rapamycin and regulates the temporal aspect of cell growth and metabolism. TORC1 stimulates anabolic processes such as ribosome biogenesis, protein synthesis, transcription upon nutrient signal, and repressing catabolic processes such as macroautophagy. TORC2 is insensitive to rapamycin and regulates the spatial aspect of cell growth such as cell cycle dependent polarization of the actin cytoskeleton. Due to the absence of specific inhibitors for TORC2. It has become very challenging to understand TORC2 downstream signalling cascades.

To identify novel, direct, and indirect targets of TORC1 and TORC2, we employed Auxin Inducible Degron (AID) system. We degraded TORC1 and TORC2 complexes in a time- dependent manner with the help of the auxin and analysed changes in the phospho-proteome and proteome. We generated a comprehensive dataset of potential targets for TORC1 and TORC2. With very stringent criteria and in-depth analysis, we identified potential TORC1 and TORC2 specific targets. Plus, we could also identify common potential targets of TORC1 and TORC2. In total, we identified 379, 178, and 45 phospho-sites as potential targets regulated solely by TORC1, TORC2, or by both TORC1 and TORC2, respectively. The detailed characterizations of some of these candidates are undergoing.

P23: Modulation of protein translation by inositol pyrophosphates

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Keywords: Inositol pyrophosphates, Protein pyrophosphorylation, Protein translation, Translation initiation factor

Inositol pyrophosphates (PP-IPs) are energy-rich small molecules present in all eukaryotic cells. The major PP-IPs that regulate cellular activities are 5-IP7 (5-diphosphoinositol pentakisphosphate). 5-IP7 is synthesized from IP6 by IP6 kinases, of which there are three isoforms in mammals - IP6K1/2/3. The cellular functions of IP6Ks are governed by three mechanisms: (1) protein pyrophosphorylation by 5-IP7; (2) 5-IP7 binding to proteins; and (3) IP6K binding to proteins independent of their ability to synthesize 5-IP7. Protein pyrophosphorylation occurs when the high-energy beta-phosphate group is transferred from 5-IP7 to a prephosphorylated target serine residue. Here we discovered the 5-IP7 mediated pyrophosphorylation of three translation initiation factors eIF3B, eIF3C, and eIF4E. As per the literature, in eukaryotes, more than twelve initiation factors are responsible for the onset of protein translation. Among them eIF3 is a large complex containing 13 subunits, that facilitates mRNA recruitment, 40S ribosome scanning, and dissociation of the 80S ribosome. Out of the 13 subunits, eIF3A, eIF3B, and eIF3C play an important role in the structural and functional integrity of the eIF3 holocomplex. Apart from this, the eIF4E (mRNA cap binding protein) promotes in the formation of many translation preinitiation complexes and also in mRNA recruitment. Primarily, to test the role of pyrophosphorylated initiation factors in protein translation, we performed [S35] methionine incorporation assay and luciferase assay in IP6K1 knockout HEK 293T cells. Since we did not see any significant change in global protein translation in IP6K1 depleted cells we inspected to find the role of IP6K2 and IP6K3 in HEK 293T cells. The Human Protein Atlas data reveals a high transcript level of IP6K1 and IP6K2 in most mammalian cell lines including HEK 293T. Therefore, to deplete 5-IP7 level significantly, we generated a CRISPR Cas9-mediated IP6K2 knockout in IP6K1 knockout HEK 293T cells to make it IP6K1/K2 double knockout.

P24: Exploring the immunomodulatory role of oxidized RNA and its metabolism

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Keywords: Oxidative stress, RNA metabolism, neurodegeneration, oxidized RNA, inflammation

Modern lifestyle is rapidly leading to a pandemic of obesity and cardiovascular diseases alongside a rapidly ageing population, prone to neurodegenerative disorders. Oxidative stress is a mainstay in all of these conditions, and for a healthy life, cells must be able to deal with it. It is one of the most persistent stresses faced by cells - an imbalance between the levels of reactive oxygen species and our cells' antioxidant mechanisms. RNA is one of the major cellular targets of ROS. Oxidatively damaged RNA molecules may have strand breaks, cross- links, and base lesions - one of the most common being 8-hydroxyguanine (8-OHG) (Tanaka & Chock, 2021). Oxidative modification in RNA has many deleterious effects- it stalls translation, leading to the formation of shorter polypeptides, tRNA cleavage and ribosomal inactivation. Oxidised RNA levels increase is observed pre-symptomatically in Alzheimer's disease (Nunomura *et al.*, 2004). Our study focuses on understanding various implications of increased oxidised RNA levels in cells - particularly how it would affect inflammation and RNA metabolism. Preliminary results suggest that the presence of oxidative modification on RNA may be able to modulate the inflammatory potential of RNA. This may be able to explain its role in the progression of neurodegenerative diseases like Alzheimer's and ALS. We are also interested in understanding how a potentially deleterious RNA modification like oxidised bases is metabolized in cells to maintain its homeostasis. To this end, we have explored the role mitochondrial RNases play in the metabolism of oxidatively modified RNA.

P25: Role of hypoxia regulated ncRNAs in regulation of genome instability in glioblastoma

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Keywords: Glioblastoma, hypoxia, microRNAs, DNA repair, genome instability

Glioblastoma (GB) is the most severe form of malignant glioma with a median survival rate of just 15 months post diagnosis. Early detection of brain cancer heavily depends upon reliable biomarkers that exhibit high tissue specificity and stability. miRNAs are short but stable RNA sequences, capable of regulating various signaling events within a cell. Their role as important regulatory entities in cancers is being heavily investigated. Most solid tumors exhibit hypoxia, which has multi-faceted effects on cancer cell phenotype such as promoting drug and IR resistance. Hypoxic cell populations within tumors gain a survival advantage by modulating their DNA repair and cell death mechanisms. Thus, over generations such cellular populations predominate in a tumor and make the tumor even difficult to treat. Here we describe a novel hypoxia regulated microRNA in GB, poised to have a capability of regulating a newly understood DNA repair axis in cancer, with a role in promoting apoptosis, affecting cell viability with a probable role in regulating DNA repair and even affecting certain aspects of cancer cell stemness. Overall, this miRNA-mRNA axis could be crucial towards understanding the role of hypoxia in regulating genome instability in glioblastoma.

P26: Investigating the non-canonical role of cytoplasmic capping enzyme in regulating cellular metabolism

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Keywords: Cytoplasmic CE, metabolism, VDAC3

Capping enzyme (CE) adds cap to the 5'-end of the nascent messenger RNA (mRNA) in the nucleus. It plays a pivotal role on the stability of mRNA, transport from nucleus to cytoplasm and also in translation process. Decapping was thought to be an irreversible decay process until the identification of cytoplasmic CE (cCE). It maintains a cyclic process of decapping and recapping of specific targets in the cytoplasm called 'cap homeostasis'. During stress condition, non-translating mRNAs and proteins form aggregates termed as stress granules (SGs). cCE localizes to these SGs. When the stress is lifted, SG are almost completely dissolved within 2hrs and cCE is dispersed into cytoplasm and recaps its target transcripts which are decapped during stress. In addition, overexpression of inactive form of cytoplasmic CE, shows reduced cell viability when introduced to stress and thus confirming role of cCE in cellular stress response.

To identify the genome wide cCE targeted transcripts during stress and recovery, RNA seq experiment has been conducted with cytoplasmic RNAs isolated before and after sodium arsenite treatment as well as 6 hr from removal of arsenite from U2OS osteosarcoma cells expressing dominant negative form of cCE and vector control. Differential expression of genes and pathway enrichment analyses with the transcriptomic data revealed differential expression of the genes associated with diabetes mellitus in cellular recovery from stress. A few candidate transcripts are selected for their further validation in osteosarcoma cells using qPCR. Also, recently we found cCE interacts with VDAC3, a mitochondrial outer membrane protein which is involved in mitochondrial metabolism through ATP and other small molecules transport. Thus, this indicates non canonical role of cCE in regulating specific pathways of metabolism. Future studies will demonstrate the function of cCE in controlling cellular metabolism., to interact with cCE.

