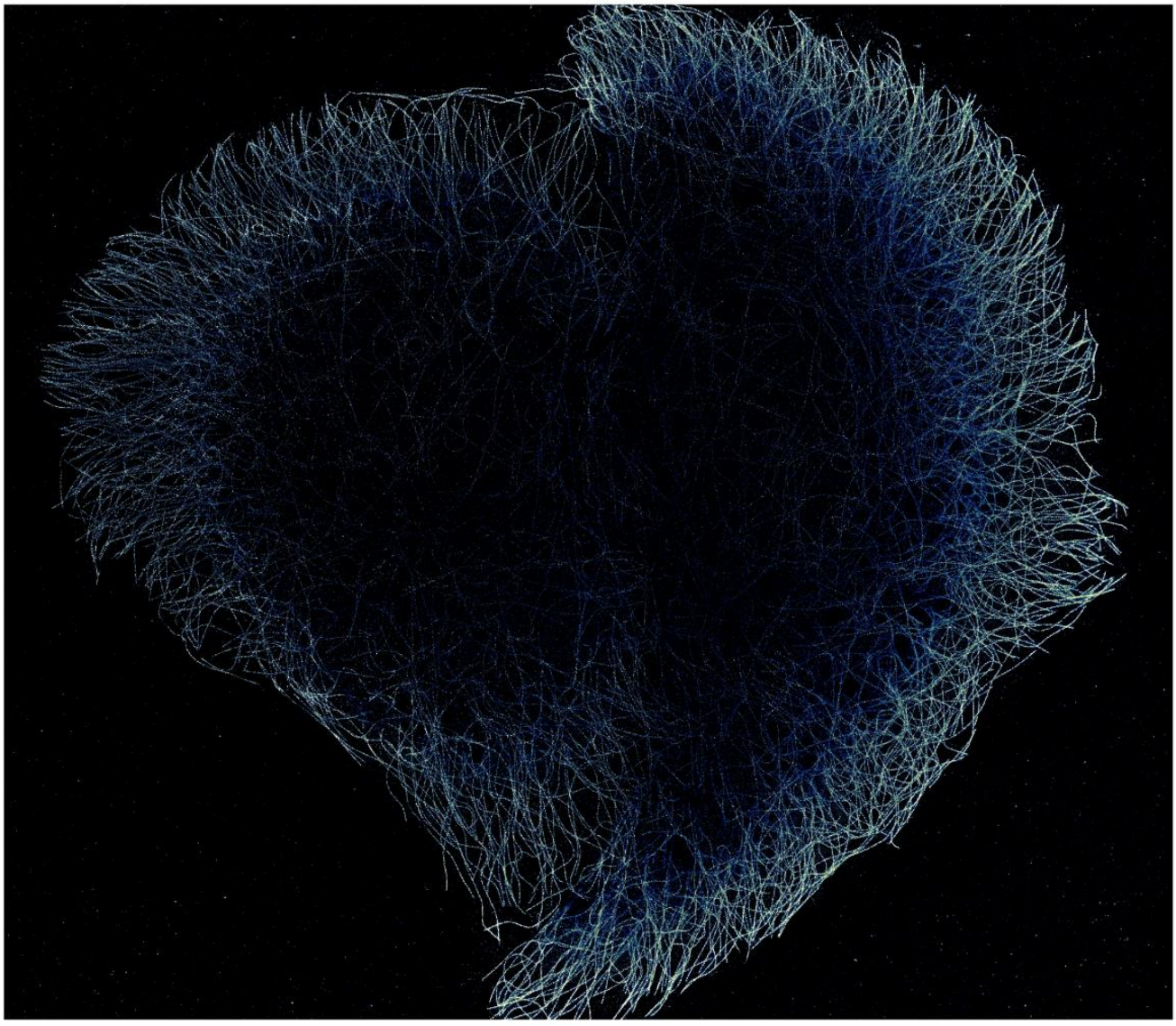
VOLUME 46, ISSUE 2/2023

CELL BIOLOGY

NEWSLETTER

ISSN: 2349-8307



PUBLISHED BY

INDIAN SOCIETY OF CELL BIOLOGY (REGD.)

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Cover page: The microtubular architecture in a pair of chemically cross-linked HeLa cells imaged in super-resolution using DNA-PAINT on a TIRF microscope at Dr. Mahipal Ganji's lab, Department of Biochemistry, Indian Institute of Science, Bangalore by Mr. Abhinav Banerjee. Microtubules are labeled with anti-alpha-tubulin-antibody and DNA-strand conjugated secondary antibody for DNA-PAINT imaging.

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	Dr. Richa Arya, BHU, Varanasi

Presidential Remarks

It is our pleasure to present the first newsletter after the current executive committee took over in April 2023. Prof. Sathees Raghavan, Secretary of ISCB, and his team, have been very active in efficiently taking over and managing the affairs of the society.

I am happy to share with you that the response from the members of the Society to contribute to the newsletter has been nothing short of overwhelming. We have received close to 30 contributions, out of which we have included 20 in this issue of the newsletter. We hope to come out with the second newsletter by March-end. Another heartening fact is that we have received contributions from different parts of the country. The current issue includes articles received from New Delhi, Bengaluru, Mumbai, Ukrul (Manipur), Kolkata, Hyderabad, Gandhinagar, Mandi, Kharagpur and Thiruvananthapuram and these have been written by members from various Universities, IITs, IISERs and research institutes. The large number of contributions from different types of academic and scientific institutions spread all over the country are indicative of the larger reach of ISCB. The ISCB thrives to improve teaching and research in Cell Biology across the country, in which the newsletter plays an important role. The newsletter makes both members and non-members aware of the various research and teaching initiatives in cell biology, which have been enriched by many young PIs who have recently established their labs to ask interesting questions and take new pedagogical approaches. I hope that you will enjoy reading these diverse articles. The newsletter also contains report of the previous ISCB conference held at Banaras Hindu University, Varanasi, and brief information about the upcoming conference at Advanced Centre for Treatment Research and Education in Cancer, Navi Mumbai.

I urge members of ISCB to continue contributing to the newsletter.

Surendra Ghaskadbi

President, ISCB

Message from Secretary, ISCB

Dear Members of the Cell Biology Society

Indian Society of Cell Biology (ISCB) is delighted to bring out the second issue of the Society Newsletter in December 2023. The current executive committee has taken over the responsibilities from April 01, 2023 and therefore this will be the first one for the new committee.

Following the COVID pandemic affecting routine research activities and life in India and worldwide, a very successful full three-day All India Cell Biology Conference was organized at Banaras Hindu University, Varanasi, in January 2023. This helped us in getting back the momentum and now the society is back with its routine full-fledged activities. The 46th All India Cell Biology Conference is being organized at ACTREC, Mumbai, from January 10-12, 2024.

The present newsletter has a compilation of activities of All India Cell Biology Conferences in 2023 at Banaras Hindu University, Varanasi. In this issue of newsletter, we have included the Cell Biology meeting conference report and the details of society lecture awards delivered by eminent scientists. Several awards were also given to the young students for both oral and poster presentations.

Besides, we received very encouraging responses for the Newsletters from both junior and senior members of the society as they submitted several short research reviews/perspectives/articles/poems. It is also important to highlight the overwhelming response that we received from both universities and research institutes across India. Although, we could not publish all the received articles this time, I am optimistic that we will have a more elaborate newsletter coming up in the early half of 2024.

This issue covers articles on topics like DNA Structure Function, RNA Biology, Cell Cycle, Infectious Disease Biology, Cancer Biology, and many more. Besides, there is a general article connecting Societies and Cell Biology, a Historical perspective of the development of modern tools, which are used in Cell Biology. An article from DBT, India also outlines funding opportunities for Cell Biology Research for PIs of different levels.

I take this opportunity to thank all the contributing authors for their timely and prompt response. I extend my thanks to the reviewers for their suggestions and edits. I also wish to thank Dr. Meghana Manjunath and Ms. Susmita Kumari for their time and effort during designing and production of the newsletter.

We hope to have the next edition of the Newsletter by March 31, 2024. Active participation from all Society members, including research students, will be highly appreciated. Looking forward to meeting you all at the forthcoming annual meeting at ACTREC, Mumbai, January 10-12, 2024.

Wishing you all a very happy new year, 2024!!

Sathees C Raghavan

Secretary, ISCB

Message from Executive Secretary

Dear Members of the ISCB

It gives me immense pleasure to pen down a few sentences for the ISCB newsletter. Having been associated with the Indian Society of Cell Biology, since the beginning of my research career, I have witnessed the society grow on all forums. The annual meetings were something that we all looked forward to and especially its announcement during the GBM was received with great enthusiasm.

Cell Biology, as it permeates into different fields of biology, has now become truly multidisciplinary. The array of different themes has overridden the limit of participation thus encouraging researchers in various fields to attend. The Society encourages participation from teachers and researchers of universities, colleges, and institutes.

I am delighted to see the second issue of the Society Newsletter in 2023. I see that this issue has focused on providing glimpses of novel, interesting cell biology research done in India and, in most cases, young faculties in the early part of their careers. It is also thrilling to see that article from investigators across the India both from universities and institutes. It would be nice to have more participations from young research students in coming days.

I congratulate the newly elected secretariat of the ISCB for bringing out their first newsletter, and I encourage the members of the society to participate in compiling the next edition by providing their research outputs, thoughts, and ways to take forward the society.

Wishing you all a very happy 2024!!

Madhu G Tapadia

Executive Secretary, ISCB

XLV All India Cell Biology Conference & International Symposium on Biology of Development and Disease: A Brief Report

20-22 January 2023
Organized by
Indian Society of Cell Biology & Department of Zoology, Banaras Hindu University, Varanasi

The "45th All India Cell Biology Conference & International Symposium on Biology of Development and Disease" was organized at the Department of Zoology, Banaras Hindu University, from January 20th to 22nd, 2023, under the auspices of the "Indian Cell Biology Society."

The three-day conference featured national experts from India, the United States, and Europe in key life science thrusts areas such as development and differentiation, cellular biochemistry, disease biology, cell signaling, cancer biology, chromatin biology, and pathogen biology. The conference provided an excellent forum for group leaders, scientists, young investigators, researcher scholars, and students from colleges, universities, and institutes to exchange scientific ideas.

The conference was inaugurated on 20 January 2023 at 9:00 AM in the presence of Anil Kumar Tripathi, Director, Institute of Science, Banaras Hindu University (BHU), Jyotsna Dhawan, President, Indian Society of Cell Biology (ISCB) and other dignitaries at Swatantra Bhavan, BHU. Then, conference talks began with Dhawan's research findings on quiescence muscle stem cells.

Session-I on Cell signaling, chaired by Anil K. Tripathi, started at 10:30 am. The sessions deliberations with Alok Sinha, who gave an excellent talk on a MAPK cascade module working in blue light-mediated Arabidopsis seedling development. The outstanding researcher Thomas Pucadyil gave the second talk, showing recent results in his group's efforts to expand the repertoire of fission proteins and elucidate their mechanism and cellular functions. Then, our first international speaker Kristin White talk about Dichaete, a Sox2 homolog that prevents activation of cell death in multiple developmental contexts. The last talk of this session was a proffered talk by Suresh Yenugu on the Role of Sperm Associated Antigen 11 A in epididymal carcinogenesis.

A total of 262 abstracts were selected for poster sessions. In the post lunch Poster session-I opened, and the enthusiastic budding researchers presented the poster on cell signaling, chromatin biology, disease biology, and development and differentiation topics.

After tea time, Session – II on Chromatin Biology was chaired by Rajiva Raman. Ganesh Nagaraju gave the first talk on finding an early activation of XRCC3 by ATR, which promotes cell survival and genome integrity and unravels new functions of RAD51 paralogs in genome maintenance and tumor suppression. Then, Luisa Di Stefano talked about her group's recent results regarding the role of the histone demethylase LSD1 in Transposable Element silencing in *Drosophila*. Next, Swati Saha talked about *Leishmania donovani* SET1 protein plays a role in mediating the cell's response to an oxidative environment. The fourth speaker was Ullas Kolthur, and his topic was Memory and Architecture of cell signaling: kinetics dictates topology when a century is not enough! Then two proffered talks by Manisha Sachan and Sreenivasulu Kurukuti.

Sathees C. Raghavan delivered the 45th AICBC Prof S P Ray-Chaudhuri's 75th Birthday Endowment Lecture on DNA Breaks to Repair: Insights into Oncogenesis and Cancer Therapy from 6:40-7:40 PM. Jyotsna Dhawan and Bhupendra N Singh chaired the lecture. Further, Beckman Coulter and BD gave technical talks on Advance Technologies from 7:40 to 8:10 PM and also had an Executive Committee Meeting of the Indian Society of Cell Biology conducted.

On day two at 9:00 AM, Session III – Disease Biology Part-I was chaired by BK Thelma. Srikanth Rapole gave the first talk of the session on the Identification and functional characterization of potential targets and biomarkers for Multiple Myeloma. Next, Patrick D'Silva discussed the Role of DJ-1 members in

combating carbonyl stress: Implications in Parkinson's Disease progression. After that, Abhijit De presented his recent work on Imaging Molecules at Work: Inventing Cancer Biology from a Live Cell Context. Then another fantastic talk on cancer was given by Bushra Ateeq on A Tale of Body Patterning Gene: From Diagnostics to Therapeutic Target. Finally, Ritu Trivedi talked about the Epigenetic regulation of microRNA to exert Skeletal Anabolic Effects During Weaning by Suppressing Hdac1.

After a tea break, Session IV – Development and Differentiation began at 11:30 AM, and SC Lakhotia chaired this session. Andreas Bergmann delivered an excellent talk on the Mechanism of Apoptosis-induced Proliferation in *Drosophila.* Then, Surendra Ghaskadbi talked about his recent work on the DNA repair repertoire of hydra. Saikat Chowdhuri talked about Resolving actin filament nucleation mediated by the Arp2/3 complex.

The Poster session-II was from 2:30 to 4:30 on the second day after lunch. It was an excellent poster session with a lot of enthusiasm from budding scientists to interact with national and international delegates at the conference to discuss their research work.

After tea, Anirban Banerjee delivered Dr. Rita Mulherkar Award Lecture on Search for a new habitat: The pneumococcal odyssey. This session's chairpersons were Srikanta Rath and Bhupendra N Singh.

Session V – Biology of Pathogens part- I chaired by Parimal Das. Rajesh Pandey delivered the first talk on Are Co-infections plus Primary pathogen KEY to Differential Disease Severities: ONE HEALTH the Key, and the second talk was by Jagreet Kaur on Transcriptome Analysis Provides Insights into the Mechanisms Underlying Arabidopsis Resistance to Alternaria leaf blight. Then General Body meeting of the Indian Society of Cell Biology was conducted from 6:45 to 7:30 PM. A cultural program was conducted from 7:30 to 8:30 PM, and then Dinner.

On Day 3, the conference's Session VI on Biology of Pathogen Part II began at 9 AM and was chaired by Pramod K Tiwari. Supriya Chakraborty delivered a talk on the Diverse roles of beta-satellites in regulating geminivirus infection.

For the BEST platform presentations 20 Ph.D. students from India and abroad were selected. This was conducted in three parts. Raj Kamal Tripathi chaired the first part and after tea time, Jagat Kumar Roy chaired the second part and after lunchtime, Debasmita Pankaj Alone chaired the third part of the BEST talks. It was an excellent sight to grasp the young researcher's enthusiastic presentation and question and answer session.

Session VII – Disease Biology Part- II was chaired by Racheal AJ. In this session, all the talks were proffered talks. First, a talk was given by Manjusha Dixit on the Noncanonical Function of EEF1A2 in Regulating HIF1A-mediated Breast Cancer Angiogenesis in Normoxia and Hypoxia. Next, Seema Mishra presented her work on Understanding the role of long non-coding RNAs in pan-cancer gene expression regulation and drug sensitivity through Systems Biology approaches. After that, Richa Arya presented her recent finding on Cut is required for growth and induces polyploidy in cortical glial cells of *Drosophila* CNS. Urmi Chatterjee on Obliterating cancer stem cells: an obligation towards reducing the risk of tumor Recurrence. The last scientific talked given by Anand K Singh on RNA quality control in amyotrophic lateral sclerosis (ALS) and polyQ diseases in *Drosophila*. We also had a technical talk from Cactus Communication presented by Dina Mukherjee.

After tea during the Valedictory function, prizes were given to some selected posters and BEST platform presenters. The conference programs end with the announcement that the next AICBC will be held at BARC, Mumbai, in 2024. The conference dispersal happened after dinner.

By: Bama Charan Mondal
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Glimpses of 45th AICBC 2023 at Banaras Hindu University, Varanasi

Inaugural function of AICBC BHU 2023 conference

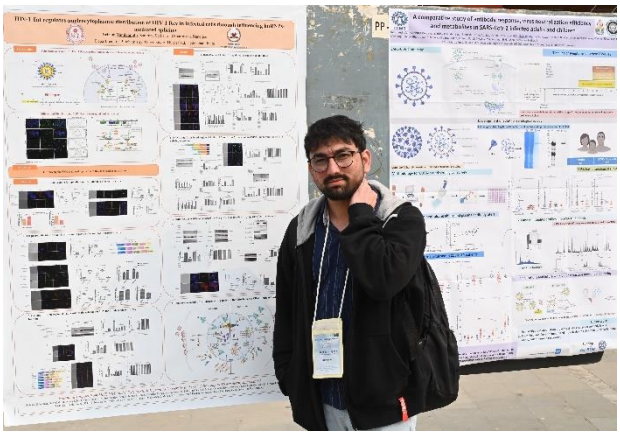
AICBC Newsletter release at 2023 conference

Presidential orientation at AICBC BHU conference 2023

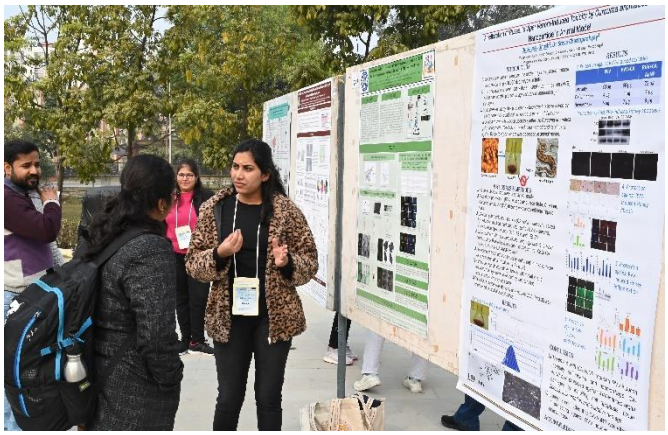
Ongoing session at AICBC BHU conference 2023

Prof. S P Ray-Chaudhuri 75th Birthday Endowment Lecture Award (Year 2022)- Prof. Sathees C. Raghavan

Moments of AICBC BHU conference 2023



Poster sessions at AICBC BHU conference 2023



Poster sessions at AICBC BHU conference 2023

Moments of AICBC BHU conference 2023

Moments of AICBC BHU conference 2023

Student awardees with ISCB executive members

Award Lectures at 45th AICBC 2023 at Banaras Hindu University, Varanasi

The 19th Professor S P Ray-Chaudhuri 75th Birthday Endowment Lecture

The Indian Society of Cell Biology created an endowment fund on the 75th birthday of Professor S P Ray-Chaudhuri and initiated the above lecture series. Prof. S P Ray-Chaudhuri was the first President of the Indian Society of Cell Biology when it formally came into being in 1976. He was doyen among the cytologists of that time in India. His contributions to teaching and research are beautifully summarized by Prof. Rajiva Raman (Sachi Prasad Ray-Chaudhuri: The First President of the ISCB) in the December 2017 issue of Cell Biology Newsletter. Having trained under Prof. H. J. Muller in Edinburgh, he initiated research in the areas of radiation genetics and comparative cytogenetics at Calcutta University. He moved to Varanasi as the Head of the Department of Zoology at Banaras Hindu University. After his retirement, he returned to Calcutta University and continued working till mid-1980s. He breathed his last in the year 1994 at 87 years of age.

Since its inception there has been 19 lectures till date including Dr Obaid Siddiqi (1984), A T Natarajan (1986), H Sharat Chandra (1990), A N Bhisey (2000), S Chattopahyay (2016). The complete list is available on the website of the society,

The 19th Prof. S P Ray-Chaudhuri 75th Birthday Endowment Lecture Award (Year 2022)- Prof. Sathees C. Raghavan, IISc, Bangalore

Prof. Sathees C. Raghavan is currently a full Professor in the Department of Biochemistry, Indian Institute of Science, Bangalore. He is well known for his contributions to the areas of DNA damage and repair, chromosomal instability and cancer, and cancer therapeutics.

Born on 10th May 1970 in Kerala, Prof. Raghavan received his M.Sc degree from Calicut University, Kerala, India, in 1992. He spent his doctoral stint at the Department of Zoology, Banaras Hindu University, India, working on DNA double-strand break repair in mammalian germ cells, followed by postdoctoral studies at the University of Southern California, Los Angeles, USA. After his post-doctoral studies in the area of molecular cancer genetics, he joined the Indian Institute of Science (IISc), Bangalore, as an Assistant Professor in 2006, where he was later got promoted to Associate Professor in 2012 and Professor in 2018.

Professor Raghavan's research group at the Department of Biochemistry, IISc, focuses on deciphering the mechanism of chromosomal translocation in leukemia and lymphoma. Besides, his group also explores the role of the immune system in the genesis of chromosomal abnormalities, DNA double-strand break repair, and its regulation in different cell types and cancer therapeutics. His group was the first to identify a novel NHEJ inhibitor with potential in cancer therapy and genome editing, published in "Cell" (2012; Chem Biol, 2015; FEBS J, 2018, 2022; Oncogene, 2020). The group also identified a BCL2-specific inhibitor, Disarib, with a prospect of being developed as a chemotherapeutic drug (FEBS J, 2015; Biochemical Pharmocol 2016 a,b; Sci Report, 2020, 2021). Some of the other notable work from the team led by Prof. Raghavan include understanding mechanism and regulation of classical nonhomologous end joining (NHEJ) and microhomology mediated end joining (MMEJ) in repair of double-strand break (DSB) in both nuclear

and mitochondrial genome and their contribution to genome maintenance (JBC, 2010; CMLS, 2011, 2018; JMB, 2012; MBoC, 2015, Cell Death Dis, 2015; Carcinogenesis, 2016; DNA Res, 2018; TIGS, 2021; JMB, 2022; FEBS J, 2022; Elife 2022), deciphering the role of DNA structure, and identification and characterization of factors responsible for the generation of chromosomal translocations in various cancers and their role in radioprotection (NAR, 2011, MCB, 2013; BBA, 2018; iScience, 2019; Plos Genetics, 2022), and biochemical characterization of nonstandard functions of RAG complex, the protein responsible for the generation of diversity in the immune system and its regulation, both in lymphoid and nonlymphoid cells (JBC, 2010, 2013; JMB, 2012; FEBS J, 2015; Cell Death Dis, 2017; Cell Reports, 2021). He has published over 170 research articles in internationally peer-reviewed journals and has obtained several Indian and International patents.

Prof. Raghavan has also reviewed scientific articles for various international journals, scientific projects for various national and international funding agencies. He is a member of various national and international committees and editorial board member for scientific journals.

Dr. Raghavan is the recipient of several awards including Shanti Swarup Bhatnagar prize, 2013; Leukemia Research Foundation (USA) 2010; Kobayashi Foundation Award (Japan), 2016, NASI-Reliance Industries Platinum Jubilee Award, 2015, National Bioscience Award, 2012. He is also a "Fellow" of National Academy of Sciences, Allahabad and Indian Academy of Sciences, Bangalore. He is currently an Editor of FEBS Journal, UK.

Prof. Raghavan also holds the additional responsibility as "Chair" of the Central Animal Facility (CAF) at the Indian Institute of Science, Bangalore.

The Society feels privileged to have Prof. Sathees C. Raghavan, as the speaker of the 19th "Prof. S P Ray-Chaudhuri 75th Birthday Endowment Lecture Award" for year 2022 at Banaras Hindu University, Varanasi.

The 4th Professor Rita Mulherkar Lecture Award

Prof. Rita Mulherkar has contributed extensively to the area of cellular and molecular biology of cancer. She worked as a Senior Scientist at the erstwhile Cancer Research Institute, now ACTREC, Tata Memorial Centre until January 2014. She specialized in the area of oncology, working mainly on head and neck cancers using genetic and genomic approaches, and she was also interested in gene therapy. She made the first transgenic mouse in the country with a distinct phenotype and is still used as a model to study hair follicles and skin carcinogenesis. She has also actively contributed to the growth of ISCB, being a member of its executive committee on several occasions and its President from 2009 to 2011. Her students instituted the Professor Rita Mulherkar Lecture Award as a mark of their respect for her and recognizing her immense contributions to the Cellular and Molecular Biology of Cancer. The first lecture was delivered by Dr. Amit Mishra in 2016.

The 4th Prof. Rita Mulherkar Lecture Award (Year 2022)- Dr. Anirban Banerjee, IIT, Mumbai

Dr. Anirban Banerjee is a Professor in the Department of Biosciences and Bioengineering, IIT Bombay. He completed his B. Sc from University of Calcutta and M. Sc from Punjabi University. He received his PhD from NIPER, Mohali and following post-doctoral stint at San Diego State University, San Diego, USA, he joined IIT Bombay in 2012 where he has established his own research group working on host-pathogen interactions. Utilising Streptococcus pneumoniae as a model pathogen, Dr. Banerjee's group have discovered novel mode of host-pathogen interactions which promises to sow the seed of immunotherapy based therapeutic approaches to tackle multidrug resistant bacterial infections. His research has demonstrated how a well-orchestrated symphony of autophagy and ubiquitin-proteasomal pathways constitute sentinel functions of host cells which not only could be boosted for pathogen clearance, but also exploited for inflammation mitigation leading to tissue regeneration. He has established ubiquitination as a novel strategy for pathogen sensing, decoded the generic principles adopted by the host to ubiquitinate phylogenetically diverse microbes, identified the first microbial surface protein as ubiquitin substrate as well as deciphered the detailed mechanism of ubiquitination driven bacterial killing. These unique discoveries promise to significantly advance the paradigm, tipping the balance towards the host during infection which could be exploited for development of novel therapeutic tactics.

Dr. Banerjee's work has been recognized in both national and international forums. His publications are selected as "Featured article" and "Spotlight Article" by leading peer reviewed journals and awarded the "Best Life Science Paper published from India in 2018" by Cell Press and TNQ Technology. He is recipient of several awards which includes Early Research Achiever Award (2017) and Research Publication Award (2021) from IIT Bombay, Fast Grant Award by Ignite Life Science Foundation (2021) and has been selected as a member of the prestigious Guha Research Council (2022).

Apart from being a researcher, Prof. Banerjee has been a successful teacher and has been actively involved in teaching both undergraduate and postgraduate courses at IIT Bombay. He was awarded the Excellence in Teaching Award (2017) from IIT Bombay, which is a testament for his teaching skills.

The Society feels privileged to have Prof. Anirban Banerjee as speaker of the 4th Prof. Rita Mulherkar Lecture Award for year 2022 at the 45th AICBC 2023, Banaras Hindu University, Varanasi. We wish him success in all his future endeavours.

Students Awards for presentation at 45th AICBC 2023 at Banaras Hindu University, Varanasi

Best paper presentation in poster session: Five Awards

Prof. V. C. Shah Award: Tanisha Sharma (Poster No. 07), NCCS, Pune

Attenuation of glycosylated PD-L1 by SCF E3 ligase can modulate the immune checkpoint

Tanisha Sharma and Manas Kumar Santra
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Tumour cells are known to adopt various strategies to evade immune surveillance. They exhibit higher levels of PD-L1, which help them to disarm the tumour cells targeting T-cells. Therefore, various drugs have been approved against PD-L1 for betterment of therapeutic intervention. However, these immuno-therapeutic agents do not provide desirable effects in many cancer patients. Hence, there is an urgent need to shed new light on regulation of PD-L1 to improve therapeutics for effective treatment. Interestingly, PD-L1 undergoes extensive glycosylation in higher grades of cancer, which facilitates its oncogenic activity. However, proteasomal regulation of glycosylated PD-L1 remains elusive. In the present study, we aim to decipher the post-translational regulation of glycosylated PD-L1 in breast cancer. We found that AKT prevents attenuation of glycosylated form of PDL-1 by SCF E3 ligase. We found that SCF E3 ligase facilitates K48-linked polyubiquitination of glycosylated PD-L1 to direct its proteasomal degradation through the 26S proteasome. We believe that deciphering the mechanism involved in glycosylated PD-L1 regulation by SCF E3 ligase can pave the way for various alternative strategies to inhibit its expression and enhance the antitumor immune response.

Prof. B. R. Sheshachar Memorial Award: Dipti Verma (Poster No. 28), BHU, Varanasi

Non-muscle myosin II Zipper positively regulates Notch signaling in *Drosophila melanogaster*

Dipti Verma and Ashim Mukherjee
Department of Molecular and Human Genetics, Institute of Science, Banaras Hindu University, Varanasi 221005

The Notch pathway is a highly versatile, evolutionarily conserved signaling system that is intricately regulated at multiple levels and it influences different aspects of development. In an effort to identify novel components involved in Notch signaling and its regulation, we carried out two independent protein-interaction screens using immunoprecipitation followed by mass spectrometry and yeast two-hybrid system. Both the screens identified non-muscle myosin II Zipper (Zip) as an interacting partner of Notch. Physical interaction between Notch and Zip was further validated by co-immunoprecipitation studies. Immunocytochemical analyses revealed that Notch and Zip co-localise within the same cytoplasmic compartment. Different alleles of zip also showed strong genetic interactions with Notch pathway components. Downregulation of Zip resulted in wing phenotypes that were reminiscent of Notch loss-of-function phenotypes and a perturbed expression of Notch downstream targets, Cut and Dpn. Further, synergistic interaction between these two genes resulted in highly ectopic expression of Cut and Dpn. Activated Notch-induced tumorous phenotype of larval tissues was enhanced by the overexpression of Zip. This synergy resulted in the activation of JNK pathway that culminated in MMP activation and proliferation. Taken together, our results suggest that Zip may play an important role in regulation of Notch signaling.

Dr. Manasi Ram Memorial Award: Namrata Kulkarni (Poster No. 83), IISER, Pune

Circadian Dysfunction in a *Drosophila* model of Amyotrophic Lateral Sclerosis

Vidyadheesh Kelkar, Namrata Kulkarni, Aparna Thulasidharan and Girish Ratnaparkhi
Indian Institute of Science Education and Research, Pune 411008

Behavioural changes like circadian rhythm dysfunction and disruption in sleep-wake cycle are a common feature of most neurodegenerative diseases. Whether these behavioural changes are a cause or an effect of neurodegeneration and their underlying molecular mechanisms remain to be identified. Amyotrophic Lateral Sclerosis (ALS) which causes progressive loss of motor neurons is one such human neurodegenerative disease with more than 30 causative gene loci being identified.

Vesicle-associated membrane protein associated protein B (VAPB); an endoplasmic reticulum (ER) membrane protein is coded by the 8th ALS locus identified in humans. We have developed a CRISPR/Cas9-engineered *Drosophila* model of ALS with a proline to serine mutation in the fly homolog, dVAP33A. Our *Drosophila* model phenocopies the disease showing reduced lifespan and age-related motor deficits in the mutant flies. Additionally, our study shows that the mutant flies have a disrupted sleep-wake cycle and become arrhythmic with age. Introduction of a single copy of wildtype VAP is able to rescue motor defects, sleep-wake cycle as well as circadian rhythm. We plan to utilize this model to further understand the molecular mechanisms connecting neurodegeneration and behaviour.

Indian Society of Cell Biology (ISCB) Award: Diksha Pathak (Poster No. 27), RCB, Faridabad

Delineating the Role of an RNA-binding Protein in Tunneling Nanotube Biology

Diksha Pathak and Sivaram V S Mylavarapu
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Tunneling nanotubes (TNTs) are novel cellular structures that mediate intercellular communication by making direct connections between distantly located cells. These tubes are tunnel-like, supported by an F-actin cytoskeleton and transport a variety of cargoes between the connected cells. Despite their demonstrated importance in health and disease, the mechanistic understanding of the biogenesis, growth, maintenance, and function of TNTs is rudimentary. The protein MSec has been shown to be essential for TNT formation in multiple cell types. The cellular interactome of MSec determined by our lab reveals the presence of RNA binding proteins (RBPs). Our current efforts are focused on understanding the role of the RBP poly (rC) binding protein 2 (PCBP2) in TNT formation. Here, using immunoprecipitation and confocal microscopy studies, we report that PCBP2 interacts with MSec and both proteins colocalize in TNTs. PCBP2 decorates TNTs and the cell cortex along with its reported population in nucleus. PCBP2 knockdown significantly reduces TNT formation, whereas its overexpression induces TNT formation in mammalian cells. These observations suggest that the RBPs bring some additional activity that is required for TNT biogenesis. Our future experiments aim to generate important molecular mechanistic knowledge regarding the importance of RBPs in TNT biogenesis.