P27: Validating the Prediction of G-Quadruplex RNA Structures**Subhajit Mondal**, Sumita Sengupta, and Ansuman Lahiri

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Prediction of correct RNA folding and structure(s) currently proves to be a very difficult problem. This is true not only for prediction of RNA tertiary structures, but also for non-canonical secondary structures. Among non-canonical RNA structures, G-Quadruplexes (G4s) are formed from guanine-rich sequences. Recently, ViennaRNA web services has incorporated a new algorithm for predicting G-quadruplex secondary structures along with canonical secondary structures in their RNAfold software and web server. Here we report the results of validating their predictions using the experimentally determined G4 structures curated and stored in the ONQUADRO database. We observed that for many sequences occurring in the database, the RNA fold algorithm failed to correctly predict the G4 structures. We so far noted that the RNA fold algorithm showed a high propensity for false negative prediction: (i) if the length of the sequence is less than 22 nt and (ii) if the consecutive guanine (maximum number) stretches are repeated less than four times. Finding limitations of the present algorithm could lead to development of a better algorithm for prediction of G4 structures.

P28: LNC896 regulates cell cycle and is required for maintenance of genome integrity

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Keywords: lncRNAs, cell cycle progression, DNA damage and repair

Long noncoding RNAs (lncRNAs) are regulatory RNA molecules that are similar to mRNAs in 5' capping, polyadenylation and splicing but do not code for proteins. It is established now that lncRNAs function in much diverse ways to modulate key events in a cell thereby regulating various biological processes and their aberrant expression has been implicated in multiple disorders including cancer. Several lncRNAs may exert their function as oncogenes or tumor suppressors. In an attempt to characterize novel lncRNAs regulating cellular proliferation, we have identified an RNA, LNC896, that is temporally expressed in a cell type and stage specific manner indicating its potential function in regulating events associated with cell cycle progression. Interestingly, LNC896 is highly expressed in transformed and cancer lines with significantly low expression in primary cells; suggesting an oncogenic role of this RNA. Quantitative RT-PCR analysis for LNC896 in cells synchronized at various cell cycle stages revealed that LNC896 is enriched at G1 phase of the cell cycle. To further understand the functional relevance of cell cycle regulated expression of LNC896, examined the effects of depletion of this RNA. PI flow cytometry data revealed that LNC896 depleted cells displayed a significant increase in S and G2/M with a concomitant decrease in G1 population of cells. Furthermore, LNC896 depleted cells also showed a significant increase in p53 and γH2AX levels suggesting DNA damage response upon LNC896 depletion. Additionally, LNC896 depleted cells revealed aberrant levels of various genes associated with DDR, DNA repair, mitotic dysregulation and cell cycle progression. To summarize, our results indicate that LNC896 is a pro-proliferative RNA and might play an important role in regulating cell cycle progression. Further studies will unravel the molecular function of LNC896 and the pathways regulated by this RNA. Altogether this study will improve our understanding of lncRNA-mediated regulation of DNA damage and repair and its significance in cancer therapeutics.

P29: Deciphering the molecular mechanism of ciTRAN induction by HIV-1 VPR.

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Circular RNA (circRNA) expression is ubiquitous in all eukaryotes. Generated by backsplicing, circRNAs are protected from exonucleases, leading to a stable form of RNA circle that can modulate cellular processes. The functions of circRNA during retroviral infection remain, however, poorly understood. Recently, leveraging the Nanopore Direct-RNA sequencing (CircDR-Seq) pipeline, we identified ciTRAN (a circRNA modulator of HIV- 1 Transcription). ciTRAN regulates HIV-1 transcription by sequestering SRSF1, a negative regulator of HIV-1 transcription. Moreover, HIV-1 Vpr was uncovered as an inducer of ciTRAN. In the present study, we aim to decipher the mechanism of induction of ciTRAN by Vpr. We employed the Vpr mutants with functionally defined characteristics and found that a Q65R (deficient in major activities like cell cycle arrest and DNA damage) failed to induce ciTRAN. Whereas the mutants specifically deficient in arresting cell cycle did not fully recapitulate the magnitude of induction by WT Vpr, we hypothesised that DNA damaging activity of Vpr might be the primary stimulus for the induction of ciTRAN. This is because treatments with etoposide could induce ciTRAN, but small molecule inhibitors of the cell cycle did not. Our work thus provides key insights into Vpr-mediated ciTRAN induction.

P30: Understanding the mechanism and physiological relevance of D-aminoacyl-tRNA deacylase 2

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Keywords: D-aminoacyl-tRNA deacylase (DTD), Chiral proofreading, Translation quality control, D-aminoacyl-tRNA adducts

Homochirality is unequivocally an essential characteristic of life. Macromolecules such as proteins are made exclusively of L-amino acids, with the exception of achiral glycine. D- aminoacyl-tRNA deacylase (DTD) is a unique chiral checkpoint that removes D-amino acids mischarged on tRNAs and recycles the tRNAs back for uninterrupted protein biosynthesis. Three distinct types of DTDs have been reported so far: DTD1 in bacteria and eukaryotes, DTD2 in archaea and land plants and DTD3 in cyanobacteria. Earlier, we have shown that DTD2 is a unique trans-editing module as it removes acetaldehyde-modified D-aminoacyl- tRNA adducts in addition to unmodified D-aminoacyl-tRNAs. Very recently, we identified that this protection offered by DTD2 is not just restricted to acetaldehyde but also to various other aldehydes, which includes formaldehyde and methylglyoxal. Using Arabidopsis as a model system, we demonstrated that dtd2 null plants are vulnerable to formaldehyde and methylglyoxal as they generate stable alkyl modifications on D-aminoacyl-tRNAs, which are recycled exclusively by DTD2. As a part of our ongoing efforts, we are currently working on delineating the mechanistic basis of adduct specificity and D-chiral specificity of DTD2. Furthermore, we are setting up plant callus as well as archaeal culture systems to characterize D-aa-tRNA adducts in vivo.

P31: Investigating the role of ribosome heterogeneity during nutrient stress in yeast**Pooja Mourya**^{1,2}, and Sunil Shetty^{1,2}¹Advance Centre for Treatment, Education and Research in Cancer ,Maharashtra²Homi Bhabha National Institute, Mumbai, Maharashtrapooja.mourya@actrec.gov.in**Keywords:** Ribosome heterogeneity, TOR signaling, Ribosome Associated Factors, protein synthesis

The ribosome is a central and key element in the production of proteins and the regulation of growth & development. It has a unique arrangement of proteins and RNA molecules and is conserved from prokaryotes to eukaryotes. The eukaryotic ribosome (80S) is made of four RNA molecules and approximately 80 ribosomal proteins (RPs). Even though the functionally critical regions of ribosomes are highly conserved, there is a heterogeneity in the composition of the ribosome due to ribosomal protein content, ribosome-associated factors, protein modifications, rRNA sequence, and rRNA modifications. Recent studies suggest that these heterogeneous ribosome populations play a role in the regulation of protein synthesis during development, senescence, and cancer. It has been shown that depending on cell type and environmental conditions, there are alterations in the expression of various paralogues of ribosomal proteins and their modifications. Whether these changes in expression lead to functionally distinct heterogeneous ribosome populations in a cell is yet unclear.

TOR (target of rapamycin) complexes are one of the best-characterized sensors of nutrients and environmental conditions that determine cellular growth. TORC1 promotes ribosome biogenesis and translation under nutrient-sufficient conditions. Inhibition of TORC1 by nutrient deprivation or rapamycin treatment results in a drastic reduction in global protein synthesis. Interestingly, we also observed the alterations in the ribosome composition upon inhibition of TORC1. Whether these ribosome associated factors have any role in translation regulation during the stationary phase or nutrient stress is the question we are following up.

P32: Regulation of translation initiation and its importance in pathogenicity in *Mycobacterium tuberculosis*

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Keywords: initiator tRNA, *metU*, knockdown, translational stress, reporter assay

Tuberculosis (TB) pathogenesis is a remarkable phenomenon, especially because its causative agent, *Mycobacterium tuberculosis* (*Mtb*), uses intricate escape mechanisms to survive under the stressful conditions and evade the host immune mechanisms. *Mtb* possesses a single copy of the gene (*metU*) for initiator tRNA (i-tRNA). Initiation is a major regulatory step in protein translation. It is also known that the relative levels of i-tRNAs, elongator tRNAs and their aminoacylated forms change under stressful conditions. In *Escherichia coli*, we showed that under the conditions of i-tRNA deficiency, translation is initiated by the elongator tRNAs. Based on our previous findings, we hypothesise that because of a single copy of the i-tRNA gene, *Mtb* is likely to respond to translational stresses by changing the i-tRNA expression, which may lead to production of novel polypeptides and help TB pathogen to adapt/tolerate stressful conditions. To investigate this hypothesis, we have generated a recombinant *Mtb* strain with regulated expression of i-tRNA by making use of CRISPRi technology. In this strain, *metU* guide RNA is expressed under ATc inducible promoter. We are now developing and standardizing a GFP reporter-based assay system to assess translation initiation by the wild-type and the mutant versions of i-tRNA in the i-tRNA depleted *Mtb*. Further, we wish to examine the physiology of the i-tRNA hypomorphic strain of *Mtb* and its interaction with the host.