Indian Society of Cell Biology (ISCB) Award: Pratham Phadte (Poster No. 201), ACTREC, Mumbai

Autophagy regulates chemoresistance by controlling the turnover of ID1 protein in Ovarian Cancer Stem Cells

Pratham Phadte[1,2], Megha Mehrotra[1,2], Aniketh Bishnu[3], Pranay Dey[1,2], Bharat Rekhi[4], Abhijit De[1,2] and Pritha Ray[1,2]
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The mechanisms of autophagy regulated chemoresistance in ovarian cancer stem cells (CSCs) remain unclear. Herein, we report the autophagy signature of cancer associated spheroids (enriched with CSC population) isolated from malignant ascites of treatment naïve and relapsed epithelial ovarian cancer (EOC) patients. A high autophagy flux and high SLC31A1 (platinum influx transporter) expression was observed in the spheroids of those relapsed patients who responded again to platinum treatment after relapse. Further, using cellular models of chemoresistance, we found that CSCs have a high basal autophagy and further promotion ofautophagy led to increased differentiation of CSC into non-CSC population and SLC31A1 expression. Mechanistically, we observed that induction of autophagy led to degradation of Inhibitor of Differentiation 1 (ID1) protein that keeps CSCs in an undifferentiated state. In-silico analysis of ID1 predicted E family of proteins (TCF3, 4 and 12) as its binding partners. Enhanced TCF12 binding to SLC31A1 promoter was associated with increased SLC31A1 expression after forced degradation of ID1. Currently, the role of ID1-TCF12 axis in CSC differentiation and chemoresistance is being investigated.

Indian Society of Cell Biology (ISCB) Award: Sriram Yandrapalli (Poster No. 245), Hyderabad

HIV-1 Tat regulates nucleocytoplasmic distribution of HIV-1 Rev in infected cells through influencing hnRNPs-mediated splicing

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Multiple RNA splicing sites in the HIV genomic RNA permit alternate splicing to transcribe various regulatory, accessory, and structural proteins of the virus. We earlier reported that unlike in CD4+T-cells, HIV-1 Rev primarily remained in the nucleus of an infected astrocyte, which correlated with lower viral-RNA nuclear export and virions. Since Rev-nuclear export signal (Rev-NES) is exposed only when Rev-nuclear localization signal (Rev-NLS) is masked by binding of Rev-Responsive-Element (RRE) containing viral-RNA (RRE-RNA), we investigated if the availability of RRE-RNA in astrocytes regulated Rev nucleocytoplasmic trafficking. We observed, that compared to CD4+T-cells, the RRE-RNAs were low in astrocytes, which correlated with the low abundance of HIV-1 Tat in the nucleus. We deciphered that Tat regulated Rev distribution in infected cells by influencing hnRNPs/SR splicing regulatory factors that determined the levels of RRE-containing unspliced/partially spliced RNA, and completely spliced-RRE-deficient RNA. Further, Tat regulated the expression of hnRNPs/SR, thereby impacting viral RNA splicing, with Tat's Cys/Pro-rich region being crucial for the same. We propose that Tat participates in a feedback mechanism that maintains an adequate ratio of completely-spliced and unspliced-viral RNA, influencing Rev trafficking and virion production. Tat nucleocytoplasmic distribution may have implications for HIV transcription and latency in reservoir cells.

Indian Society of Cell Biology (ISCB) Award: Akancha Mishra (Poster No. 260), CDRI, Lucknow

Autophagy proteins are essential and druggable for maintaining malaria parasite cellular homeostasis and organelle biogenesis

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Plasmodium parasites have a complex life cycle that transitions between mosquito and mammalian host and undergoes continuous cellular remodeling to adapt to various drastic environments. Following hepatocyte invasion, the parasite discards superfluous organelles from hepatocytes for intracellular replication. Previous studies have shown that *Plasmodium berghei* ATG8 localizes to the membranes of the apicoplast and cytoplasmic vesicles. With the limited repertoire of autophagy-related genes found in the apicomplexan parasite, molecules and cellular mechanisms that regulate this process is poorly understood. Here, we show that *P. berghei* autophagy proteins are indispensable for the clearance of micronemes, organelle biogenesis, and the development of hepatic stage parasites. We disrupted Atg4 and OTU-like cysteine protease in *P. berghei* blood stages directly, however, after failing to disrupt Atg7, a yeast-based Flp/FRT conditional mutagenesis system was employed. Atg7 conditional knockout (Atg7 cKO) sporozoites failed to initiate the blood-stage infection in mice and Atg4 and OTU showed delayed patency. Through *in vitro* and *in vivo* analysis, we found that mutant sporozoites invade and develop into early liver stages normally, but showed severely hampered nuclear division and failed to mature into hepatic merozoites. We also demonstrate that micronemes clearance and organelle biogenesis was severely impaired in Atg7 cKO liver stages. We also engineered a transgenic *P. berghei* strain expressing PbAtg7-mCherry in the cytosol of blood and the liver stage forms of the parasite, supporting the potential role it plays in conjugating Atg8 to the membranes. Further, we identified the compounds from the Maybridge library with a high docking score against *Pf*Atg7 and show that these compounds inhibit apicoplast biogenesis and parasite growth. Overall, this study establishes the essential functions of autophagy in *Plasmodium* blood and liver stages and highlights the potential of using Atg7 as a drug target against malaria.

Organizer's Award by Beckman Coulter: Shatarupa Bhattacharya (Poster No. 236), IIT-Kharagpur

HLA typing influencing the clinical outcome of Apicomplexan infections in HbE/B-thalassemic patients

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Intracellular single-celled pathogens belonging to the phylum Apicomplexa are amongst the most prevalent and disease-causing, worldwide. Apicomplexan parasites like *Plasmodium* and *Toxoplasma* play a critical role in shaping the pathophysiology of various haematological diseases including Sickle Cell Anaemia and Thalassemia. Thalassemic patients from sub-Saharan region have been reported to be protected from malaria, and are susceptible towards toxoplasmosis mostly due to their dependence on repeated blood transfusion. In the contrary we have identified 3 key Human Leukocyte Antigens (HLA A*33, HLA-C*07, HLA-DQB1*02) which play a pivotal protective-role in regulating the clinical outcome towards *Plasmodium* and *Toxoplasma* susceptibility in HbE/β-thalassemic co-hort of Eastern India with high co-incidence of malaria. Next-Generation-Sequencing for HLA-A, B, C, DRB and DQB loci in 71 HbE/β-thalassemic patients, combined with qRT-PCT based High-Resolution-Melting–Curve-Analysis revealed significant presence of these protective-alleles in the cohort as compared to healthy controls. Interestingly, a significant co-relation was also observed between the serum ferritin level and the incidence of parasitic infections. The Indian sub-continent being a hub for HbE/β-thalassemia, as well as protozoan infections, this study will provide key insights into how genetic constitution might influence clinical heterogeneity towards prevalent protozoan infections and impact selective fitness of HbE/β-thalassemia mutations in the community.

Best paper presentation in oral session: Four Awards

Prof. S. R. V. Rao Award: Shruti Apte (Abst. No. 12), IIT, Mumbai

Ubiquitination of bacterial surface proteins aids in pathogen clearance

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Ubiquitination, a key cellular homeostatic process, plays a pivotal role in the surveillance of intracellular milieu and elimination of a variety of pathogens. However, the detailed mechanism for bacterial ubiquitination, including the identity of a proteinaceous substrate on pathogen remains elusive. Here, we identify bacterial ubiquitination substrates BgaA and RlpA, surface-exposed proteins present on phylogenetically diverse bacteria, Streptococcus pneumoniae (SPN) and Salmonella enterica serovar Typhimurium (STm), respectively. We reveal a strategy utilized by the host to recognize such targets based on sensing of degron-like motifs. Early death of mice when infected with degron deleted strains as compared to WT strain of bacteria strengthens our observation. Such consensus degron-like motifs resemble intra-cytosolic PAMPs (Pathogen associated molecular patterns) to aid in the recognition of diverse microbes. Remarkably, by applying this approach, we could remodel and stimulate non-ubiquitin targets to be identified by host ligases. We also showcase an anti-microbial role of SCFFBXW7 complex supported by a regulatory host kinase, GSK3β in pathogenic elimination for the first time. This could provide a molecular explanation to enhanced risk of infections in CLL (Chronic Lymphocytic Leukaemia) patients, bearing mutations in FBXW7 component of SCF complex. In conclusion, our study suggests a universal set of rules for recognizing bacterial ubiquitination substrates to conserve cellular resources and boost anti-microbial immunity.

Prof. A. S. Mukherjee Memorial Award: Jayasree P J (Abst. No. 4), BITS-Pilani, Hyderabad

Role of m6A RNA methylation regulated miRNAs in oral cancer

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OSCC is the most common malignant tumor of the oral cavity. A multitude of gene regulatory processes impact OSCC progression. One such process is reversible m6A RNA methylation, which influences a plethora of biological processes, by modulating various facets of cellular RNA metabolism like splicing, stability, translation, and biogenesis of ncRNAs i.e. miRNAs. The m6A regulators (m6A

writer, eraser, and reader proteins) have been reported to be dysregulated in various cancer types. Mechanistically, m6A writer METTL3 has been reported to modulate miRNA biogenesis by recruiting DGCR8 to the primary miRNA and facilitating its processing. Studies exploring the role of m6A regulated miRNAs in the context of oral cancer are lacking. We examined the expression of various m6A regulators in OSCC cell lines and found it to be significantly deregulated in most of the OSCC cell lines. Interestingly, METTL3 is significantly upregulated OSCC cell lines and siRNA mediated knockdown of METTL3 severely reduced the cancer properties. To interrogate the role of m6A regulated miRNAs in OSCC, we carried out miRNA sequencing in OSCC cells depleted of METTL3 and found 85 differentially expressed miRNAs. Further validation and characterization of these candidate miRNAs are in progress and will provide novel insights into OSCC pathophysiology.

Indian Society of Cell Biology (ISCB) Award: Saurabh Rai (Abst. No. 22), BHU, Varanasi

An insight into the Immunomodulatory aspects of Hsc70-4 using Drosophila melanogaster as a model system.

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Immunity is the balanced state in which any organism has an adequate defense to combat any unwanted invasion of foreign antigen while also having passable tolerance to not mount any response against their own molecules. It is mediated in Drosophila by the humoral and cellular components of the innate immune response. Chaperones are one of several molecules that are involved in the regulation of this process. While their role in immune response regulation is well understood, several uncharted parts of the immune regulation pathway have yet to be explored. One of these segments is the regulation of NF-κB activation, which is critical for the execution of the humoral immune response. As a result, our current research has concentrated on the role of hsc70-4 in the regulation of NF-κB.

We found that hsc70-4 regulates NF-κB activation and thus affects the level of innate immune response in both neuronal and non-neuronal tissues in Drosophila. Since the immune response is hyperactivated in neurodegenerative diseases like Alzheimer's, Parkinson's, Huntington's, and many other polyglutamine (PolyQ) disorders, we investigated the effect of Heat Shock Cognate 70-4 (Hsc70-4) on transgenic flies carrying a stretch of 127 glutamine repeats was expressed in compound eyes to obtain Huntington-like neuropathy in the neural retina. Its downregulation resulted in improved ommatidial arrangement and eye color, implying that hyperactivated immune function was restored. The interaction of Hsc70-4 with NF-κB with the assistance of polyglutamine aggregates may cause immune dysregulation. In contrast to hsc70, neither downregulation nor overexpression of hsp70 improves the circumstances for PolyQ, despite the fact that stress-inducible Heat Shock Protein 70 is increased in backgrounds where PolyQ is overexpressed. However, while downregulating hsp70 results in a significant increase in Relish under normal conditions, no such effect has been observed when PolyQ is overexpressed. Thus, we show that PolyQ hyperactivates the immune response in a way that is unique to Hsc70-4. So, a detailed investigation into the regulation of the NF-B pathway by Hsc70-4 will be presented.

Organizer's Award by Beckman Coulter: Navneesh Yadav (Abst. No. 1), DUSC

Deletion induced splicing in RIC3 drives nicotinic acetylcholine receptor regulation with implications for endoplasmic reticulum stress in human astrocytes.

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Nicotinic acetylcholine receptor (nAChR) dysregulation in astrocytes is reported in neurodegenerative disorders and their modulation through agonists confers protection from stress but regulation of chaperones is unclear. Resistance to inhibitors of cholinesterase 3 (RIC3), a potential chaperone of nAChRs is poorly studied in humans. We characterized RIC3 in astrocytes derived from

isogenic wild-type and Cas9 edited 'del' hiPSC line harboring a 25bp homozygous deletion in exon2. Altered RIC3 transcript ratio due to deletion induced splicing with unexpected increased α7nAChR expression were observed in 'del' astrocytes. Transcriptome analysis showed higher expression of neurotransmitter/G-protein coupled receptors mediated by cAMP/calcium/calmodulin-dependent kinase signaling. Functional implications examined using tunicamycin induced ER stress in wild-type astrocyte showed cell cycle arrest, RIC3 upregulation, reduction in α7nAChR but increased α4nAChR membrane expression. Conversely, tunicamycin treated 'del' astrocytes showed comparatively higher α4nAChR membrane expression/upsurged cAMP signaling. Furthermore, reduced expression of stress markers CHOP, phospho-PERK and lowered XBP1 splicing, validated by proteome-based pathway analysis indicated lowered disease severity. Findings indicate a complex RNA regulatory mechanism via exonic deletion induced splicing and RIC-3 as a disordered protein having contrasting effects on co-expressed nAChR subtypes under basal/stress conditions. Cellular rescue mechanism through deletion induced exon skipping encourages ASO based therapies for tauopathies.

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DBT: Fostering Innovation and Nurturing Talent

Dr. Niloo Srivastava
Department of Biotechnology, New Delhi, Government of India

The Department of Biotechnology (DBT) is a Department under the Ministry of Science and Technology that empowers and enables biotech innovation & nurturing future leaders in the field, creating a workforce that is ready to adopt, develop & deploy the new and emerging technologies for the welfare of the society.

The Department, through its various schemes and programmes, continues to promote skill upgradation, skilling for the future, and building a solutions-driven approach to address national problems. The India BioEconomy Report 2023 provides an in-depth analysis of the economic impact of biotechnological advancements across diverse sectors where the Department along with its Public Sector Undertakings (PSUs) and 14 autonomous institutions across the country had played a significant role. Recently, DBT has amalgamated its 14 autonomous institutions into a single unified entity known as the Biotechnology Research and Innovation Council (BRIC). This is to enhance foster research synergies and outcomes of the institutes and implement new education programs in line with the National Education Policy (NEP), and improve human resource structures.

In addition to supporting Junior/Senior research fellowships and research associateships for UG/PG students, the Department also has remarkable opportunities in the form of fellowships available for early and mid-career researchers/investigators. These are detailed below:

Har-Gobind Khorana Innovative Young Biotechnologist Fellowship (IYBF): This is an attractive research fellowship to identify and nurture outstanding young scientists with innovative ideas who are desirous of pursuing research in the frontier areas of Biotechnology. The applicants must be below the age of 35 and desire to pursue research in biotechnology or any other biotechnology-related field. The research work can be carried out at any recognized research institution/university in India having adequate facilities.

The applicant should be an Indian citizen with excellent academic credentials and track record, high impact peer reviewed publications or should have developed technologies, Indian and/or international patents, with proposed research work to be of very high scientific quality with innovativeness. Nominations for the applicants need to be forwarded through Head/Executive authority of the institutes/scientific organizations/ departments/approved scientific academies or societies. Nominations from individuals sponsoring their own names are not considered. The applicant must have a PhD in any branch of Life Sciences, Computational Sciences (applied to Biotechnology, Medicine or Biological Sciences), Veterinary Sciences, Pharmaceutical Sciences or Agricultural Sciences. Master degree holders in Medicine or Dentistry (MD/MS/MDS or IMC designated equivalent) and Engineering/Technology (M.Tech or AICTE designated equivalent) are also eligible to apply.

MK Bhan-Young Researcher Fellowship Program (MK Bhan-YRFP): The Department has instituted M K Bhan–Young Researcher Fellowship Programme in the area of Biotechnology / Biological Sciences with an aim to encourage young bright researchers to continue their research in the country after PhD. It offers an independent research grant along with

fellowship to young Post-Doctoral Fellows, so as to enable them to emerge as independent researchers and pursue research on issues of national priorities. Like a regular faculty, MK Bhan-Young fellows will be eligible for regular research grant through extra mural and other schemes of various S&T agencies of the Govt. of India, provided that Co-PI is a regular faculty. The fellowship is awarded for 3 years for research work to be carried out at DBT-autonomous institutions only.

Ramalingaswami Re-entry Fellowship (RLS Fellowship): The Department of Biotechnology instituted "Ramalingaswami Re- entry Fellowship" for Indian Nationals below 45 years of age who are working overseas in various fields of biotechnology and life sciences and are interested in taking up scientific research positions in India. Ramalingaswami Re-entry Fellows are able to work in any of the scientific institutions/universities in the country. The aim of the programme is to encourage scientists of Indian nationality working outside the country and are looking to return to various institutes in India to pursue mainstream research.

DBT- Tata Innovation Fellowship: Tata Innovation Fellowship is provided to recognize and reward scientists with excellent track record and have made significant scientific contributions in life sciences and biotechnology. The major emphasis is on innovation and translational research with a potential towards commercialization.