P33: To understand the role of the uS19 protein in ribosome biogenesis and translation

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Key Words: Ribosome, Biogenesis, 18S rRNA, uS19

Ribosomes are responsible for decoding a mRNA to bio-synthesized protein, a process known as translation. In eukaryotes, the 80S ribosome comprises of a large 60S and a small 40S subunit. The 60S subunit comprises of three rRNAs, that are 25S, 5.8S, and 5S rRNA, and 46 proteins, while the 40S subunit comprises a single 18S rRNA and around 33 proteins. The biogenesis of the ribosome begins in the nucleolus and completes in the cytoplasm with the help of several biogenesis factors. Proper ribosome biogenesis is essential for maintaining fidelity during all three steps of translation – initiation, elongation, and termination. Our lab previously identified a C1209U mutation in helix 32 of 18S rRNA, which can suppress the translation initiation fidelity defect of the eIF5G31R mutant. The 18S rRNA C1209U mutation shows a slow-growth phenotype. Structural analysis of mature ribosomes showed us that a small subunit protein uS19 interacts with the 18S rRNA helix 32 region. Reports suggest that the uS19 protein may have a role in the ribosome biogenesis. We hypothesize that the C1209U mutation in the 18S rRNA helix 32 may lead to ribosome biogenesis defect, potentially mediated via uS19 protein. To understand the role of the uS19 protein in biogenesis, we plan to screen suppressor mutations in the uS19 gene that can restore the slow growth defect of the 18S rRNA C1209U mutation.

P34: Ubiquitin-Like Protein Hub1 Facilitates Optimal Splicing Efficiency in Rapidly Synthesised Transcripts

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Gene expression is intricately regulated across various stages of RNA metabolism, encompassing processes such as RNA synthesis, splicing, and degradation. The efficacy of RNA synthesis is notably influenced by the strength of the promoter, thereby dictating the rate of transcription. Given the predominantly co-transcriptional nature of splicing events, a heightened splicing kinetics becomes crucial to ensure the efficient splicing of transcripts synthesized at an accelerated pace. In this context, our research reveals the indispensable role of the unconventional ubiquitin-like protein Hub1 in facilitating the efficient splicing of such rapidly transcribed transcripts in *Schizosaccharomyces pombe*. Hub1 is an evolutionarily highly conserved protein that functions in alternative splicing through its interaction with splicing factor Snu66 and DEAD-box helicase Prp5 in *Saccharomyces cerevisiae*. When SRC1 is the only reported splicing target in *S. cerevisiae*, we show that Hub1 is required for splicing of a large number of transcripts in intron-rich *S. pombe*, which are further identified to be synthesised faster. Genetic interaction with subunits of the transcription elongation complex and RNA polymerase proposes Hub1 as a putative mediator bridging the interface between transcription and splicing.

Structural analysis of spliceosomal B-complex shows Hub1 binds to 5' exonic region of its target intron, and many other splicing factors. We further identified the interaction residues and found their importance in Hub1 function. Collectively, our findings propose that Hub1, through its physical interaction with the upstream region of an intron, coordinates the recruitment of additional splicing factors, thereby ensuring the efficient splicing of fastly synthesised transcripts in intron-rich organisms.

P35: The role of RNA on the phase behaviour of RNA binding proteins in senescence

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Keywords: senescence, stress granules, RBPs, RNA metabolism, dynamics

Cellular reactions are spatially compartmentalized into membrane-bound organelles and membrane-less condensates. RNA and RNA-binding proteins (RBPs) constitute most cellular condensates. Interestingly higher eukaryotes have many RBPs with low complexity (LC)- unstructured domains, which enable them to undergo spontaneous phase separation. In neurodegenerative diseases- Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTLD), a special class of LC domain-containing nuclear RBPs known as prion-like RBPs (e.g. FUS, TDP43 and hnRNPA1) often aggregate to form solid-like aggregates in the cytoplasm (Mori *et al.* 2008; Lim *et al.* 2016). During cellular stress, these prion-like RBPs transiently mis-localize to the cytoplasm and get enriched in stress granules (Streit *et al.* 2022; Baron *et al.* 2013). Physiological stress granules are liquid-like condensates which rapidly exchange the constituent RNA and RBPs with the surrounding cytoplasm. It is not clear how the liquid-like RBP constituents of stress granules, phase transition into solid-like aggregates, and if the aggregation of these RBPs occur in stress granules. Although aggregation of RBPs is found in more than 90% of ALS and FTD cases, only 5-10% of the familial cases of ALS and FTD show mutations in these in RBPs, suggesting the contribution of additional cellular factors in the solid-like aggregation (Sultan *et al.* 2016). Further, mutations in the ALS genes only increase the probability of getting the disease and the disease phenotype manifests in the age group of 55- 80 years (Wolfson *et al.* 2023; Logroscino *et al.* 2005). These observations suggest that the cytoplasmic aggregation of FUS and TDP43 in the cytoplasm is induced by the additional cellular changes associated with ageing. We aim to study the implication of ageing on the RNA-RBP localization, metabolism, and ability to form condensates. We are studying stress granule dynamics in cellular senescent models and skin fibroblasts from young and aged individuals. We observed alteration in stress granule formation (Lian and Gallouzi 2009) and persistent stress granules upon removal of stress in senescent cells. Interestingly, we also noticed that the total RNA levels and polyA RNA levels were decreased in senescence with concomitant changes in concentrations of stress granule scaffold proteins (G3BP1 and G3BP2) and other prion-like RBPs (FUS and TDP43). In future, we aim to delineate the altered RNA metabolic pathways and implications of altered stress granule dynamics towards the aggregation of prion-like RBPs in senescence.

P36: Canonical vs. Non-canonical 3'-end formation – role in hypertrophy gene program in the heart

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Polyadenylation at the 3'-end is crucial for eukaryotic gene expression that is carried out by poly (A) polymerases (PAP) in a multi-protein complex. There are two major PAPs - canonical PAP (α/γ) and the non-canonical Star-PAP (Speckle targeted PIPKI α regulated PAP) that shows distinct preferences in target mRNAs regulating diverse functions. Yet, how different PAP complexes regulate mRNA targets in distinct cellular functions is unclear. To understand the uniqueness of two PAP complexes, we analyse core components using mass spectrometry followed by immunoprecipitation. Interestingly, canonical components, CPSF subunit WDR33 (required PA-site recognition), CstF-64, and PAP α are absent in the Star-PAP complex, whereas Star-PAP complex has additional proteins including RBM10 or PIPKI α . To further analyse the specificity, we perform RNA sequencing of WDR33 (along with PAP α), to compare with global transcriptome profile of Star-PAP. Approximately 80% of WDR33 targets overlapped with PAP α , whereas <5% of are overlapped with Star-PAP targets revealing distinctness of the two complexes. We then validate six target mRNAs of respective PAP complexes by qRT-PCR and Western blot. Knockdown of WDR33 or PAP α resulted in the loss of expression (GATA6, GATA4, MSRB1, NME3, FBLN2 and SIKE1) that are not affected by Star-PAP depletion. Similarly, Star-PAP target expressions (NQO1, HO1, HAND2, CDH13, FEZ1, PTEN) were reduced specifically on Star-PAP depletion but not on WDR33/PAP α knockdown indicating dispensability of these factors for non-canonical processing. The loss was rescued only by FLAG-Star-PAP re-expression but not by PAP α or WDR33 and vice versa. Functional analysis of WDR33 common targets shows enrichment in cardiovascular and cancer. WDR33/PAP α predominantly controls pro-hypertrophy genes, while Star-PAP regulates anti-hypertrophy genes. Inducing hypertrophy in rat hearts model and H9C2 cells by isoproterenol up regulates canonical components (WDR33, CstF64, CPSF73, CPSF30) while down regulating non-canonical components (Star-PAP, RBM10, CPSF160), emphasizing the balance between these complexes regulates cardiac hypertrophy.

P37: Unraveling the Time Dependent Progression of Alzheimer's Disease in**Mouse Cerebral Cortex****Satakshi Bagchi**, Zhumur Ghosh

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Introduction: Alzheimer's Disease (AD) is the most common neurodegenerative disease characterized by progressive cognitive decline, memory impairment, and behavioral changes. The cerebral cortex is responsible for a wide range of higher-order cognitive functions, including memory, language, reasoning, and perception. AD profoundly affects these functions, making it a key region to investigate. Numerous neurons and synaptic connections make up the cerebral cortex, which undergoes atrophy as the disease progresses. Investigating gene expression changes in the cortical region and finding its relation with clinical outcomes can help in early disease diagnosis, which is essential for timely intervention and potential disease-modifying treatments.

Objective: The aim of this investigation is to perform an exhaustive and careful transcriptional profiling analysis to elucidate the time-dependent progression of AD pathology in the cortical region of the brain. This study has also looked upon gender specific disparities in the AD mouse models.

Results: Bulk RNA-sequencing data of specific AD mouse models corresponding to specific time points since AD initiation have been analysed. Interestingly, a distinct difference have been observed in male and female AD models with respect to the gene expression profile across the disease progression. The enriched pathways across the initial, middle and advanced stages of AD progression too reflected a gender specific distinct pattern which could be correlated with relevant clinical outcomes.

Conclusion: Overall these findings provide a better understanding regarding the common and distinct cellular events contributing towards AD progression in both male and female mouse systems; demanding special attention towards churning out the contribution of sex-specific factors in AD pathogenesis. Apart from this, the future goal of this work is to look into the role of long non-coding RNAs (lncRNAs) in AD pathology, as lncRNAs have reported role in neuronal development and maintenance

P38: Role of m6A RNA methylation-miRNA axis in oral squamous cell carcinoma

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Keywords: m6A RNA methylation, microRNA, Epitranscriptomics, ncRNAs, Oral Cancer.

Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of the oral cavity. Of the plethora of gene regulatory processes which impact OSCC pathophysiology, reversible m6A RNA methylation has emerged as an important player in the recent years. m6A RNA methylation modulates various facets of cellular RNA metabolism and very often m6A regulators i.e. m6A writers, erasers, and readers have been reported to be ectopically expressed in multiple cancers and influence tumor progression via aberrant m6A methylation of various RNA targets. Interestingly, m6A RNA methylation have also been reported to regulate processing of primary miRNAs and thus mature miRNA levels. Comprehensive studies exploring the role of m6A regulators and m6A regulated miRNAs in OSCC are sparse and hence needed. We examined the expression of m6A regulators, and quantified the global m6A levels in multiple OSCC cell lines and a control esophageal epithelial cell line. Expression of various m6A regulators was derailed and the global m6A level was significantly enhanced in OSCC cells. Further, we interrogated the role of the m6A regulators in OSCC by siRNA mediated knockdown followed by a variety of in vitro pathophysiological assays to examine cell proliferation, apoptosis, colony formation, migration, and invasion of OSCC cells. Our data conclusively shows a role of these m6A regulators in various facets of OSCC pathophysiology. Further to delineate the role of m6A regulated miRNAs in OSCC, we carried out small RNA sequencing of OSCC cells depleted of the primary m6A writer i.e. METTL3. Bioinformatic analysis revealed a number of differentially regulated miRNAs. Further validation and functional analysis of these candidate miRNAs is in progress. Taken together, our work indicates a crucial role for m6A RNA methylation in OSCC pathophysiology, with a potential to develop novel therapies in future.

P39: Understanding the mechanism of mRNA cap recognition by eukaryotic initiation factor eIF(iso)4E in *Arabidopsis thaliana*

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Keywords: Translation initiation, eukaryotic initiation factors (eIF), mRNA cap recognition, eIF4E isoform (eIF(iso)4E), Structural understanding

Translation initiation in eukaryotes is a complex and multistep process that starts with the formation of the 43S translation initiation complex, which comprises many eukaryotic initiation factors (eIF), met-tRNA_i, and the 40S ribosome. One of the most important regulatory steps in translation initiation is the recruitment of the mRNA transcript over the 40S ribosome, which involves recognition of the 5' mRNA cap by the eukaryotic initiation factor eIF4E. In plants, along with this factor, its isoform, eIF(iso)4E, is also present, which recognizes a distinct set of mRNAs under stress conditions and also provides resistance during viral infections. The structural understanding of this preferential mRNA recognition is yet to be understood. In this study, we aim to crystallize eIF(iso)4E from *Arabidopsis thaliana* and determine a high-resolution structure of eIF(iso)4E with and without an mRNA 5' cap analog. This study will help us understand the aspects of the eIF(iso)4E structure that specify translation control under stress conditions and resistance to viral infections

P40: Mouse brain hippocampal transcriptome revealing a distinct gender specific pattern in Alzheimer's Disease Progression

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Keywords: Alzheimer's Disease, hippocampus, stress, gender specific factors, long noncoding RNAs

Introduction: Alzheimer's Disease(AD) is one of the most prevalent neurodegenerative diseases and the most common form of dementia in elderly adults. It leads to deterioration of brain tissue, memory impairment, lack of logical reasoning and decision making, serving as a chronic debilitating mental inconvenience. AD is reported to be almost twice more frequent in women than in males, the cause of this disparity is yet to be elucidated. AD has been reported to initiate in the hippocampus region of the brain, but the zoomed view of AD progression within the hippocampus and its associated regions are yet to be deduced.

Objective: Our main focus is to unveil the hippocampal gene expression signatures in AD. We have analysed the transcriptomic data corresponding to different AD mouse models viz ArcticAD, 5xFAD , APP/PS1 and humanized APOE4 (both males and females).Our logic behind choosing these models lies in encompassing all possible types of AD i.e. early onset, late onset, familial and sporadic forms to elucidate important biological and cellular pathways for every model separately as well as deducing a holistic idea about AD progression irrespective of the model to model variations in both the sexes.

Results: We have obtained significant set of differentially regulated genes for different AD models with respect to different time points and mapped the relevant biological pathways with reported associated clinical manifestations, indicating a common picture of AD progression enriched with significant variations between males and females.

Conclusion: Association of AD with stress, Diabetes, Rheumatoid Arthritis and cancer were prominent in both systems. Despite such similarity, certain distinct gender specific differences in gene signatures associated with predisposition towards AD have been observed. Our future work will lay special emphasis towards deciphering the contribution of gender specific factors along with the role of long noncoding RNAs(lncRNAs) in AD progression.

P41: Restoration of the expression of miR-185-5p rewires cisplatin chemosensitivity in cisplatin resistant OSCC cells through downregulation SOX9

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Keywords: Oral squamous cell carcinoma, Cisplatin, miR-185-5p, Chemoresistance, miR- 203a-3p

Resistance to platinum drugs like Cisplatin, is a major difficulty to effective Oral Squamous cell carcinoma (OSCC) treatment, linked with tumor relapse. Compelling evidence has demonstrated that dysregulated expression of microRNAs (miRNAs) influences the development of cancer chemoresistance and tumorigenesis. Although couple studies have shown the involvement of miRNAs in OSCC chemoresistance, the exact molecular mechanisms in many cases are not clearly understood. In the current study, we intend to track down miRNA signature(s) and its associated targets imparting chemoresistance in OSCC. We analyzed array profiles of drug resistant OSCC cells. We obtained 101 significantly dysregulated mRNAs targeted by 83 differentially expressed miRNAs, out of which only 36 target genes are functionally enriched in tumorigenic and drug resistance-related pathways. Among the enriched miRNA-target pairs, we found miR-185-5p as a signature miRNA modulating chemoresistance in OSCC. Our qRT-PCR analysis validated that miR-185-5p is significantly downregulated in the cisplatin-resistant SCC9 (CIS-R-SCC9) and H357(CIS-R- H357) cell line, consistent with our findings from the array analysis. Subsequently, the gain of function study of miR-185-5p by mimic transfection increases cisplatin sensitivity by decreasing cell viability in CIS-R-SCC9/H357 cell lines. Furthermore, miR-185-5p treatment in combination with cisplatin increases drug induced DNA damage, intracellular ROS production and apoptosis in CIS-R-SCC9 cells. Mechanistic studies revealed that miR-185-5p sensitizes cisplatin by decreasing drug efflux and DNA repair by promoting the expression of miR-203a-3p through negative regulation of SOX9. This study will pave the way for employing this miRNA in therapeutic strategies to combat chemoresistance in oral cancer.