The scheme aims at rewarding interdisciplinary work where major emphasis is on innovation and translational research with a potential towards commercialization. The applicant should possess a PhD degree in Life Sciences, Agriculture, Veterinary Science or a Master's degree in Medical Sciences, Engineering or an equivalent degree in Biotechnology / related areas and must have a regular permanent position in a university/ institute/ organization with active involvement in research and development having outstanding contribution and publication in the specific area and must be below 55 years of age. Also, the applicant should have spent at least 5 years in India before applying for the fellowship. The duration of the fellowship is for 3 yrs, extendable further by 2 yrs on a fresh appraisal.

DBT/Wellcome Trust India Alliance (India Alliance), established in the year 2008 as a charitable trust, funded by the Department of Biotechnology (Government of India) and Wellcome Trust (UK) is advancing India's capacity for conducting ground-breaking research to build excellence in Indian science, establish a globally-recognized research ecosystem, and empower researchers to drive policy and solve critical health problems for India.
India Alliance primarily offers three types of Fellowships based on career levels of applicants – Early Career, Intermediate and Senior Fellowships. These Fellowships are offered under both, the Basic Science stream and the Clinical & Public Health stream. The focus of the Fellowship programmes is on setting the funded researchers on a leadership track through a continuous system of engagement and mentoring. Generous and flexible research funds, support for international mobility, and an efficient and transparent application process are among IA Fellowship hallmarks. Moreover, the fellowships are not restricted by age or nationality of the applicant. However, the research must be done at a not-for-profit institution in India. The Fellowship period is for five years. India Alliance has a pan-India reach through its programs, with Fellows present at 133 organizations across 47 cities.

DBT-Biotechnology Career Advancement and Re-orientation Programme (BioCARe) This fellowship is provided to promote the participation of women scientists in technology and innovation or bring them back into the mainstream of research. The support is for 3 years and extendable by two more years. Areas supported are Medicine / Life sciences / Biotechnology or allied domain There are two categories:

Research Grant Opportunity (RGO) Women scientists with M.D./M.Tech./PhD./or equivalent degree in any branch of Life Sciences and post PhD. experience of more than 2 years can apply.

Early Career Scientists (ECS) Women Scientists having experience less than two years post PhD. are eligible. Women applicants who are employed, unemployed, or are desirous of coming back after a break or for whom it is the first grant will be given preferences.

References

1. https://dbtindia.gov.in/sites/default/files/Revised%20Guidelines%20of%20IYBF%20Program.pdf
2. https://dbtindia.gov.in/sites/default/files/Advertisement%2C%20Application%20proforma%20and%20Guidelines%20for%20MKB-YRFP-2023-24.pdf
3. https://rcb.res.in/RRF/
4. https://www.indiascienceandtechnology.gov.in/nurturing-minds/scholarships/faculties-scientists/tata-innovation-fellowships
5. https://www.indiaalliance.org/applicants/fellowships-at-a-glance
6. https://rcb.res.in/DBTBioCARe/
7. https://birac.nic.in/webcontent/India_BioEconomy_Report_2023.pdf

Whispers of Life

A Cell's Odyssey Through Cancer's Veil

Amrita Mondal

Once upon a time, a cell began its journey,
Simple and normal, with no cause for worry.
It divided and grew, just as it should,
A building block of life, doing what it could.

In ancient oceans, life took its start,
Tiny beings emerged, playing their part.
Simple cells, the pioneers of life's creation,
Where it all began, a cellular foundation.

Yet whispers of change stirred deep within,
A mutation emerged, a tale to begin.
From a normal cell to a rebellious flight,
It turned rogue, lost in a relentless fight.

Cancer's shadow loomed, spreading its gloom,
Unchecked growth, like a silent monsoon.
Invading tissues, disrupting life's chore,
A once-harmonious cell, now seeking more.

From a cell's genesis to cancer's sway,
A journey through life, a turbulent display.
Disrupting the body, causing strife,
A once-normal cell now in a chaotic life.

In the body's battle, it tried to defend,
But cancer's force seemed to have no end.
Tissues ravaged, the body grew frail,
The cancerous cell, an unyielding tale.

Till eventually, worn out and tired,
The body gave in, its strength expired.
The cancer's journey met its last bend,
As life bid adieu, reaching its end.

Value each moment, cherish each day,
For life's a fleeting beauty, here then away.
In the saga of existence, a truth so clear,
Value life's balance, hold it dear.

Origin and Evolution of Academic Societies and Groups: A Tale of Phase Separation

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As a student in the 1960s, I learned Cytology as the domain name for the study of cells through a microscope. Studies that examined chromosomes in some genetic contexts were grouped as Cytogenetics while Cell Physiology included physiological/biochemical studies at cellular levels. During the 1960s, Cytology, Cytogenetics, Cell Physiology and related discipline names were replaced by all-encompassing Cell Biology as the common domain for all cell structure and function studies. As I recall my first encounter with Cell Biology at the 3rd Cell Biology Conference held at the Zoology Department of Delhi University (see https://iscb.co.in/history/), several years before the Indian Society of Cell Biology was established and the AICB Conference series started, the major sessions in the 1969 meeting were organized around Cell structure and function, Cytogenetics, Ultrastructure, Embryology, Cancer, etc. Around this period, Embryology also metamorphosed into Developmental Biology and a few years later, the Indian Society of Developmental Biology was established.

Over the decades since the 1960s, many other societies/groups have emerged with an emphasis on specialized and limited domains. I have been more closely associated with meetings of the Indian Society of Developmental Biology, Stress Biology, Drosophila community, RNA group, etc. Each of these, and other similar groups, has displayed remarkable expansion, not only in the diversity and numbers of participating researchers but also in the molecular biologists in recent years. Interestingly, like the emergence of cytology and embryology in their new avatars, 'phase separation' also finds its roots in ideas propounded more than 100 years ago. E. B. Wilson's (1899) description of nature of questions asked, and the approaches taken to address them. The past two decades have been especially remarkable in India because of the significant increase in the numbers of younger researchers at the many newly started institutions across the country. The remarkable advances in methodologies and analytical instruments in recent decades have indeed revolutionized all domains of life sciences. Researchers looking at dynamic cells with diverse perspectives and seeking answers to ever more specific questions at finer scales have exploited the technological explosion very well. While attending the various meetings and becoming overwhelmed with the quality of presentations, I have felt that the specific domains with which researchers tend to associate themselves more closely often overlap with other domains as well. As expected from the dynamicity of cells and the living systems that they organize, it is inevitable that a significant overlap exists in the questions that are sought to be addressed and the ways different researchers with varying specializations attempt to peep into the complex lives of cells. Consequently, several of the research works presented at meetings of the specialized groups could be equally relevant in other domains, including the larger cell or developmental biology groups.

The distinct identities of these academic groups with overlaps in their themes and participants, remind me of the phenomenon of 'phase-separation' in cells. Liquid-liquid phase separation (LLPS) has become very exciting for cell and protoplasm as a "mixture of liquids, in the form of a fine emulsion consisting of a continuous substance in which are suspended drops of two general orders of magnitude and of different chemical nature" and his prophecy "It is impossible

to doubt that powers still higher than any at our command would reveal the existence of granules still smaller and that what appears as 'continuous' or 'homogeneous' substance is itself an emulsion beyond the range of vision", and Oparin's idea of coacervation leading to the origin of life, foretold the biological phenomena that physicists recognized later as phase-separation (see Lakhotia, 2019). Physicists describe LLPS as spontaneous de-mixing of two liquid phases which results in higher concentration of certain molecules in one phase but less abundance in the other liquid phase although the interface between the two phase-separated liquid droplets permits diffusion of some, but not all, molecules. In cell's dynamic environment, spontaneous and rapid phase separation is triggered (nucleated) by critical concentrations and/or modifications of certain proteins and their. interactions with other molecules under specific conditions of temperature pH, osmolarity etc.

New academic societies and groups phase out from the larger groups in an analogous manner. As the knowledge and number of researchers in a sub-area expand, smaller intra-group interactions become more frequent. This leads to the emergence (phase-separation) of new 'aggregates' of smaller and more specialized groups of like-minded researchers. As in healthy LLPS, it is necessary for the maintenance of a dynamic homeostasis that the interfaces between the different aggregates do not become impervious to 'diffusion'. If they become 'closed' groups and lose their permeability, they may become 'pathogenic' like the various proteinopathies.

The phase-separation events are essential in a healthy cell's dynamic life. Likewise, the emergence of new academic groups that provide greater intra-group interactions but with permeable interfaces is necessary for academics. It is indeed greatly satisfying to note that over the more than five decades, the Cell Biology group has remained open and permeable. Obviously, lessons from what happens within our cells can make our academic societies stronger and more vibrant.

Discoveries that Shaped Cell Biology: Unraveling Morphological to Molecular Features *in situ*

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The technological innovation that drove cell biology was the development of microscopes by Hans and Zacharias Janssen in 1595. Robert Hooke developed the Compound microscope, and the initial studies in cell biology began in the seventeenth Century with the discovery of cells by Hooke and van Leuwenhoek. In the 1880s, Walther Flemming discovered the presence of chromosomes inside the cell. The detailed structure of the cell, the organelles, and macromolecules was driven by Electron microscopy. The discovery of Phase Contrast microscopy allowed for visualization of the chromatin in the cell.

Further, advancements in cell biology were governed by the discovery of DNA structure by Watson and Crick in 1953 and the Central Dogma of Molecular Biology. The discovery of the Confocal microscope in 1955 and Scanning Electron microscopy (SEM) in 1963 provided a 3D view of the tissues. The discovery of Haematopoetic Stem Cells by Till and McCulloch in 1963, coupled with Flow cytometry in 1965, led to the identification and characterization of the immune cells. The flow cytometry and confocal microscopy fields benefitted from understanding the cell-specific proteins against which antibodies were developed. Further, cloning of GFP-tagged proteins helped localize the proteins inside the cell. To understand the type of mutations in the DNA that impacted cellular function, the mutations were characterized by Sanger sequencing technology established in 1977. The isolation of Embryonic stem cells from mice in 1981 was a breakthrough that led to the establishment of mouse models of human diseases. At the same time, visualization and capturing cellular events got a boost with the discovery of the CCD camera in 1982. The discovery of organelle-specific markers and vital dyes and super-resolution microscopy (1991) aided in studying cellular components in greater detail. Advancements in microscopy have led to single-molecule and single-cell imaging, allowing the study of the variability among apparently identical cells in situ. These studies have already resulted in discoveries about the role of noise in gene expression (Elowitz et al., 2002). The introduction of laser technology to measure forces has allowed the study of fundamental processes in cell biology, such as the nature and magnitude of forces during muscle contraction (Tyska and Warshaw, 2002). The interaction of the molecules can be studied by measuring fluorescence resonance energy transfer between engineered GFP variants, in vivo (Pollok and Heim, 1999). Microscopy along with techniques such as Hematoxylin and Eosin staining, Immunohistochemistry (IHC), Fluorescence in situ hybridization (FISH), RNA in situ hybridization (RISH), and Fluorescence resonance energy transfer (FRET), In cell western and cytotoxicity using cell culture helped in localizing proteins and studying single gene expression studies which evolved to multiplexing overtime but did not assess the changes globally at the level of RNA or protein. The advent of Omics technology (Genomics, Transcriptomics, Proteomics and Metabolomics) has paved the way for analyzing cellular changes and connecting with the altered states of the cells and has led to the identification of altered pathways. In the 21st Century, two critical discoveries have changed how we study cells in the context of diseases: the development of disease-specific iPSCs and the next-generation sequencing technologies. The most used next-generation sequencing technology to understand differences between the cell types or tissue types is to perform RNA-sequencing using Illumina sequencing, which was established as a technology with the discovery of

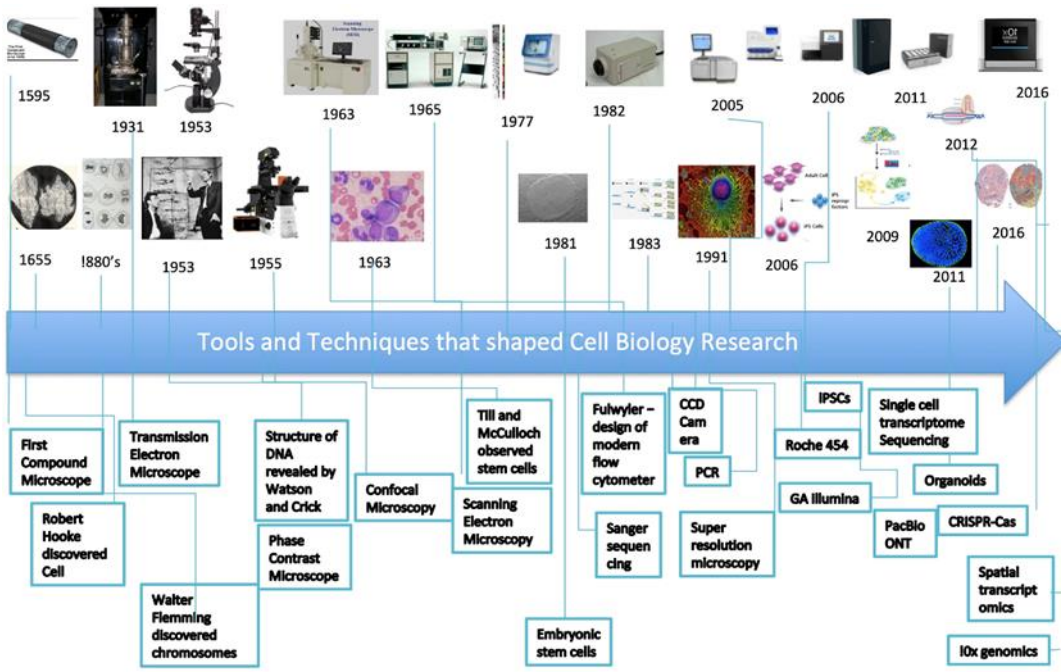

Figure 1. Timeline of Tools and Technologies that shaped Cell Biology Researc

next-generation sequencing technologies and instruments such as 454 from Roche and GAIIx from illumina. Newer machines, Next Seq and Nova Seq from Illumina have already replaced this equipment. The variants/mutations in the genome of the cells/individuals can be sequenced and analyzed. The epigenome can be sequenced using Whole Genome Bisulfite Sequencing (WGBS) and Chromatin-immunoprecipitation and sequencing (ChIP-seq). The third generation of sequencers (PacBio and ONT (Oxford Nanopore Technology)) can sequence repetitive genome regions, analyze variants, and study epigenome simultaneously and identify isoforms of RNA. The difference between the two technologies is the length of the reads/ sequences it generates. Illumina generates short reads (50-500bp length) vs >20 Kb by PacBio and ONT. The limitations of the above sequencing technologies have been overcome by single-cell sequencing and understanding the genome and transcriptome of each cell in a tissue using single-cell transcriptomics (2009). Gene editing technology, the CRISPR-Cas system (2012), has allowed the editing of genes in situ and added to the knowledge base of mutations and their impact on cellular function. To understand how gene expression is guided by the cell-cell communication and arrangement of the cells in space in tissue, a revolutionary technology, spatial transcriptomics using 10X genomics, has been established. The next few years will be dedicated to understanding and regulating gene expression in tissue in healthy and diseased states.

We started with morphological analysis of a single cell, followed by global profiling of a mixture of cells, and back to single cell analysis using microscopy. The merger of microscopy with RNA sequencing in situ is the way forward in advancing our understanding of the contribution of cellular milieu in disease progression and pathogenesis.

We have progressed immensely in cell biology in over 400 years due to the continuous discovery of techniques and technology. It is important to note that if we have to continue to be able to utilize recent techniques and technologies, we have to be ready to adapt, and that would require having fundamental concepts in physics, chemistry, mathematics, and computational methods.

References

1. Rust M. J., Bates M., Zhuang X. Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM) Nat. Methods. 2006;3:793–795.
2. Elowitz M. B., Levine A. J., Siggia E. D., Swain P. S. Stochastic gene expression in a single cell. Science. 2002;297:1183–1186.
3. Tyska M. J., Warshaw D. M. The myosin power stroke. Cell Motil. Cytoskeleton. 2002;51:1–15.
4. Pollok B. A., Heim R. Using GFP in FRET-based applications. Trends Cell Biol. 1999;9:57–60.

A Stack of Secrets: How Base Stacking Shapes Life's Blueprint

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Literally every life form known to mankind carries DNA as its genetic information. This polymeric molecule in each of our cell is about a meter in length, which is comprised of four unique bases – A, T, G, and C. These basic building blocks are ordered in a myriad of combinations to form the blueprint to the vast diversity of life we see around us. Thanks to the ground-breaking work of Rosalind Franklin, James Watson, and Francis Crick that came to light in the year 1953, we learnt that DNA is helical in structure where two anti-parallel strands that are complementary are held together like a twisted ladder (Watson and Crick, 1953). This revelation resulted in a plethora of tools for performing molecular biology experiments and transformed the way scientists carried out routine biological research, leading to unprecedented biomedical breakthroughs over decades. After this seminal work, a wealth of information was generated around structure-function relationship of DNA.

One would wonder what brings about the iconic helical nature of this complex yet universal molecule. While base-pairing helps in programming our DNA, the helicity is a resultant of the molecule trying to achieve energy minimization between coulombic repulsive forces and hydrophobic attractive stacking interactions between neighboring bases. In contrary to the in-depth knowledge regarding base-pairing (A hybridizes to T forming two hydrogen bonds, and G hybridizes to C forming three hydrogen bonds), little is known about the stacking interactions between neighboring nucleotides in the DNA molecule. Stacking interactions are known to arise from the hydrophobic interaction between neighboring bases. These bases carry degenerate **π**-orbitals that can interact with those from the adjacent bases. Such interactions are usually attractive in nature due to the nitrogenous bases. Though the nature of these forces were predicted and established several decades back, the strength of these weak interactions has been hard to measure on individual molecules at the single base level due to the absence of sensitive techniques. Early endeavors managed to unravel the variable effects of purines (A and G) and pyrimidines (T and C) on stacking interactions and have also measured these interactions in bulk assays, albeit under non-equilibrium conditions (Yakovchuk et al., 2006). Researchers have also hinted at the implications of base-stacking interactions on almost all DNA metabolic process, from replication to repair. The knowledge of base-stacking interactions and the stabilizing forces that they bring about is crucial to better understand the DNA molecule.

In our recent study, published in Nature Nanotechnology, we aimed to understand the stacking interactions between individual di-nucleotides such as A|A, A|T, A|C… etc., (a total of 16 combinations) (Banerjee et al., 2023). To explain our experimental approach, we will now introduce you to the tools that our study leveraged – DNA-PAINT super-resolution imaging and DNA Nanotechnology (Figure 1).

"DNA-PAINT super-resolution microscopy relies on transient binding of short single-stranded DNA sequences that carry a fluorophore (imagers) on their complementary sequences that are attached onto the target of interest (docking strand) (Jungmann et al., 2010). These short binding events result in 'blinks' that are detected under a

fluorescence microscope with a camera (Figure 1a). These blinks can then be fitted with the gaussian function to obtain the exact position of the fluorophore with nanometer precision. This technique allows us to breach the diffraction limit of light and obtain super-resolved images of various in situ and *in vitro* structures. The difference is clear when comparing the two images of microtubular network within a cell (Figure 1b)."

"DNA nanotechnology, as the name suggests, uses DNA as building blocks to assemble nanostructures of any predefined, arbitrary shape (Rothemund, P. W. K., 2006). The assembly of such structures are highly programable, thanks to the complementary nature of DNA. One can build any shape they can envision with DNA by designing short single stranded DNA oligomers with specific sequences that would self-assemble into a predetermined shape (Figure 1c). We exploit this technology for its modularity, flexibility in forming different shapes and forming a neat workbench for measuring base-stacking energetics at single-molecule level."

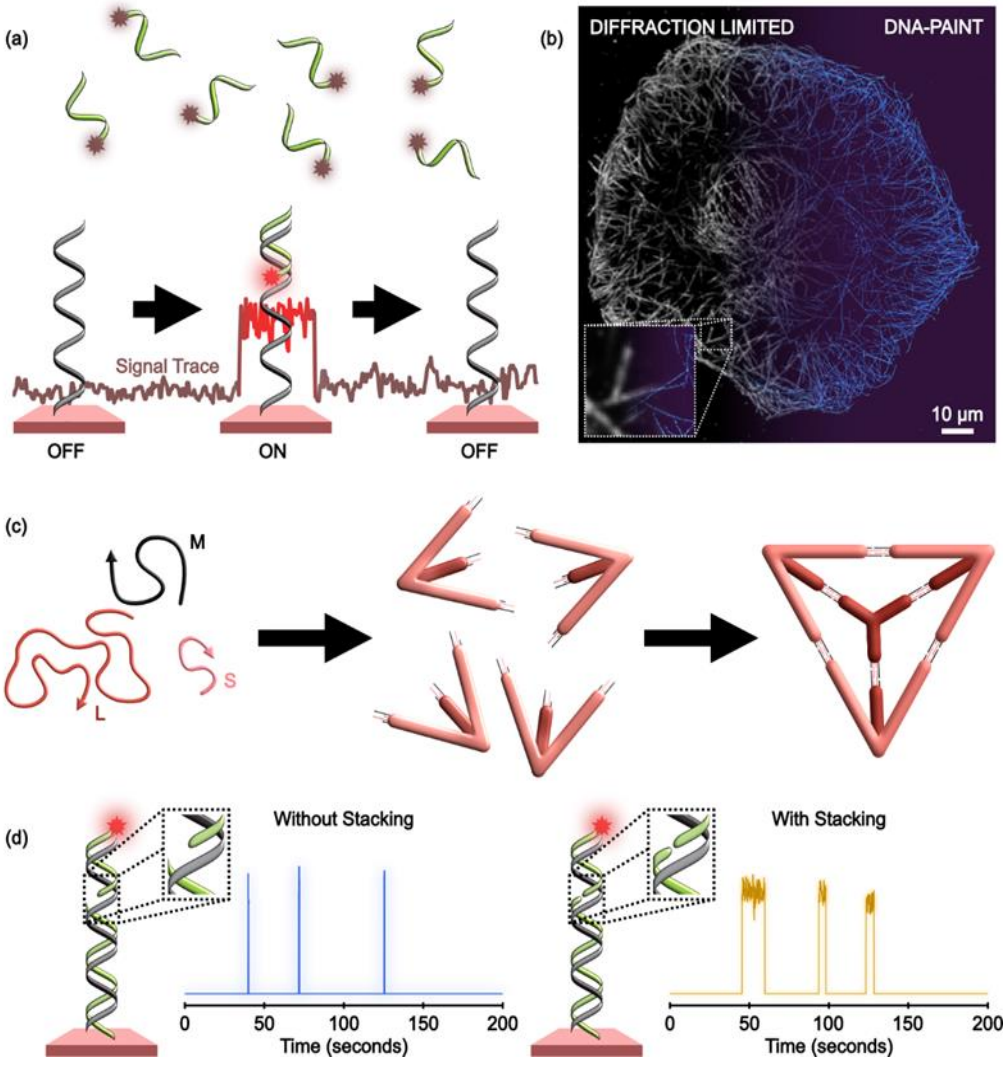

Figure 1: (a) Graphical representation of the principle behind DNA-PAINT. Transient hybridization of the fluorophore-labelled short ssDNA (imagers) with its complementary strand gives rise to a signal that can be fit with Gaussian function to obtain a super-resolved image. (b) Comparison of diffraction limited image (left side) and the super-resolved image of microtubules within a cell using DNA-PAINT (right side). (c) Graphical representation of a simple tetrahedron folding scheme. The programmability of DNA makes the field of DNA nanotechnology highly diverse. (d) The two configurations used in our assay. Left: The configuration without stacking interactions shows short binding events. Right: The configuration with stacking interactions shows much longer binding events.

Our research lies in the crossroads of both these techniques, thus allowing us to peer into the unknown forces of DNA base-stacking. The basic principle of DNA-PAINT super-resolution imaging has been exploited as we can now visually see the hybridization and dissociation of two complementary DNA strands in real time. The target DNA strand present on a DNA origami nanostructure can be easily manipulated to facilitate different interaction with the imager, thanks to the programmability of the structures using DNA nanotechnology. We designed two assay configurations where a single-stranded DNA (docking strand) sticks out from double stranded DNA (Figure 1d). The imager binding on the docking strand leaves either a nick or gap, nick facilitates additional stacking interactions compared to gap. By comparing the binding kinetics of the imager with and without additional di-nucleotide stacking interactions, we measure stabilization energetics that is provided by these weak interactions at the single molecule level. Our assay further allowed us to acquire measurements for five different species in the same imaging field simultaneously as each structure had a different shape, thus making them easily identifiable (Figure 2).

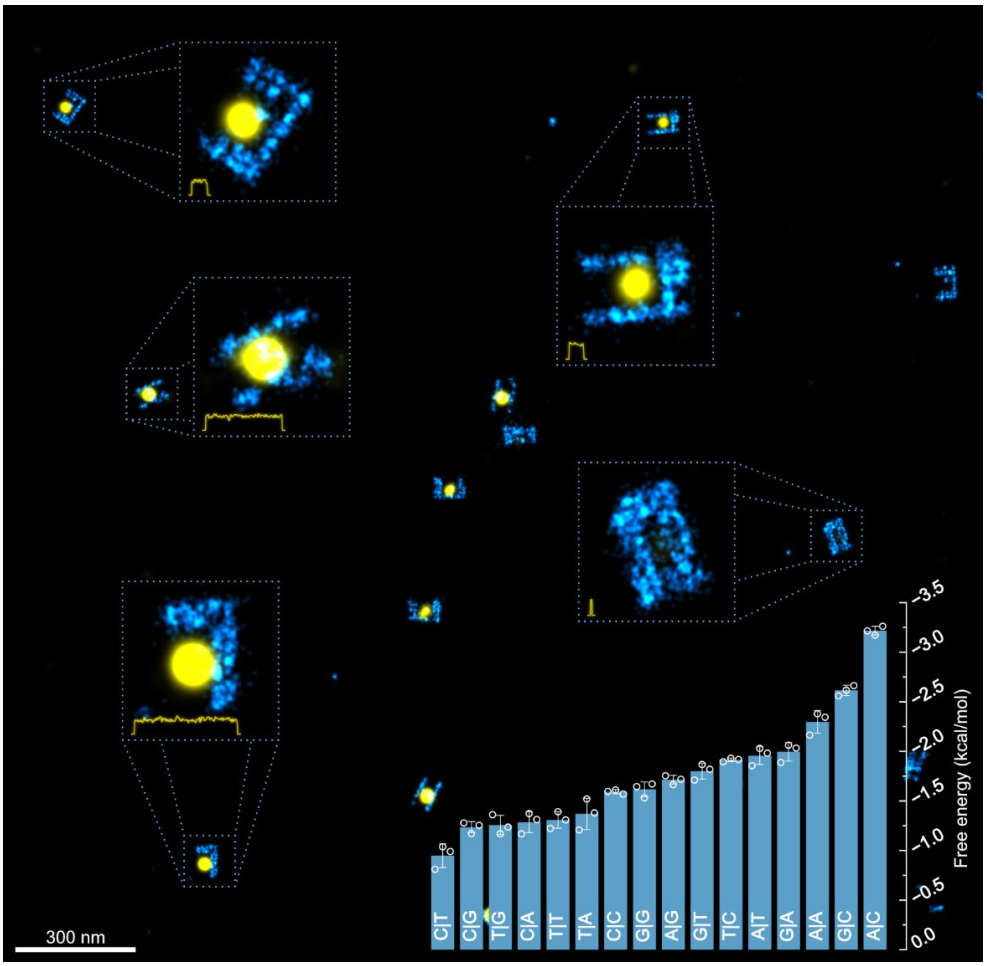

Figure 2: A representative field of view of different DNA origami structures with different stacking interactions. The inset traces on the zoomed origamis clearly show the differences in binding times. The inset graph shows the measured base-stacking values for all 16 di-nucleotide pairs.

Surprisingly we observed great heterogeneity in the di-nucleotide stacking energetics between all 16 possible combinations. For instance, an additional 5'-A|C-3' stacking resulted in a 250-folds stabilization of the DNA duplex. On the other hand, 5'-C|A-3' stack stabilized the DNA duplex by only 10-folds. Also, the reverse complement of 5'-A|C-3', i.e., 5'-G|T-3' stack stabilized the DNA duplex by around 25-folds. Enhancement in binding time due to a single di-nucleotide stacking, resulted from the comparison of imager binding on nick and gap sites, was then plugged into a simple

thermodynamic model to readily convert these values to free energy (Figure 2 inset). We find a weak correlation between molecular area overlap between neighboring nucleotides and stacking energetics.

DNA nanotechnology is becoming increasingly popular for broad range of applications including sensing the local pH and ionic strength, targeted drug delivery, and constructing synthetic nanomachines. Previous reports focusing on building hierarchical nanostructures utilized stacking energies without prior knowledge. We showcased the applicability of known stacking interactions in the efficient self-assembly of DNA tetrahedral nanocages, envisioned to use as a carry a drug molecule to specific location in the body, thus providing the field of DNA nanotechnology with an additional control to develop multimeric DNA devices.

We envision a broader range of applications for our discovery in the future. One can drastically enhance the ligation rates of various DNA fragments during routine cloning procedures by utilizing strongly stacking nucleotides at the nick site. The knowledge on stacking interactions would also be of great significance to understand the repair of certain DNA abnormalities where bases need to be flipped out for their detection and repair.

In conclusion, the combination of DNA nanotechnology and super-resolution imaging techniques has opened a new horizon for understanding the fundamental forces that stabilize DNA structure. By measuring the intricate stacking interactions between individual di-nucleotides, we have provided a comprehensive picture of the molecular forces resulting in the helical structure. This knowledge enhances our basic understanding about the building blocks of life and paves the way for future endeavours in broader fields ranging from molecular cell biology to DNA nanotechnology for enhancing scientific understanding and technological innovation.

References

1. Watson, J. D.; Crick, F. H. C., Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid. Nature 1953, 171 (4356), 737-738.
2. Yakovchuk, P.; Protozanova, E.; Frank-Kamenetskii, M. D., Base-stacking and base-pairing contributions into thermal stability of the DNA double helix. Nucleic Acids Research 2006, 34 (2), 564-574.
3. Banerjee, A.; Anand, M.; Kalita, S.; Ganji, M., Single-molecule analysis of DNA base-stacking energetics using patterned DNA nanostructures. Nature Nanotechnology 2023, 18 (12), 1474-1482.
4. Jungmann, R.; Steinhauer, C.; Scheible, M.; Kuzyk, A.; Tinnefeld, P.; Simmel, F. C., Single-molecule kinetics and super-resolution microscopy by fluorescence imaging of transient binding on DNA origami. Nano Lett 2010, 10 (11), 4756-61.
5. Rothemund, P. W. K., Folding DNA to create nanoscale shapes and patterns. Nature 2006, 440 (7082), 297-302.

Writings on Chromosomes: A Novel Role of Nuclear Hormone Receptors in Mitotic Genome Bookmarking

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Mitosis is a natural cellular process marked by immense structural changes in the nuclear membrane. This is accompanied by the modification and re-arrangement of the chromatin architecture that undergoes enormous condensation, dislodging majority of the cellular proteome. Despite the mitotic cell trespassing in a transcriptionally silent state, it has been found that some transcription factors and chromatin-remodeling proteins remain associated with the mitotic chromatin. The preservation of mitotic accessibility is a crucial function of such bookmarked genome regions for the robust post-mitotic reactivation of genes. 'Mitotic genome-bookmarking' is a natural phenomenon associated with transcription regulation and faithful inheritance of cellular proteome, traits, and phenotype from the progenitor to the progeny cells (Rizvi et al., 2023). This requires the implementation of several converging mechanisms like histone modifications, association of co-regulatory proteins and transcription factors, and promoter methylation. Several studies suggest that bookmarked genes, upon exiting mitosis, are poised for gene reactivation in progeny cells to re-establish their progenitor's proteome (Raccaud M et al., 2019).

The first evidence of mitotic accessibility came in the 1990s when DNase1 hypersensitivity sites were found in the condensed chromatin.

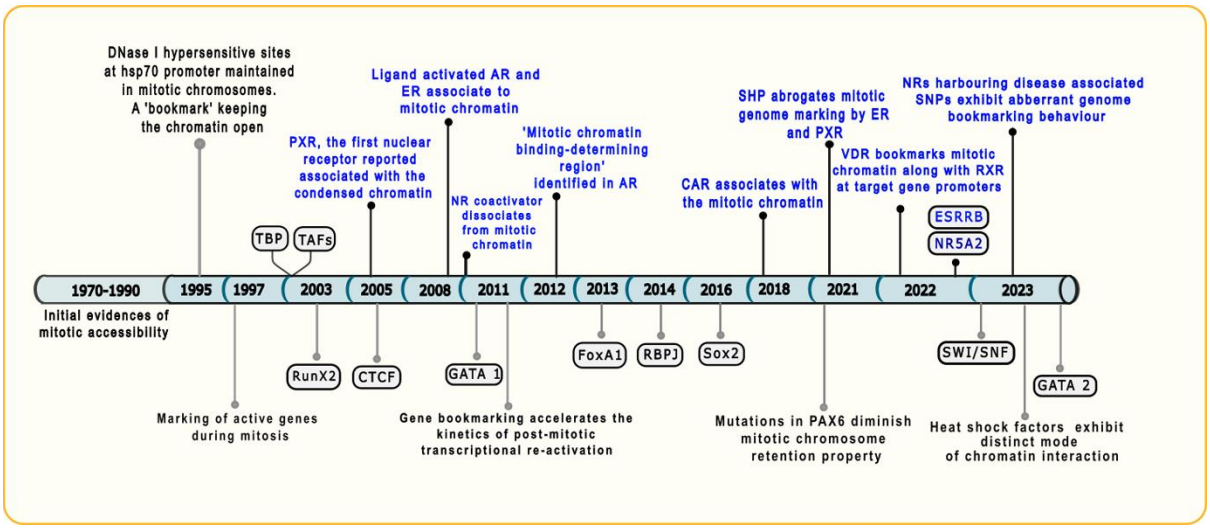

Figure 1. Timeline illustrating key events in the discovery of the genome-bookmarking phenomenon and revelation of a novel role of nuclear receptors as 'bookmarks.' Pioneer transcription-associated factors reported to associate with the mitotic chromatin are shown in black. Key nuclear receptors and their associated events are highlighted in blue colour (Rizvi et al., 2023).