P42: Identification of novel long non-coding RNA (lncRNA) signatures in meningioma: path to biomarker discovery and therapeutic intervention

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Meningioma is one of the most common primary brain tumours, classified into 3 malignancy grades by WHO. Surgical resection and radiotherapy are the mainstays of treatment and often inadequate to tackle higher grade tumors and those that are inaccessible for gross or subtotal resection and subsequent clinical management. Complex histology of meningiomas coupled with lack of reliable genetic and epigenetic markers impacts accuracy of tumour grading, disease prognosis, and therapeutic discourse. Distinct interactions between the coding (mRNAs) and noncoding (ncRNAs) transcriptomes have been shown to drive regulation of gene expression at multiple levels, with significant impact on disease development, including cancer. Long noncoding RNAs (lncRNAs) act as competing endogenous RNAs (ceRNAs) that titrate miRNA levels through direct binding, impacting downstream miRNA-based regulation of target mRNAs. For the first time, through transcriptome sequencing of meningioma patient tumour samples (N = 75) inclusive of the three WHO grades, we characterized the expression profiles of lncRNAs in meningioma associated with each tumor grade. Several lncRNAs such as H19, BLACE, MIR124-2HG, SOX2-OT, FOXP1-AS1 among others showed significant dysregulation across different meningioma grades. LncRNA-mRNA expression correlation and lncRNA-miRNA-mRNA interaction analyses revealed key lncRNA- mRNA inter-relationships that drive meningioma pathogenesis. The differentially expressed lncRNAs (DE- lncRNAs) through their targets were shown to impact key functional pathways such as mTOR signalling pathway, AMPK signalling pathway, Neutrophin signaling pathway, cell cycle, insulin resistance, focal adhesion implicated in meningioma. LncRNA-mRNA expression correlation analysis provided insights into the probable functional roles of both well-characterized and novel unannotated lncRNA transcripts in meningioma. Furthermore, DE-lncRNA-miRNA-mRNA interaction networks from our data revealed key regulatory axes that impact meningioma pathogenesis at different levels. Overall, our results for the first time, have identified long noncoding RNA signatures in meningioma and revealed important ncRNA-mRNA inter-relationships that may impact meningioma pathogenesis and serve as potent biomarkers and therapeutic targets.

P43: MicroRNA-874 regulates nonsense-mediated mRNA decay factor UPF3B

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Keywords: Nonsense-mediated mRNA decay, UPF3B, microRNA, Gene regulation, Neurodevelopmental disorders

Nonsense-mediated mRNA decay (NMD) is a critical surveillance mechanism that protects cells from accumulating aberrant transcripts. The UPF3B protein is considered an essential factor that governs NMD and is implicated in neuronal development. Mutations in UPF3B are found in XLID patients and are strongly associated with schizophrenia, attention deficit hyperactivity disorder (ADHD), and autism spectrum disorder (ASD). Differential gene expression analysis of Upf3b-null mice and CRISPR cas9 mediated UPF3B knockout in HEK-293 cells identified UPF3B regulated RNA. In UPF3B knockout, we have identified the transcripts associated with neuronal processes, including CNS development and axon guidance. Thus, supporting the possible role of UPF3B in neuronal processes; however, the regulation of UPF3B remains unclear. Recent studies show the importance of microRNAs in modulating the post-transcriptional network essential for the development and function of the nervous system. In this study, we demonstrate that miRNA-874 is a bonafide regulator of UPF3B in human neuronal cell lines (IMR32 and SH-SY5Y). miRNA-874 binds to the microRNA response element in 3'UTR of UPF3B, leading to its mRNA degradation and down regulating the expression of UPF3B at the mRNA and protein levels. These results indicate the existence of an RNA circuit linking microRNAs and NMD. The deregulation of microRNA expression has been identified in various human diseases, including cancers. Thus, the identified microRNAs may prove to be the biomarker for diagnosing UPF3B-linked neurodevelopmental disorders. Our work may open the door for developing the microRNA-based therapy to return aberrantly expressed microRNA to their physiological level by administering the microRNA mimics or the antisense oligos named anti-miRs.

P44: Aberrant CD19 splicing in Pediatric B-cell Acute Lymphoblastic Leukemia (B-ALL) Patients

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Keywords: B-ALL, CD19, CART therapy, alternative splicing

B-cell acute lymphoblastic leukemia (B-ALL) is characterized by an uncontrolled deleterious burgeoning of B-cell lymphocytes in the bone marrow, posing a major threat to our immune system, eventually culminating in death. In recent years, chimeric antigen receptor-T cell (CAR-T) therapy targeting the CD19 antigen on B-cells has emerged as a promising treatment modality for relapsed and incurable forms of B-ALL. However, a significant percentage of the patients experience relapse post-therapy, potentially due to the loss of CD19 antigen on B-cells. Aberrant splicing and mutations in CD19 are thought to be the major contributors to therapy relapse. Herein, we investigated CD19 splicing patterns in primary paediatric B-ALL patient samples using RT-PCR-based splice assays. We observed that a significant percentage of patients exhibit the presence of CD19 splice-isoform responsible for CAR-T resistance in primary stage itself. Additionally, through in-silico and qRT-PCR analysis in patient samples, we have identified dysregulated RNA-binding proteins (RBPs) responsible for the aberrant splicing of these exons. Taken together, our findings suggest that the aberrantly spliced CD19 isoforms associated with CAR-T resistance may exist in primary patients possibly due to dysregulation of certain RBPs.

P45: The molecular function of B-Complex pre-mRNA splicing factor Snu66

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Transcripts from most eukaryotic genes are synthesized as precursor mRNAs (pre-mRNAs) from which non-coding introns are removed and exons are ligated back to produce mature mRNAs. These mature RNAs are then exported to the cytoplasm and translated into proteins. The process is carried out by a dynamic multi-megadalton RNA-Protein complex, the spliceosome. It consists of five small nuclear RNAs (snRNAs) and ~200 proteins. These proteins stably associate with snRNAs and form RNA-protein particles, termed as small ribonucleoproteins or snRNPs, which include U1 and U2 SnRNP and U4/U6.U5 tri-snRNP. Components of spliceosome are assembled and disassembled during each round of splicing.

The U2/U6.U5 tri-snRNP complex of the spliceosome forms the catalytic centre. It consists of core splicing factor Prp8 including Snu66 which appears to function like a scaffold. Deletion or specific mutation in the protein, gives rise to splicing defects in *S. cerevisiae* and *S. pombe*. Snu66 appears to be responsible for conversion of pre-B to B-complex via assembling a B-complex-specific ternary complex of Prp38-Spp381-Snu23. In condition when the U4/U6 RNA helicase Brr2's activity is compromised, Snu66 become critical. This result suggests that Snu66 might function together with Brr2 for the conversion of the tri-snRNP U4/U6.U5 into catalytically competent splicing complex with U2/U6.U5.

P46: Global translation initiation with non-methionine amino acid in *E. coli***Divya Nashier**¹ and Umesh Varshney^{1,2}¹Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore²Jawaharlal Nehru Centre for Advanced Scientific Research, Bangaloredivyanashier@iisc.ac.in, varshney@iisc.ac.in**Keywords:** initiation, tRNA, Met, alanine-based initiation.

Across all domains of life, the process of mRNA translation unanimously begins with methionine (Met) as the initiating amino acid. While bacteria, archaea and eukaryote showcase domain specific evolution of the translational apparatus, the use of Met (or fMet) as the initiating amino acid has remained ubiquitous across all domains of life. Such a phenomenon raises a question of why the translation apparatus evolved to initiate protein synthesis with Met and whether *E. coli* can be recoded to initiate with any amino acid other than Met to support global translation initiation in the cells. We use G3:U70 acceptor stem mutant of i-tRNA having the ability of alanine charging while retaining conserved determinants of i-tRNA for its exclusive initiator function. By overexpressing alanine tRNA synthetase (AlaRS), we successfully aminoacylated G3:U70 mutant initiator tRNA with alanine. To elucidate G3:U70 mutant i-tRNA can perform function of global translation initiation, we generated *E. coli* strain (Δ metZWV and Δ metY) which is supported on G3:U70 mutant i-tRNA. Further, with in vivo reporter based assay we showcase participation of G3:U70 i-tRNA towards alanine-based translation initiation. Using this systems biology approach towards recoding global translation initiation with non-Met amino acid, we ought to elucidate potential regulatory and evolutionary aspect for choice of Met towards translation initiation.