This provided initial clues to the existence of a 'bookmarker' keeping the chromatin open, and subsequently, promoters of active genes were found to possess similar sites. Hereafter, several transcription-related proteins were found to be associated with the mitotic chromatin, like TBP, RUNX2, GATA1, FOXA1, RBPJ, CTCF, etc. (Figure 1). These included factors from the basal transcriptional machinery, chromatin-modifying proteins, insulator proteins that maintain chromatin boundaries and domains, etc. Although the term 'genome-bookmarking' was initially used to describe the nuclease hypersensitivity of gene promoters in mitotic cells, it has been used thereafter to describe the retention of specific transcription factors at target gene loci on mitotic chromosomes, a scenario also observed with nuclear receptors.

Nuclear receptors are ligand-modulated transcription factors that regulate several physiological processes involved in growth and development (Frigo et al., 2021). The macromolecular enrichment of nuclear receptors on mitotic chromosomes was first reported in 2005, constitutively associated with the condensed chromatin. Thereafter, diverse modes of nuclear receptor-chromatin interactions operating during mitosis were unveiled. The ligand-inducible nature of androgen receptor interaction with the mitotic chromatin was the first reported bookmarking phenomenon where hormones appeared to be essential to mediate the act (Figure 2). Moreover, disease-associated nuclear receptor polymorphisms were thereafter shown to exhibit perturbed receptor-chromatin interactions (Kashyap et al., 2023).

Assays measuring site-specific interactions between chromatin and transcription factors during mitosis elaborate their bookmarking function contrary to live-cell imaging of fluorescently tagged TFs that display the overall global decoration of the mitotic chromosomes. The phenomenon appears to be a summation of specific and non-specific interactions. The site-specific interactions of NRs on their target genes through ChIP analysis have already been published for PXR and VDR, supporting their

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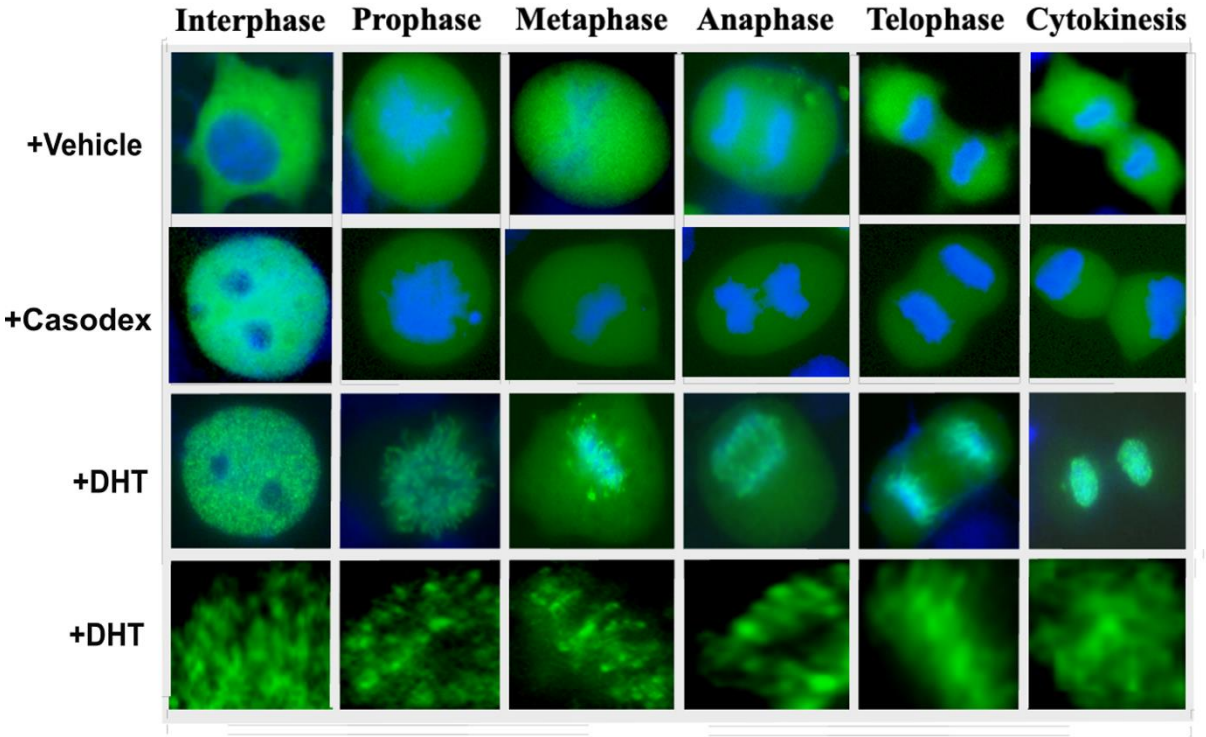

Figure 2: Representation of genome-bookmarking by a nuclear hormone receptor: evidences from live cell imaging. Dihydrotestosterone-activated receptor translocates to the nucleus and promotes receptor association with the condensed mitotic chromatin during all the stages of mitosis. Such a process of retention and transmission was absent in cases where the receptor was bound to a pure anti-androgen, casodex. The last row shows the magnified images of the portions derived from the third row, revealing the retention and transmission of transcriptionally active sites or 'nuclear foci' from the progenitor to the progeny via mitosis. (Rizvi et al., 2023).

bookmarking function. Additionally, transcription factors beyond the NR superfamily have been shown to exhibit similar interactions. For e.g., bookmarking by GATA2 is essential for definitive haematopoiesis, a lineage restriction requisite of haematopoietic cells. Conclusively, the genome-bookmarking function, in part, has been well-established to be associated with fundamental processes like pluripotency, lineage commitment, haematopoiesis, etc., through multi-approach, state-of-the-art studies elaborated in stem cells.

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In conclusion, our laboratory is currently investigating this phenomenon to unravel the missing links between the genome-bookmarking phenomenon with normal cellular acts and perturbed occurrences with diverse diseases. In the near future, breakthrough technologies are anticipated to reveal finer aspects of this phenomenon that can help achieve normalcy in receptor malfunction-led disease outcomes.

References

1. Rizvi S, Chhabra A, Tripathi A, Tyagi RK. Mitotic genome-bookmarking by nuclear hormone receptors: A novel dimension in epigenetic reprogramming and disease assessment. Mol Cell Endocrinol. 2023;578:112069. doi:10.1016/j.mce.2023.112069.
2. Raccaud M, Friman ET, Alber AB, et al. Mitotic chromosome binding predicts transcription factor properties in interphase. Nat Commun. 2019;10(1). doi:10.1038/s41467-019-08417-5.
3. Frigo DE, Bondesson M, Williams C. Nuclear receptors: From molecular mechanisms to therapeutics. Essays Biochem. 2021;65(6):847-856. doi:10.1042/EBC20210020.
4. Kashyap J, Kumari N, Ponnusamy K, Tyagi RK. Hereditary Vitamin D-Resistant Rickets (HVDRR) associated SNP variants of vitamin D receptor exhibit malfunctioning at multiple levels. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms. 2023;1866(1):194891. doi:10.1016/j.bbagrm.2022.194891.

Deciphering the Chromosomal Harmony: Unveiling the Order of Chromosomes in the Nucleus!

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The Human Genome Project marked a significant milestone in the field of genomics, culminating in the publication of the human genome sequence. The project was launched officially in 1990 as a collaborative international effort that used various techniques combining automated DNA sequencing and mapping methods. This involved breaking the genome into smaller, more manageable fragments, sequencing those fragments, and assembling them to reconstruct the genome. A decade later, in 2001, a nearly complete version of the human genome was published providing a reference map for understanding the genetic basis of health and disease, facilitating advancements in fields such as medicine, genetics, and personalized healthcare (Lander et al., 2001; Venter et al 2001).

Unlike the linear reading of text in a book, the genome operates not merely in a sequential manner but is intricately folded in three-dimensional (3D) space. This spatial configuration enables distant genomic elements to interact and influence each other. Recognizing that genome function is no more solely a linear process, we now appreciate the imperative role of comprehending spatial genome organization in gaining a holistic understanding of genomic functionality. Beyond the DNA double helix and nucleosomes, there exists a hierarchy of higher order structures of chromatin. This includes chromosome territories, compartments and Topologically Associating Domains. 'Chromosome territories' are the distinct regions in the nucleus occupied by chromosomes. Each chromosome occupies a defined territory in three-dimensional space with very little intermingling between them. Further, the chromosomal loci are organized into two megabase sized 'compartments': A and B that comprise of active euchromatin and inactive heterochromatin regions respectively. Sub-chromosomally, the chromatin is organized into megabase-sized structures called 'Topologically Associating Domains (TADs)', and the genomic loci within a TAD exhibit a higher frequency of interaction when compared to loci outside of it. TADs are separated from each other by insulated boundaries. The higher order structures play a crucial role in coordinating gene expression within the nucleus (Wit E et al., 2012; Denker et al., 2012).

The field of chromatin organization was reliant on microscopy techniques until the advent of Chromatin Conformation Capture (3C) techniques that coupled proximity ligation and high throughput sequencing. The 3C techniques investigate the physical interactions between distant genomic regions. The chromatin is 'fixed' in its native three-dimensional state by chemically cross-linking the interacting segments. This captures the spatial relationships between different DNA regions that are close together in three-dimensional space. The cross-linked cells are now lysed, chromatin digested by restriction enzymes and ligated to join the fragments. The cross-links are then reversed and the ligated DNA is analyzed using techniques such as quantitative PCR or high-throughput sequencing. This helps identify and quantify the specific interactions between different genomic regions. The evolution of 3C techniques represents a series of methodological advancements including Circular Chromatin Conformation Capture (4C), Copy Chromatin Conformation Capture (5C) and Hi-C. These chromatin conformation capture techniques collectively contribute to our understanding of how the spatial organization of chromatin influences

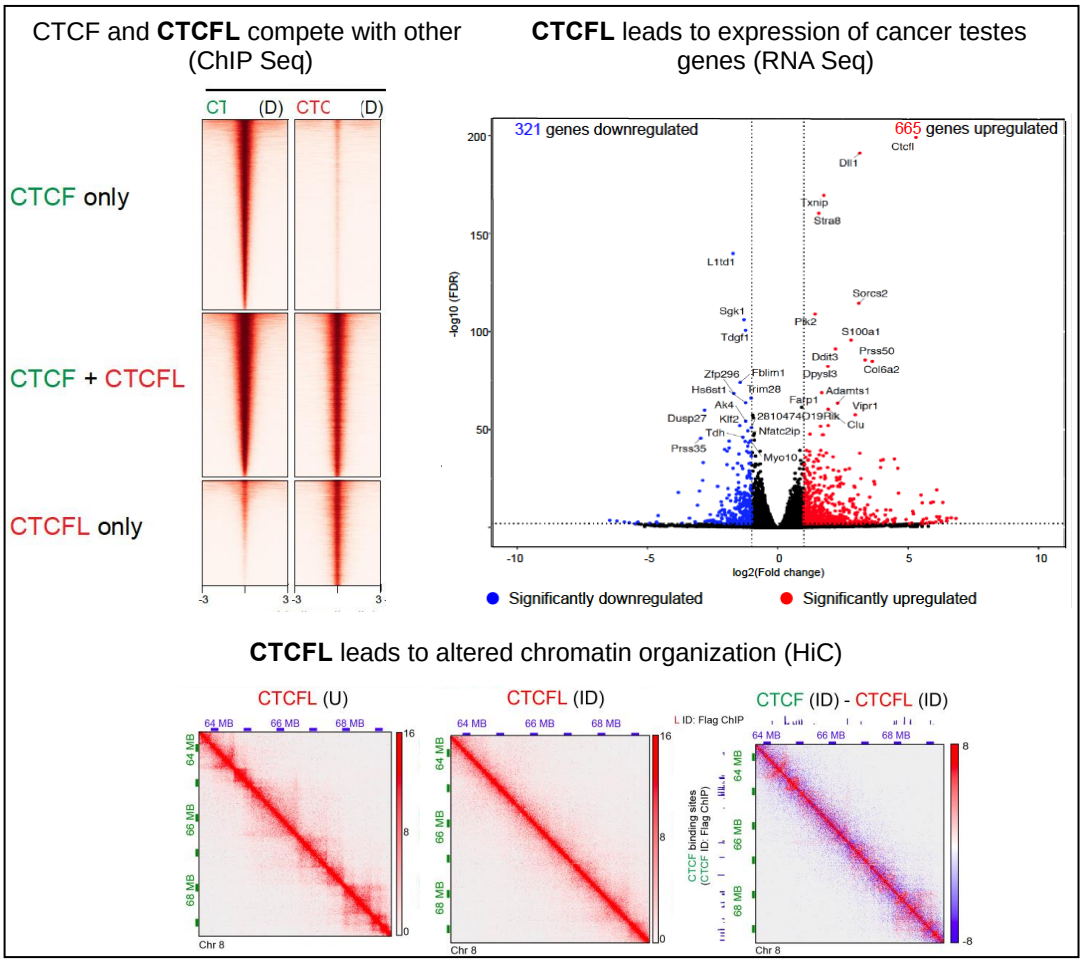

Figure 1: The panels show that on expression of CTCFL in presence of CTCF. a. CTCF and CTCFL compete with each other (as shown by ChIP seq). b. CTCFL leads to expression of Cancer Testes Antigens (as shown by RNA seq) and c. leads to altered chromatin organization (as shown by HiC).

gene expression, regulation, and other cellular processes the spatial organization of chromatin influences gene expression, regulation, and other cellular processes (Wit E et al., 2012; Denker et al., 2012).

CTCF along with the ring-shaped protein complex; cohesin is responsible for the formation of the Topologically Associating Domains (TADs); the basic unit of chromatin organization. CTCF and cohesin are responsible for the "loop extrusion" that is responsible for TAD formation.

CTCF is a ubiquitously expressed, essential, architectural protein that plays a key role in chromatin organization. CTCF has a paralogue, CTCFL that was evolved by gene duplication and transposition across evolution. Unlike the ubiquitously expressed CTCF, CTCFL is normally expressed ONLY in the testes. However, it is expressed in several cancer types and is classified as a cancer testes antigen. CTCF binds to ~50,000 sites in the genome with the help of its 11 zinc fingers. CTCFL has a similar DNA binding region with 11 zinc fingers (74% homology) though the N and C terminals are different between the two. We hypothesized that, when expressed together in cancer, CTCF and CTCFL would compete for binding sites owing to the similarity in the DNA binding region. However, following the binding, the functions would not be recapitulated as the protein terminals are different (Nishana et al., 2020). We used novel auxin inducible complementation degron system in mouse embryonic stem cells where both alleles of endogenous CTCF was tagged with AID (auxin inducible degron) that primes its degradation when auxin is added to the cell growth manner. This allowed us to express CTCF or CTCFL or both or neither in the engineered cells solely by addition of auxin or doxycycline to the growth

media. We then modified the cells to express transgenic CTCFL in a doxycycline inducible Under the conditions, using ChIPmentation we showed that CTCF and CTCFL do compete for the DNA binding sites (Figure 1a). RNA seq showed how CTCFL resulted in expression of cancer testes genes (Figure 1b). Immunoprecipitation showed that CTCFL failed to interact with cohesin: the major interacting protein of CTCF. HiC showed that CTCFL binding disrupted the chromatin organization globally (Figure 1c). We also swapped the N and C terminals of CTCF and CTCFL and these CRISPR knock-ins revealed the specific roles played by the N and C terminals as well as the DNA binding regions of CTCF and CTCFL. By analyzing CTCF and CTCFL binding in tandem we identified phenotypically distinct sites with respect to motifs, targeting to promoter/intronic intergenic regions and chromatin folding. Finally, we revealed that the N, C and zinc finger terminal domains play unique roles in targeting each paralog to distinct binding sites, to regulate transcription, chromatin looping and insulation (Nishana et al., 2020).

This study clarifies how competition between CTCF, the master weaver of chromatin organization and CTCFL; its paralogue resulted in alteration of chromosome organization and transcription leading to tumorigenesis. CTCFL when expressed along with CTCF in cancers, evicted CTCF from a certain set of its DNA binding sites, resulting in re-organization of the three-dimensional chromatin organization and alteration in gene expression. Our study implies that reduction in CTCF anchoring on DNA by its altered expression, competition with CTCFL, loss of cofactors or mutations in the protein itself can alter nuclear organization and gene expression.

References

1. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, et al. 2001. Initial sequencing and analysis of the human genome. Nature 409: 860-921.
2. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, et al. 2001. The sequence of the human genome. Science 291: 1304-1351.
3. de Wit E, de Laat W. 2012. A decade of 3C technologies: insights into nuclear organization. Genes Dev 26: 11-24.
4. Denker A, de Laat W. 2016. The second decade of 3C technologies: detailed insights into nuclear organization Genes Dev. 30: 1357-1382.
5. Nishana M., Ha C., Rodriguez-Hernaez J., Ranjbaran A., Chio E., Nora E.P., Badri S.B., Kloetgen A., Bruneau B.G., Tsirigos A. and Skok J.A. 2020. Defining the relative and combined contribution of CTCF and CTCFL to genomic regulation, Genome Biology, 21:108

Regulation of Mitochondrial Fission: Novel Insights into the Master Regulator Dnm1

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Mitochondrial division is a complex and finely tuned process crucial for organelle, cellular and organismal health. At the heart of the regulatory machinery required for this process lies the multi-domain dynamin-like protein (Dnm1 in yeast) that belongs to the dynamin family of large GTPases (Otsuga et al., 1998; Bleazard et al., 1999). Dnm1 self-assembles into spirals at the mitochondria and drives the organelle division. GTP binding and hydrolysis induce conformational changes, resulting in constriction of the spirals that generates mechanical force, which culminates in membrane scission (Mears et al., 2011). Several interacting proteins that aid in this process are known and the mechanism is mostly deciphered. While signalling mechanisms that tune Dnm1 activity are unknown in yeast, the mammalian ortholog DRP1 is modulated by various post-translational modifications, especially phosphorylation (Banerjee et al., 2022). Any alteration in this regulation of DRP1 results in altered mitochondrial function, a key hallmark of several human diseases.

The budding yeast, *Saccharomyces cerevisiae* is a crucial model organism for studying fundamental cellular processes relevant to human health and disease. Its high degree of genetic and functional conservation with humans enables us to study the proteins and pathways associated with human diseases. Interestingly, yeast has been instrumental in identifying the mechanism of mitochondrial fission and fusion and the various proteins involved in these processes, including Dnm1, the protein of interest in our study. At OBCAL in IITG, we aim to understand how Dnm1 is regulated in yeast cells using a comprehensive approach that includes molecular biology, biochemistry, biophysics, and computational methods. In one of our recent studies, we identified Threonine 62 and Serine 277 as two important residues for the structure and function of the protein. Though both these residues are present in the GTPase domain of the protein, the cellular changes observed were remarkably different. This is very interesting in terms of human disease conditions as several mutations in DRP1 are reported, where the exact alteration caused by the mutation is still unknown. In this study, we used the classical site-directed mutagenesis to mutate T62 and S277, which are both reported as putative phosphorylation sites in the protein, to the non-phosphorylatable alanine (A) and phosphomimetic aspartic acid (D), respectively. The mutant variants T62A/D or S277A/D rendered the protein non-functional *in vivo*, confirmed by the mitochondrial morphology analysis (Figure 1; Banerjee et al., 2023). However, the effect of the mutation on the protein distribution in the cells was very different. T62 variants formed a few large cytosolic aggregates that failed to associate with mitochondria, contrasting to this, puncta in S277 mutants were localized to mitochondria albeit non-functional (Figure 1). *In vitro* experiments with purified proteins showed no major changes in the secondary structure. Interestingly, severe impairment of GTP hydrolysis despite retaining the ability to form higher-order structures was observed. Molecular Dynamics simulations revealed disruption of local hydrogen bonds in the mutant variants and altered residue fluctuations distant from the mutation loci. This most likely destabilizes the interaction between the different domains of the protein and hampers the generation of the protein conformation required for membrane scission.

Our work provides an understanding of the conserved GTPase domain residues T62 and S277 in modulating the Dnm1 structure-function relationship. The study also highlights a new mitochondrially targeted yet inactive Dnm1 variant (S277A/D), which exerts a dominant negative effect even in the presence of endogenous functional Dnm1. Interestingly, all variants retain overall protein structure but exhibit reduced GTP hydrolysis, suggesting subtle alterations in Dnm1 higher-order assembly and dynamics. Phosphorylation at these sites may offer spatiotemporal control over mitochondrial division. The significance of our study in understanding the role of residues important for the structure-function of the protein is highlighted as mutation of DRP1 T59 (corresponding to Dnm1 T62) has been implicated in neurodegenerative disorders (Lhuissier et al., 2022). Ongoing efforts in this direction will enable us to decode this regulation and will aid in targeting mitochondrial dysfunction in human diseases. Overall, our findings not only advance our understanding of mitochondrial division regulation but also contribute valuable insights into the broader landscape of protein dynamics and function.

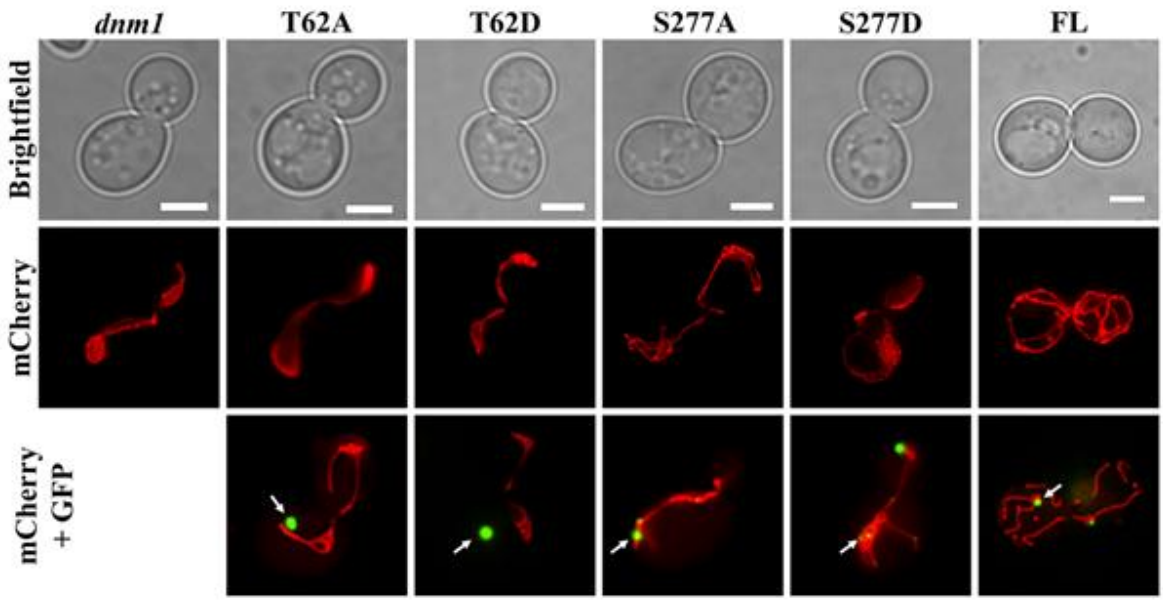

Figure 1: Dnm1 T62 and S277 mutants exhibit defective mitochondrial fission and altered protein distribution. Mitochondrial morphology in dnm1 cells upon the expression of FL (full length) and variants of Dnm1-GFP. preCOX4-mCherry was used as a marker to visualize mitochondria. Maximum intensity projections of the z-axis are used to depict the images (panel 2). In panel 3, representative fluorescence image (single z plane) of dnm1 cells expressing Dnm1-GFP variants and preCOX4-mCherry is depicted. Randomly selected Dnm1-GFP puncta (white arrow) were evaluated for colocalization with mitochondrial marker and the extent of colocalization. Scale bar - 5 μm.

References

1. D. Otsuga, B.R. Keegan, E. Brisch, J.W. Thatcher, G.J. Hermann, W. Bleazard, J. M. Shaw, The dynamin-related GTPase, Dnm1p, controls mitochondrial morphology in yeast, J. Cell Biol. 143 (2) (1998) 333–349. W. Bleazard, J.M. McCaffery, E.J. King, S. Bale, A. Mozdy, Q. Tieu, J. Nunnari, J. M. Shaw, The dynamin-related GTPase Dnm1 regulates mitochondrial fission in yeast, Nat. Cell Biol. 1 (5) (1999) 298–304.
2. W. Bleazard, J.M. McCaffery, E.J. King, S. Bale, A. Mozdy, Q. Tieu, J. Nunnari, J. M. Shaw, The dynamin-related GTPase Dnm1 regulates mitochondrial fission in yeast, Nat. Cell Biol. 1 (5) (1999) 298–304.
3. J.A. Mears, L.L. Lackner, S. Fang, E. Ingerman, J. Nunnari, J.E. Hinshaw, Conformational changes in Dnm1 support a contractile mechanism for mitochondrial fission, Nat. Struct. Mol. Biol. 18 (1) (2011) 20–26.
4. R. Banerjee, A. Mukherjee, S. Nagotu, Mitochondrial dynamics and its impact on human health and diseases: inside the DRP1 blackbox, J. Mol. Med. 1-21 (2022).
5. R. Banerjee, A. Mukherjee, A. Adhikary, S. Sharma, S. Hussain, E. Ali, S. Nagotu, Insights into the role of the conserved GTPase domain residues T62 and S277 in yeast Dnm1, IJBM (2023).
6. C. Lhuissier, B.E. Wagner, A. Vincent, G. Garraux, O. Hougrand, R. Van Coster, V. Benoit, D. Karadurmus, G. Lenaers, N. Gueguen, Chevrollier I, Maystadt, case report: thirty-year progression of an EMPF1 encephalopathy due to defective mitochondrial and peroxisomal fission caused by a novel de novo heterozygous DNM1L variant, Front. Neurol. 13 (2022), 937885.

Illuminating Cell Biology Through Innovative Imaging Tools

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Fluorescent Proteins (FPs) are indispensable for cell biology research. The pioneering work by Chalfie et al., introduced GFP as a marker for protein localization and expression, marking a pivotal moment in cell biology. This breakthrough (Chalfie et al., 1994) initiated a revolutionary shift in bioimaging techniques and forever altered the trajectory of cell biology (Crivat & Taraska, 2012). FPs fused to proteins of interest (POI) have allowed us to "see" cellular and subcellular events such as cargo transport, chromosome segregation, motility, division, and more. FP-POI fusions can also reveal levels of expression, subcellular localization, interacting partners, *in vivo* reaction kinetics and dynamics. A palette of FPs is now available to genetically "paint" a protein *in vivo* for microscopy providing choices in FPs excitation and emission spectra, brightness and pH sensitivity, while allowing to visualise multiple proteins/structures simultaneously.

The growing significance of bioimaging using FP-POI fusions in cell biology is evidenced by many advancements and innovations in microscopy, highlighting its integral role in documenting dynamic cellular and subcellular processes. The shift from fixed cell imaging to "Live cell imaging" has proved to be instrumental in observing subcellular processes under physiological and perturbed conditions at previously unthought of spatial and temporal resolutions. These superpowers, though, come with a catch. Live-cell microscopy often involves a compromise between obtaining high-quality images and the duration of imaging due to effects on cell health. Increased exposure to excitation light causes phototoxicity via increased Reactive Oxidative Species (ROS) production (formed as a by-product of fluorescence) and photobleaching (irreversible loss of fluorophores), which limits the utility of fluorescent protein fusions. Many times, post-translational modifications at either C- or N- or both of the terminals of the POI render functionality, thus making it unavailable for tagging with FPs (Jensen, 2013). Thus, a major drawback of FPs is that many proteins lose functionality upon C- or N-terminal fusion with a FP. But this has occasionally been overcome by inserting specifically designed long linkers between the FPs (Bisson-Filho et al., 2017) or by placing the FPs at a structurally feasible position in the POI (Bisson-Filho et al., 2018; Hussain et al., 2018).

Tagging the Untaggable

Our research group focuses on cell division, particularly studying the cytoskeletal proteins that form polymers and subsequent higher-order structures. Visualising cytoskeletal proteins has been notoriously difficult as FP fusions disrupt their oligomerization and interactions, thus, causing loss of their function. One such protein is Tropomyosin (Tpm), which envelopes F-actin filaments, protecting it from actin-severing proteins such as cofilin. Tpm also regulates the interaction of the actin filament with other actin-binding proteins, such as the motor protein (i.e., myosin). Tpm exists as multiple isoforms in various model systems that differentially fine-tune the functionality of spatially distinct actin filament networks. A major challenge in understanding the complex regulation of Tpm isoforms in cells is the lack of functional fluorescent fusion Tpms, which can be used to collect dynamic information about their localization and kinetics from live-cell imaging (Hatano et al., 2022).

To tackle this problem, we along with three other labs collaborated to construct novel functional

fusion proteins of Tpm with FPs. This feat was achieved by designing fusion proteins having a N-terminal fluorophore (mNeonGreen; mNG) connected via a flexible (40 amino acid) peptide linker to the Tpm protein, reporting tropomyosin dynamics in multiple model organisms that include *S. cerevisiae*, *S. pombe*, *S. japonicus*, and mammalian cells (Hatano et al., 2022). In this study, along with clear subcellular localization of Tpm on linear actin filaments, the presence of Tpm on the branched actin patches in yeast was also noted. This approach was also used to investigate tropomyosin dynamics in mammalian cells, using immortalised human retinal cells (RPE1) as an example. These constructs have now enabled us to observe the dynamics of Tpm isoforms in live-cells during cell cycle progression and promise to reveal novel undiscovered aspects of Tpm and actin cytoskeleton.

ALFA-NbALFA Duo: Pioneering Precision in Live-Cell Imaging

Our findings were further corroborated using Camelid nanobodies - an emerging powerful tool for investigating protein localization and function. A nanobody, Nb5, was screened and validated to bind S. pombe Tpm – Cdc8 and used to visualise Cdc8 localization in interphase and mitotic cells. However, screening and generating Camelid nanobodies (Nbs) that bind POIs effectively without affecting function can be laborious and may take a long time to optimise. While FPs are valuable epitope tags for reporting localization, their large size can create steric hindrance, and are not particularly useful for biochemical applications. We have attempted to address these challenges by utilising the newly-developed ALFA tag-NbALFA system (Götzke et al., 2019). ALFA tag is a 13 amino-acid long helical peptide that is recognised by its nanobody binding partner-NbALFA. We have created a plasmid toolkit ALIBY: ALFA Nanobody-Based Toolkit for Imaging and Biochemistry in Yeast (Akhuli et al., 2022), particularly for *Saccharomyces cerevisiae*. The toolkit consists of tagging and detection plasmids that work parallelly as a single tag-based system to create endogenous C-terminal ALFA tag fusion to the POI (POI-ALFA) along with a fluorescently-labelled NbALFA to detect the POI-ALFA fusion. The study extensively demonstrates the versatility and convenience of this toolkit for a diverse range of applications such as live-cell imaging with an excellent signal-to-noise ratio and clean biochemical detection in immunoblotting and immunoprecipitation. We have successfully utilised ALFA tag fusions to visualise POI at various subcellular locations and organelles, demonstrating its potential as a universal single-tag based workflow (Figure 1). Moreover, the small size of the ALFA tag allows terminal as well as internal fusions on a POI and the modular nature of the NbALFA can be used for innovative experimental approaches.

ALFA Tag and IntAct Unleash Actin's Secrets

One of the major challenges in cell biology is tagging cytoskeletal proteins without loss of function. The ALFA tag addresses this by allowing tagging at permissive internal sites within a protein without perturbing its function. In a recent collaborative study with Dr. Koen's group (Radboud University, Netherlands), we used our engineered ALFA toolkit to directly visualise the major cytoskeletal protein- actin in live cells without loss of function; a feat which has eluded actin researchers for many decades.

This study, titled "IntAct: a non-disruptive internal tagging strategy to study actin isoform organisation and function" (van Zwam et al., 2023) utilises an internal tag to study the molecular interactions and dynamics of actin isoforms. The T229/A230 residues in the subdomain four of actin was identified as the most suitable site for tag insertion. The internally tagged actin (IntAct) when expressed, integrated into the filaments, and retained native protein-protein interactions as verified by immunofluorescence, immunoprecipitation, and live-cell imaging in mammalian cells. Incorporation of IntAct variants into yeast actin patches and cables further highlights the permissive nature of the T229/A230 site for modification and use across model systems. IntAct represents the first tool to study the live-cell dynamics of specific actin isoforms and further opens avenues to understand the mechanisms of actin-mediated diseases. We believe that approaches such as IntAct and use of novel molecular tools like nanobodies will be key to unravel the hidden dynamics of polymeric cytoskeletal proteins in the near future.