P47: Multiomics approach unveils epigenetic alterations associated with cognitive dysfunction in hepatic encephalopathy model

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Keywords: Hepatic encephalopathy, Transcriptomics; Epigenomics, Cognition, Neurodegeneration

Hyperammonemia and systemic inflammation are the hallmarks of neurodegeneration that contributes to cognitive deficits in hepatic encephalopathy (HE). However, the molecular mechanisms underlying cognitive impairment are still unclear. Here we integrated transcriptomic, epigenomic and metabolomic analyses of different brain regions of Bile duct ligated rats to identify molecular pathways involved in HE. RNA sequencing analysis revealed differential expression of transcription and chromatin related genes including histone modifiers. LC-MS/MS based histone modification profiling revealed alterations in histone H3 and H4 modifications linked to transcription, chromatin and disease pathways in HE. Metabolomic analysis of brain tissues from Bile duct ligated rats suggests impairment in energy metabolism with altered tricarboxylic acid cycle, glycolysis and ketogenesis. These metabolic changes directly correspond to changes in epigenome. Co-cultured astrocytes and neurons treated with ammonium chloride also showed downregulation of genomewide H3K9ac, H3K27ac, H4K12ac. Together, these findings suggest that HE disrupt the epigenome by affecting the metabolic balance thereby dysregulating transcription and chromatin gene feedback loops. The identification of this process will highlight potential epigenetic strategies for HE treatment.

P48: Deciphering the role of host proteins in Cocksackievirus B3 infection using Systems Biology Approach

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Keywords: Cocksackievirus B3, RBPs, systems approach, pathogenesis

CVB3 is a positive sense, single-stranded, non-enveloped RNA virus of the Picornaviridae family causes acute myocarditis and pancreatitis in children and young adults. Previous studies from our laboratory have suggested the involvement of many host proteins, including La, PSF, PTB, PCBP2, hnRNP C1/C2 in various processes during CVB3 infection. To complete an infection cycle, the overall ratio of various host factors needs to be modified at a fast pace by the virus. We are interested to apply a systems approach in investigating the cellular proteins' dynamic modulation during CVB3 infection, giving us insight into the proteins that can differentially regulate the early and late phases of the CVB3 life cycle. For the same, we performed mass spectrometry with CVB3-infected cell lysate harvested 4h and 8h post-infection. Around 8000 proteins were detected. Among them, 11 proteins were upregulated 4h post-infection, and none were downregulated significantly at this early hours. However, at 8h post-infection, 669 proteins were upregulated, and 472 were found to be downregulated. Gene ontology enrichment analysis showed the different pathways and functions regulated by these proteins. Interestingly, a large number of dysregulated proteins that showed up in the screen are RNA-binding proteins. We wanted to explore different functional role of these RNA binding proteins, for which we used three different approaches. By Antisense Oligonucleotide (ASO)-mediated pulldown, we identified 44 proteins interacting with CVB3 5'UTR and 34 with CVB3 3'UTR. RBPs such as hnRNPC, PCBP2, and PTBP1, which are already reported to interact with CVB3 RNA, also showed up in our screening, validating our findings. Besides, to identify proteins whose subcellular localization is affected by the virus infection, we performed nuclear and cytoplasmic fractions of CVB3-infected cells and mass spectrometry was carried out. Also, to detect the proteins that are regulated at the level of translation, ribo-sequencing and mass spectrometry are being carried out (in collaborations with Laboratory of Dr. Mihaela Zavolan, Biozentrum). The integrated analysis would reveal interesting insights into the various pathways and cellular functions modified upon CVB3 infection as a result of dysregulated proteins.

P49: Identification of chromatin-associated circular RNAs

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Keywords: Circular RNA, chromatin, DNA-RNA hybrid, RNA-sequencing, cellular fractions

Subcellular distribution of RNAs has become a hot topic for researchers in recent years as subcellular localization of RNA molecules defines their cellular functions by associating with other biomolecules. Circular RNAs (circRNAs) are one of the newly discovered RNA families that regulate various cellular functions by interacting with proteins and microRNAs in a spatiotemporal manner. It has been reported that circRNAs localized in the cytoplasm mostly regulate mRNA translation or stability by acting as a microRNA sponge, while circRNAs in the nucleus regulate transcription and pre-mRNA splicing by associating with transcription factors and splicing factors. This provokes us to think that circRNA might also directly interact with chromatin through a sequence complementary to the genomic DNA. To unravel this mystery, we have analysed the previously published RNA-sequence data from chromatin-associated fractions and discovered hundreds of chromatin-associated circRNAs. To confirm their true association with chromatin, we validated the enrichment of a subset of circRNAs in the chromatin pellet of HEK293 cells. Furthermore, the BLAST analysis of these circRNAs with the genomic sequence suggested their interactions with various regions of the genome apart from their host gene location. Interestingly, computational prediction of the functions of RBPs interacting with chromatin-associated circRNAs suggested their role mainly in RNA processing and splicing. Overall, our research depicts the association of circRNA with chromatin, a new functional aspects of the circRNAs. However, more experiments are underway to validate the exact region of circRNA-DNA interaction and its role in gene expression regulation.

P50: RNA condensates in anti-cancer drug resistance

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Keywords: Cancer, chemoresistance, RNP condensates, disassembly factors

Cancer is the uncontrolled proliferation of cells due to mutations in the genes essential in cell growth. Effective inhibitors to deal with these cancers are developed but their response is temporary. Cancer cells undergo stress when exposed to chemotherapeutic treatment. The tumor cells respond to this stress by inducing the formation of RNP condensates (conserved membrane-less dynamic RNA-protein condensates). These condensates have a protective role towards the cancer cells and lead to the development of tolerance and resistance to the inhibitors. Therefore, we intend to identify factors that would disassemble these granules and increase the efficacy of the inhibitors.

The objectives of my project are: 1) Screening of various potent disassembly factors to modulate the dynamics of the RNP condensates. 2) To elucidate the mechanism of disassembly of RNP condensates formed under chemotherapeutic stress. 3) To understand the interaction of the disassembly factors with the condensate components. 4) To analyse the role of disassembly factors in cancer progression and drug resistance. Since the RNP condensates induced by chemotherapeutic agents are protective in nature, targeting the assembly or finding factors that disassemble the granules would lead to the increase sensitivity of cancer cells to drugs.

P51: Role of circSmad1 encoded protein in skeletal muscle differentiation

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Keywords: RNA-sequencing, Circular RNA, translation, micropeptide, myogenesis

CircRNAs are a novel class of RNA molecules with functions implicated in gene regulation via RBP and microRNA interaction. A growing body of research has reported a subset of circRNAs showing cap-independent translation into polypeptides, opening a new dimension in our understanding of protein diversity. This study identified potentially translatable circRNAs expressed in skeletal muscle, an organ characterized by high protein complexity. More than 2500 circRNAs were identified in differentiating C2C12 cells, out of which 145 circRNAs were reported by riboCIRC to have protein-coding ability. Differential expression of sixteen protein-coding translatable circRNAs in differentiating C2C12 cells were validated by RT-qPCR and Sanger sequencing. Polyribosome profiling of 3-day differentiated C2C12 myotubes confirmed the enrichment of circRNAs, including circCdk8, circCdy1, circH6pd, circSmad1, circAsph, circNfix in polysome fractions. Based on polysome association, circRNA length, and mass spectrometry evidence of junction peptide, we selected the 581 nt long circSmad1 encoding a junction-spanning 192 aa protein for functional study. LNA-mediated silencing of circSmad1 in differentiating C2C12 cells showed significantly reduced myotube formation, decreased levels of Myh, MyoD, and higher expression of Id1 mRNAs. Interestingly, the circSmad1-192aa protein was predicted to fold into a 3D structure with absolute identity to the MH1 domain of SMAD1, a protein crucial for ID1-mediated myogenic inhibition via the BMP signaling pathway. In summary, we propose that circSmad1- 192aa protein may function analogously to the MH1 domain of Smad1 by binding to the ID1 BMP responsive element (BRE) region in the promoter and affecting myogenic differentiation.

P52: Characterization of the role of human DEAD box RNA helicase 3X (DDX3X) in regulating Influenza virus RNA synthesis and virus life cycle

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Keywords: Influenza B, RNA dependent RNA polymerase (RdRp), Dead Box RNA Helicase-3 X (DDX3X), Viral RNA synthesis, Nucleoprotein (NP).

Influenza viruses are negative sense RNA virus that cause respiratory infections amongst human with high morbidity and mortality rates. Virus encoded RNA dependent RNA polymerase (RdRp) and nucleoproteins, in its oligomeric form, associates with viral genomic RNA to constitute the Ribonucleoprotein particles (RNPs) which is the main RNA synthesis machinery of the virus. Viral RNPs interacts with plethora of host factors that positively or negatively regulate virus replication. Comprehending the intricate interplay between viral RNP components and host factors is imperative for elucidating the replication strategy of the virus and formulating therapeutic interventions. Human Dead Box RNA Helicase 3-X (DDX3X) has emerged as a pivotal regulator across multiple RNA virus life-cycles, including influenza A virus. DDX3x acts as an antiviral host factor which regulates stress granule formation and activates NLRP3 inflammasome to release proinflammatory cytokines, although the detail molecular mechanism behind such effects remains elusive. Here we show that human DDX3X in downregulate influenza B virus life cycle by directly impacting RNP activity. Our investigations reveal that DDX3X overexpression downregulates FluB RNA synthesis and decreases virus replication through a novel pathway which is independent of its previously reported antiviral effects. A helicase-deficient mutant (AAAD mutant) fails to curb viral RNA synthesis. Being a member of RIG-I family, DDX3X can discern viral RNA and trigger host immune responses. We have found out that, DDX3X mediated suppression of viral RNA synthesis operates independently of interferon- β response. We also unveil the interaction between DDX3X and FluB genomic RNA and Nucleoprotein (NP). In absence of RNA, DDX3X-NP interaction increases, which is an indicative of competitive RNA binding by these two proteins. Collectively, our data indicates DDX3X's dual role as both a moderator of viral RNA synthesis through interaction with NP and an initiator of host innate immune responses via recognition of genomic RNA.

P53: Adaptive signatures modulating the transcription-replication balance of Influenza polymerase

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Keywords: Influenza virus, negative-sense RNA virus, RNA-dependant RNA-polymerase (RdRP), transcription-replication switch, cross-species transmission, molecular evolution

The Influenza virus RNA-dependant RNA Polymerase (RdRP) is an elegant machinery responsible for transcribing the negative-sense viral RNA during early stages of infection and genome replication during the later phase. The trimeric polymerase comprising of PB2, PB1 and PA subunits undergoes major structural rearrangement to form a dimer of the heterotrimer in order to switch from transcriptase to replicase mode. Apart from its pivotal role in virus replication, RdRp is also a major determinant of host tropism, driving cross-species transmission of influenza viruses. We have recently discovered a conserved “S-E-S” motif (amino acid positions 279, 282 and 286) within the PB2 subunit that plays a critical role in balancing transcription and replication, thus governing species-specific fitness of distantly related influenza viruses (Banerjee et al; 2022). The “S-E-S” motif of classical Influenza A viruses (H1N1, H3N2, H5N1, etc.) harbours a naturally occurring substitution to “S-S-T” only in case of the distantly related bat-influenza viruses (subtypes H17N10, H18N11). Swapping the human virus specific “S-E-S” motif with the bat-virus specific “S-S-T” attenuates human influenza polymerase and restricts H1N1 virus replication, Conversely, introducing “S-E-S” motif into the bat-influenza (H17N10) polymerase enhances RNA synthesis in a reporter-based RNP activity assay. Further, delving into the molecular mechanism of the “S-E-S”/ “S-S-T” motif mediated regulation of polymerase activity, we reveal a skewed balance between the two modes of viral RNA synthesis, favouring replication in H1N1 and transcription in H17N10 virus polymerases. Moreover, we show that the “S-E-S” motif allosterically regulates the structural transition and subsequent dimerization of the RdRP, thus modulating the switch from transcription to replication, a process that is suboptimal in case of the “S-S-T” containing (H17N10) polymerase. Together, our data unveils a novel mode of regulation of the transcription- replication switch, which in turn determines the species-specific fitness of influenza viruses.

P54: Identification of Human ECSCR-derived Circular RNA

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Keywords: Angiogenesis, ECSCR, Circular RNA, miRNA sponge, miR-646

Angiogenesis is a significant process that plays an important role in cancer progression and metastasis. Endothelial cell-specific chemotaxis receptor (ECSCR) is a cell surface protein expressed by endothelial cells that plays a pivotal role in endothelial cell migration and signal transduction. ECSCR also plays an essential role in the migration of angioblast. Here, we sought to investigate ECSCR expression regulation, focusing on the posttranscriptional regulation by circular RNAs (CircRNAs). CircRNAs are known to regulate gene expression by acting as transcriptional regulators, microRNA sponges, and protein templates. We have identified more than five circular RNA back-spliced from the ECSCR gene (circECSCR), as shown in the circInteractome database. We have validated the expression of a subset of circRNAs by RT-PCR and Sanger sequencing. Furthermore, TargetScan and the miRanda tools were used to identify 14 common miRNAs binding to different circECSCR. Downstream mRNA targets of circECSCR- associated miRNAs were identified using miRTarBase. Gene ontology analysis of 14 genes interacting with miR-646 showed significant enrichment in Artery morphogenesis, cell cycle, and cancer. Among 14 genes, VEGFA was found to be regulated by miR-646 and is previously reported to regulate angiogenesis of endothelial progenitor cells in pre-eclamptic pregnancy. Altogether, the study provides a novel insight into regulatory functions of the ECSCR-derived circECSCR- miR-646 regulatory axis in angiogenesis.

P55: Star-PAP-controlled lncRNA HAS2-AS1 stability enables SP1- target pro-hypertrophy gene expression in the heart

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Keywords: Star-PAP, RNA metabolism, lncRNA, transcription complex.

Star-PAP (speckle-targeted PIPKI α regulated PAP) is a non-canonical poly(A) polymerase that controls anti-hypertrophy gene program in the heart. Interestingly, Star-PAP depletion also results in the upregulation of a number of pro-hypertrophy genes, yet the mechanism is unknown. We show that many of such mRNAs are regulated by long non coding RNAs (lncRNAs) implicated in cardiac hypertrophy. We have identified a new lncRNA, HAS2-AS1 that is negatively regulated by Star-PAP and controls pro-hypertrophy gene expression in the heart. To understand the role of Star-PAP-HAS2-AS1 regulation in cardiac hypertrophy, we used in vivo animal heart model and cellular hypertrophy model using H9C2 cardiomyoblast cell line. In both the models, we observed upregulation of HAS2-AS1 lncRNA while Star-PAP is downregulated. We show that Star-PAP regulation of HAS2-AS1 is independent of 3'-end processing, and that Star-PAP controls HAS2-AS1 RNA metabolism (stability and turnover). There was 2-4 fold induction in the half-life of the HAS2-AS1 on Star PAP knockdown. Co- immunoprecipitation showed that Star-PAP binds AGO2 protein and recruits targeted miRNAs onto HAS2-AS1 RNA thus facilitating degradation. Consistently, many of the Star-PAP target pro-hypertrophy genes were compromised on HAS2-AS1 knockdown both under normal and isoproterenol treated condition. Many of this targets (FGF2, FGFR1, HCN2 and HCN4) were overlapped with a well-established hypertrophy regulator SP1 transcription factor. By immunoprecipitation experiments we showed that HAS2-AS1 binds SP1 transcription factor, and is required for SP1 promoter DNA occupancy in vivo. We demonstrated that HAS2-AS1 helps in the assembly of SP1 transcriptional complex along with and RNA-polymerase II on SP1 target promoter DNA. Further, we confirmed HAS2-AS1 strong association with chromatin at the promoter regions of SP1 target genes (FGF2, FGFR1, HCN2, HCN4) by chromatin isolation by RNA purification (CHIRP) but not with control chromatin regions. Thus, our study establish that Star-PAP-mediated HAS2-AS1 stability controls expression of SP1 target pro-hypertrophy regulators in the heart.

**P56: The paralog dilemma – characterising exon junction complex proteins
MAGOH and MAGOHB**

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Keywords: EJC, paralogs, knockout, CRSIPR-Cas9

Gene duplication provides a source of genetic material for evolution to act upon, and is a means for new genes to emerge. Paralogs arising out of gene duplication share similarity among each other, posing a challenge towards discerning their individual functions. MAGOH/MAGOHB is an interesting paralog pair conserved across mammals, differing only in two amino acids at the N-terminus. MAGOH/MAGOHB are core components of the exon junction complex, deposited on spliced mRNAs. The absence of both these paralogs is lethal to the cell, while single protein loss is tolerated albeit with different disease phenotypes. The loss of MAGOH paralogs is associated with different types of cancers and neurodegenerative disorders. Currently, MAGOH and MAGOHB are believed to have redundant functions, but lack of methods to distinguish these two proteins has hindered delineation of their independent roles. Synonymous substitutions in the coding sequence allow their endogenous distinction at the mRNA level, whereas distinguishing them at protein level by conventional techniques is exceedingly difficult. Using the CRISPR-Cas9 system we have developed cell lines to study independent roles of MAGOH paralogs, via gene knockout and endogenous tagging. We have found differences in the half-lives of the MAGOH and MAGOHB mRNAs, which does not corroborate with difference at the protein level. Additionally, our MAGOH/MAGOHB knockout cells have varied phenotypes, which can be linked to the differences in the disease etiologies upon individual paralog loss. Our data will be further helpful to analyze functional differences and overcome the abiding enigma that surrounds these paralogs.

P57: Unveiling the Molecular blueprint of Mitochondrial Phenylalanyl-tRNA Synthetase: Insights into Pathogenic Variations

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Keywords: Phenylalanine tRNA synthetase, pathogenic mutations, refolding, stability, solubility.

Introduction: In order to maintain energy production and structural as well as functional integrity mitochondria need to import several essential proteins. These proteins are encoded by nuclear DNA and synthesized in cytoplasm. Mitochondrial aminoacyl-tRNA synthetases (mtAARS) are responsible for accurately pairing specific amino acids with their corresponding tRNAs, facilitating the precise incorporation of amino acids into growing polypeptide chains during mitochondrial protein synthesis. Phenylalanine tRNA synthetase (HsmtPheRS) is one such protein which migrate to mitochondria. In this journey from cytoplasm to mitochondria HsmtPheRS have to endure many different cellular environments including pH gradient across the membrane of mitochondria. This pH gradient is a significant barrier which can cause partial unfolding of imported proteins that facilitates the entry into the matrix of the mitochondria. Inside the mitochondrial matrix HsmtPheRS must refold to regain their functionality. Thus, the unfolding and refolding is very crucial to maintain the structural integrity, solubility and activity of these proteins. It has been reported that some mutations in FARS2 gene leads to Alper's encephalopathy or spastic paraplegia. These pathogenic mutations were shown to affect the enzymatic activity, however, their effect on protein stability and unfolding/refolding is still unknown.

Method: We have selected some reported pathogenic mutations of hsmtPheRS (P136H, D142Y, G309S, D325Y, P361L) to evaluate their stability (turbidity assay, dynamic light scattering and thermofluoro assay) and unfolding/refolding (fluorescence based assays and aminoacylation assay) .

Result: We observed that wildtype HsmtPheRS can endure the whole process of unfolding and refolding in a very efficient way, without compromising its functionality, stability and solubility. On the contrary, mutants are less efficient in refolding, affecting their functionality and solubility. Wildtype hsmtPheRS has shown remarkable stability compared to the mutants. Additionally the stability of the wildtype increased substantially in the presence of the cognate substrates and reducing environment, however, in mutants their effect are minimal.

Discussion and Conclusion: In our research endeavor, we wanted to understand the mechanism that may lead to pathogenicity due to genetic mutations. We found that it depends on two critical factors: the capacity for efficient refolding and the ability to uphold solubility and stability. All the mutants studied here, fall short in meeting these criteria.

P58: Unraveling the Genetic Complexity of Non-syndromic Hearing Loss

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Keywords: NSHL, GJB2, TMC1, SLC26A4 and WFS1

Non-syndromic hearing loss (NSHL) that affects both central and peripheral auditory system, is the result of numerous gene mutations responsible for auditory system development. NSHL showcases a multifaceted genetic landscape encompassing autosomal dominant, autosomal recessive, X-linked and currently emerging mitochondrial inheritance pattern. Most notably, the GJB2 gene emerges as a prevalent factor with high occurrence, implicated in more than half of NSHL cases, while TMC1, SLC26A4, and WFS1 also shows contribution in it's progression. Adding on to that, mutation in mitochondrial genes commence a late-onset form of NSHL. From the therapeutic front, cochlear implants (CI) or auditory brainstem implants (ABI) only afford a limited benefits and doesn't cater to all affected individuals. Gene therapy introduces a radical approach, extending the potential for a personalized cure or restoration of hearing by directly labeling the genetic roots of NSHL. This abstract gives a comprehensive exploration of the genetic intricacies of NSHL, in order to map out the mutational landscape that causes this disorder. Incorporating collaborative research initiatives, our plan is to scrutinize the functionality of these genes, investigate the feasibility of latest adopted strategies of targeted gene therapy, and explore potential application of precise gene editing. The goal is to mend the gap between genetic investigation and therapeutic application, in order to develop personalized gene therapy protocols that will provide durable solutions for individuals afflicted with NSHL.

P59: Deciphering the Role of Serine/arginine-rich protein-specific kinase 1 (SRPK1) in Castration-resistant Prostate Cancer Progression

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Keywords: SRPK1, SRSF1, AR-V7, CRPC, phosphomimetic

Castration-resistant prostate cancer (CRPC) presents an alarming clinical challenge due to its resistance to androgen deprivation therapy. A comprehensive understanding of the molecular mechanisms underlying CRPC is essential for developing successful therapeutic approaches. Numerous studies have implicated the role of Serine-Arginine Protein Kinase 1 (SRPK1) in CRPC progression and aggressiveness. SRPK1 phosphorylates SR protein; SRSF1 which thereby allows it to dynamically shuttle between the nucleus and cytoplasm. Phosphorylated SRSF1 is involved in the generation of different androgen receptor (AR) isoforms, including Androgen Receptor splice variant 7 (AR-V7), which lacks the ligand-binding domain and is associated with CRPC progression and resistance to AR inhibitor (enzalutamide).

To understand the mechanistic details of how SRPK1 promotes AR-V7 generation, we have generated a series of alanine and phosphomimetic mutants of SRPK1 and evaluated their effect on p-SRSF1 and AR-V7 generation. Furthermore, we evaluated the tumorigenic potential of cell lines overexpressing the SRPK1 mutants and assessed their effect on enzalutamide resistance. Taken together, our studies provide mechanistic insights into SRPK1 mediated CRPC progression..

P60: Unravelling the Regulatory Mechanism that Connects Ribosome Biogenesis and Stringent Response with Bacterial Cell Growth

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Keywords: Ribosome biogenesis, stringency response, bacterial cell growth

Era, a Ras-like small GTPase, is essential for survival in *Escherichia coli*. This protein, conserved in both prokaryotes and eukaryotes, plays a diverse role, including the maturation of the small ribosomal subunit. On depleting Era using a tunable protein degradation system, we observed severe growth-associated defects in the bacterial cell. We could attribute this to an inactive translation state in the cells, even though the cells are in a metabolically active state with mature ribosomes devoid of any assembly defects. These are the characteristic features of persistent cells produced during stringent response. We observed that RelA, which synthesizes (p)ppGpp in *E. coli*, tends to be concomitantly expressed along with Era during the lag phase of bacterial cell growth. Furthermore, in the absence of RelA, depletion of Era tends to restore the severe growth-associated defects. This suggests that Era employs a temporal regulation upon RelA, preventing it from negatively regulating translation in the lag phase to drive ribosome biogenesis as the cell transitions to the log phase. Era is observed to exert its function in two specific ways. Firstly, it interacts with the 30S small subunit, preventing it from associating with the 50S large subunit to form 70S translating ribosomes, the substrate for RelA to activate the stringency pathway. Era also binds to RelA, preventing it from interacting with the ribosomes. Our investigation provides a novel role of Era in regulating ribosome biogenesis during the active growth phase.

P61: *In silico* solutions for accelerating RNA research in Biopharma

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Keywords: mRNA vaccine optimization, ASO target identification, Omics bot, scRNA data analysis, AI/ML

The post-pandemic Biopharma landscape is witnessing a clear and growing focus on advancing RNA research to develop innovative therapeutic candidates and utilize RNA as a targeted strategy for addressing various diseases. The recent advancements in computer algorithms and computational infrastructure have significantly accelerated RNA research by efficiently analyzing extensive datasets from high-throughput experiments and deriving valuable insights. These computational tools also offer additional recommendations to optimize experimental outcomes. In this poster, we highlight a series of transformative case studies that demonstrate the potential and effectiveness of utilizing state-of-the-art *in silico* methods in the Biopharma sector. The showcased studies address diverse challenges, including optimization of an mRNA vaccine for Lyme disease to simultaneously improve thermal stability and translation efficiency, analyzing single-cell RNA sequencing data to differentiate responders from non-responders to IVIG treatment, and accurately identifying targets for an antisense oligonucleotide-based therapy for a rare disorder. Additionally, we introduce an AI-driven Omics bot designed to streamline complex single-cell RNA data analysis, empowering scientists and researchers to extract valuable insights and make informed decisions without requiring specialized coding expertise.



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