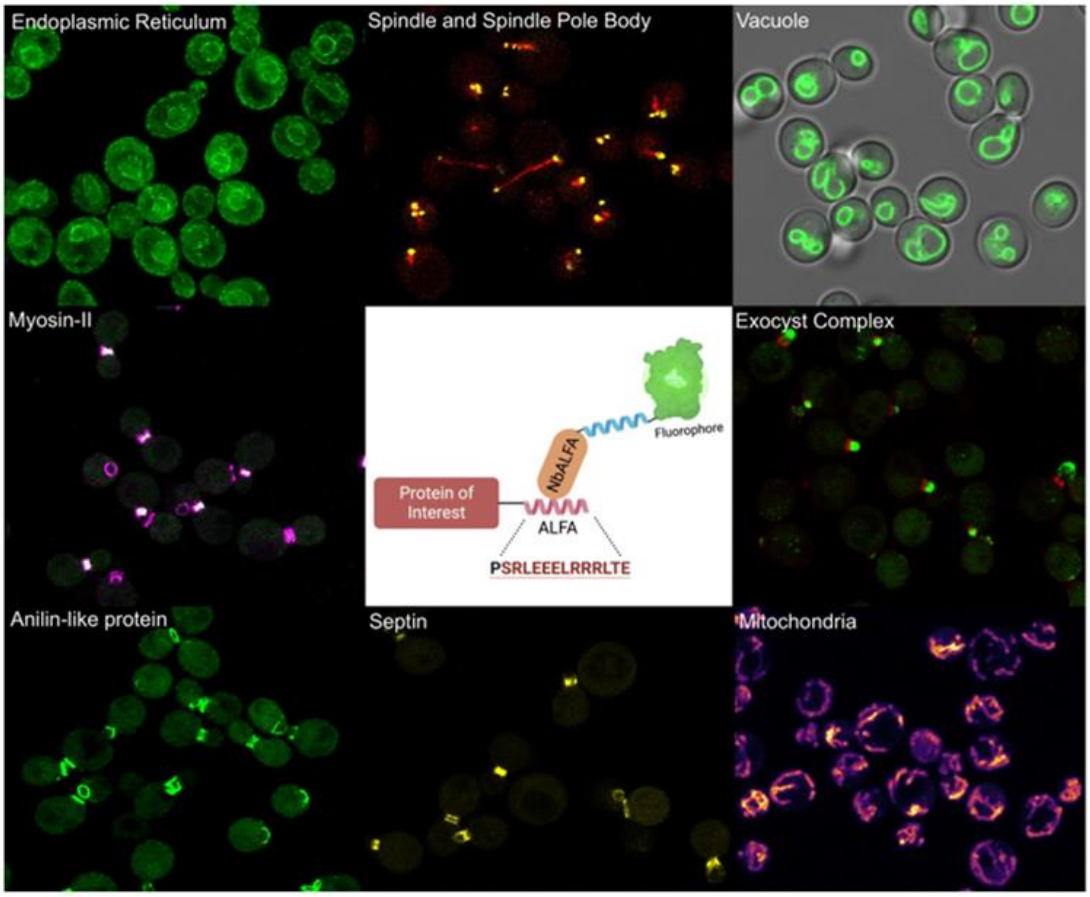

Figure 1. Mapping the Intracellular Landscape: Visualisation of ALFA-Tagged Proteins Across Diverse Subcellular Locations in Yeast Cells

Stay Gold with mStayGold

While ALFA tag and Nbs fusion to FPs resolve some major issues in imaging POIs, photobleaching of FPs remains a fundamental limiting factor in obtaining high signal-to-noise-ratio during time-lapse/live cell imaging. Photobleaching is characterized by the transition of a fluorophore to a non-fluorescent state, involving irreversible loss of fluorescence due to photon-induced chemical damage and covalent modifications. The development of photostable FPs has traditionally come with a trade-off – an increase in photostability tends to be accompanied by a decrease in quantum yield. This challenge prompted scientists to explore innovative approaches in creating derivatives of FPs, seeking improved alternatives that provide enhanced photostability for live cell imaging. Among these alternatives are Enhanced Green Fluorescent Protein (EGFP) (Zhang et al., 1996) and Enhanced Blue Fluorescent Protein (EBFP), such as Azurite (Mena et al., 2006) which offer potential solutions for improved photostability in live cell imaging. Enhanced fluorescent proteins/fluorophores are engineered using techniques such as directed evolution and rational design. These methods involve modifying the gene encoding the fluorophore through mutagenesis and selection or targeted modifications to improve brightness and photostability for optimal fluorescence performance. StayGold, the recently discovered dimeric FP, exhibits outstanding performance in parameters such as brightness and photostability (Hirano et al., 2022). Each StayGold monomer is composed of an 11-stranded beta barrel that is almost identical to the well-characterized Green Fluorescent Protein. A recent study by Hirano et al., titled "StayGold Photostability under Different Illumination Modes" (Hirano et al., 2023) compared the photostability of StayGold to FPs like EGFP, mClover3, and mNeonGreen and found that StayGold was approximately 40, 200, and 50 times more photostable inside the HeLa cell nucleus.

Despite its qualities, the dimeric nature of StayGold limits its usage *in vivo* due to effects on protein

stoichiometry and dynamics. In collaboration with laboratories at the University of Warwick, we have created a monomeric version of StayGold called mStayGold in a study titled "A monomeric StayGold fluorescent protein" (Ivorra-Molla et al., 2023). After obtaining a 1.6 Å crystal structure of StayGold, we made mutations at the dimeric interface of StayGold to disrupt oligomerization. This approach yielded a truly monomeric StayGold with a single amino acid change (E138D) without affecting its photostability and brightness. The dimeric and monomeric StayGold have near-identical excitation and emission maxima, similar extinction coefficients and quantum yields, and are stable over a broad range of pH. Furthermore, mStayGold displayed ~7 times greater half-life as compared to sfGFP under constant illumination. We also tested the applicability of mStayGold in different model systems (*E. coli, S. cerevisiae*, *S. pombe*, mammalian cells) by monitoring their photobleaching kinetics in comparison to GFP at full laser power for 30 seconds. Astonishingly, StayGold lost only 40% of its emission intensity whereas its GFP counterpart quickly photobleached, losing up to 90% of its initial emission intensity, asserting the superior performance of mStayGold in vivo over current benchmark FPs. We envision that mStayGold will be a revolutionary fluorescent protein with the potential to completely replace conventional FPs and usher us into an era of photostability (Ando et al., 2023).

Beyond Boundaries: Illuminating the Future of Imaging

In parallel to these developments in fluorescent proteins, cell biology has greatly benefitted by advancements in image acquisition hardware and image analysis capabilities. Tagging a POI is challenging, but an equally great challenge lies in effectively interpreting and quantifying the collected information for downstream analysis. Fluorescence microscopy involves finding the right balance between the suitable microscope, sample preparation method, imaging parameters, and analysis pipelines for robust and reproducible scientific outcomes.

The work done in our lab is just a tip of the iceberg, and its scope extends into "near" futuristic ideas in microscopy such as super-resolution techniques (Expansion microscopy, Structured Illumination Microscopy, Single-Molecule Localization techniques) which promise to equip us with a sub-100 nm spatial resolution in live cells. These advancements will unravel exciting new biology and we envision the future of bio-imaging to be "bright" and "everlasting".

References

1. Akhuli, D., Dhar, A., Viji, A. S., Bhojappa, B., & Palani, S. (2022). ALIBY: ALFA Nanobody-Based Toolkit for Imaging and Biochemistry in Yeast. mSphere, 7(5), e0033322.
2. Ando, R., Shimozono, S., Ago, H., Takagi, M., Sugiyama, M., Kurokawa, H., Hirano, M., Niino, Y., Ueno, G., Ishidate, F., Fujiwara, T., Okada, Y., Yamamoto, M., & Miyawaki, A. (2023). StayGold variants for molecular fusion and membrane-targeting applications. Nature Methods, 1–9.
3. Bisson-Filho, A. W., Hsu, Y.-P., Squyres, G. R., Kuru, E., Wu, F., Jukes, C., Sun, Y., Dekker, C., Holden, S., VanNieuwenhze, M. S., Brun, Y. V., & Garner, E. C. (2017). Treadmilling by FtsZ filaments drives peptidoglycan synthesis and bacterial cell division. Science, 355(6326), 739–743.
4. Bisson-Filho, A. W., Zheng, J., & Garner, E. (2018). Archaeal imaging: leading the hunt for new discoveries. Molecular Biology of the Cell. https://doi.org/10.1091/mbc.E17-10-0603
5. Chalfie, M., Tu, Y., Euskirchen, G., Ward, W. W., & Prasher, D. C. (1994). Green fluorescent protein as a marker for gene expression. Science, 263(5148), 802–805.
6. Crivat, G., & Taraska, J. W. (2012). Imaging proteins inside cells with fluorescent tags. Trends in Biotechnology, 30(1), 8–16.
7. Götzke, H., Kilisch, M., Martínez-Carranza, M., Sograte-Idrissi, S., Rajavel, A., Schlichthaerle, T., Engels, N., Jungmann, R., Stenmark, P., Opazo, F., & Frey, S. (2019). The ALFA-tag is a highly versatile tool for nanobody-based bioscience applications. Nature Communications, 10(1), 4403.

8. Hatano, T., Lim, T. C., Billault-Chaumartin, I., Dhar, A., Gu, Y., Massam-Wu, T., Scott, W., Adishesha, S., Chapa-Y-Lazo, B., Springall, L., Sivashanmugam, L., Mishima, M., Martin, S. G., Oliferenko, S., Palani, S., & Balasubramanian, M. K. (2022). mNG-tagged fusion proteins and nanobodies to visualize tropomyosins in yeast and mammalian cells. Journal of Cell Science, 135(18). https://doi.org/10.1242/jcs.260288
9. Hirano, M., Ando, R., Shimozono, S., Sugiyama, M., Takeda, N., Kurokawa, H., Deguchi, R., Endo, K., Haga, K., Takai-Todaka, R., Inaura, S., Matsumura, Y., Hama, H., Okada, Y., Fujiwara, T., Morimoto, T., Katayama, K., & Miyawaki, A. (2022). A highly photostable and bright green fluorescent protein. Nature Biotechnology, 40(7), 1132–1142.
10. Hirano, M., Yonemaru, Y., Shimozono, S., Sugiyama, M., Ando, R., Okada, Y., Fujiwara, T., & Miyawaki, A. (2023). StayGold Photostability under Different Illumination Modes. In bioRxiv (p. 2023.11.23.568490). https://doi.org/10.1101/2023.11.23.568490
11. Hussain, S., Wivagg, C. N., Szwedziak, P., Wong, F., Schaefer, K., Izoré, T., Renner, L. D., Holmes, M. J., Sun, Y., Bisson-Filho, A. W., Walker, S., Amir, A., Löwe, J., & Garner, E. C. (2018). MreB filaments align along greatest principal membrane curvature to orient cell wall synthesis. eLife, 7. https://doi.org/10.7554/eLife.32471
12. Ivorra-Molla, E., Akhuli, D., McAndrew, M., Scott, W., Kumar, L., Palani, S., Mishima, M., Crow, A., & Balasubramanian, M. (2023). A monomeric StayGold fluorescent protein. https://doi.org/10.1038/s41587-023-02018-w
13. Jensen, E. C. (2013). Overview of live-cell imaging: requirements and methods used. Anatomical Record, 296(1), 1–8.
14. Mena, M. A., Treynor, T. P., Mayo, S. L., & Daugherty, P. S. (2006). Blue fluorescent proteins with enhanced brightness and photostability from a structurally targeted library. Nature Biotechnology, 24(12), 1569–1571.
15. van Zwam, M. C., Dhar, A., Bosman, W., van Straaten, W., Weijers, S., Seta, E., Joosten, B., Palani, S., & van den Dries, K. (2023). IntAct: a non-disruptive internal tagging strategy to study actin isoform organization and function. In bioRxiv (p. 2021.10.25.465733). https://doi.org/10.1101/2021.10.25.465733
16. Zhang, G., Gurtu, V., & Kain, S. R. (1996). An enhanced green fluorescent protein allows sensitive detection of gene transfer in mammalian cells. Biochemical and Biophysical Research Communications, 227(3), 707–711.Ivorra-Molla, E., Akhuli, D., McAndrew, M., Scott, W., Kumar, L., Palani, S., Mishima, M., Crow, A., & Balasubramanian, M. (2023). A monomeric StayGold fluorescent protein. https://doi.org/10.1038/s41587-023-02018-w
17. Jensen, E. C. (2013). Overview of live-cell imaging: requirements and methods used. Anatomical Record , 296(1), 1–8.
18. Mena, M. A., Treynor, T. P., Mayo, S. L., & Daugherty, P. S. (2006). Blue fluorescent proteins with enhanced brightness and photostability from a structurally targeted library. Nature Biotechnology, 24(12), 1569–1571.

Tagging Proteins for Ciliary Removal: Ubiquitin Does it Again!

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The primary cilium is a surface-exposed organelle present in almost every cell in the human body. Primary cilia, often called "signalling antennas," function in phototransduction, olfaction, planar cell polarity, and several signalling pathways essential for development and tissue homeostasis (including the Sonic hedgehog and Wnt signalling pathways). Abnormalities in primary cilia function are linked to numerous human diseases, including Bardet-Biedl syndrome, Joubert syndrome, Meckel-Gruber syndrome, nephronophthisis, and Sensenbrenner syndrome, collectively termed ciliopathies. Primary cilia orchestrate signalling pathways by concentrating signalling molecules in a unique environment of lipids and second messengers. The unique density of proteins and receptors in the cilia is maintained through a regulated importing process governed by intraflagellar transport (IFT).

Meanwhile, the BBSome –an obligate coat-like complex of eight Bardet–Biedl syndrome (BBS) proteins mediate regulated and constitutive exit from cilia. The Hedgehog pathway provides the best-studied example of cilium-based signalling. Hedgehog signalling patterns limbs and neural tubes, and its signal transduction relies on the dynamic accumulation of signalling receptors - G protein-coupled receptors (GPCRs). Earlier studies have demonstrated that BBSome is the main retrieval complex that traffic-activated GPCRs out of cilia on demand. The critical question is how the BBSome selectively removes activated GPCRs from cilia.

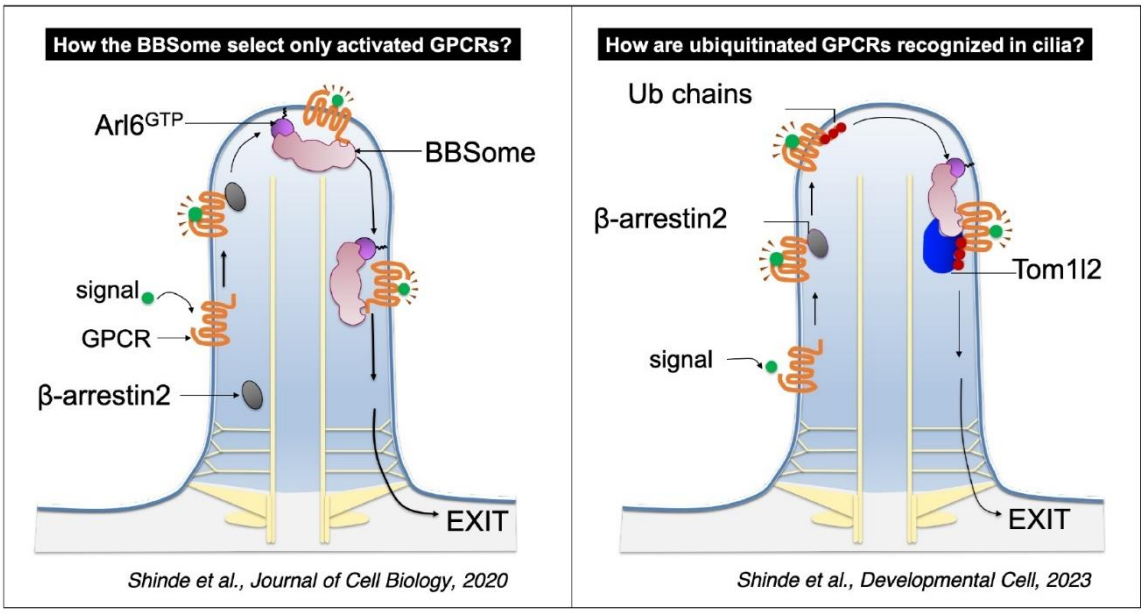
Our recent studies demonstrated that, upon activation, ciliary GPCRs are tagged with ubiquitin chains comprising Lysine-63 linkages (UbK63) in a β-arrestin manner before BBSome-mediated exit. We performed immunostaining-based assays using a well-established UbK63 antibody and biochemical ubiquitination assays to show that activated ciliary GPCRs are ubiquitinated with UbK63 polyubiquitin chains. Next, we employed two approaches to demonstrate that these UbK63 chains regulate the signal-dependent exit of ciliary GPCRs. First, we removed all ubiquitination sites from Somatostatin Receptor 3 (SSTR3) and an orphan Hedgehog signalling receptor, GPR161, by mutating all cytoplasm-exposed lysine residues to arginine (cK0 variants) and studied their exit kinetics. cK0 mutant versions of SSTR3 and GPR161showed defective exit from cilia. Second, we targeted a UbK63-specific deubiquitinase, AMSH (an associated molecule with the SH3 domain of STAM), to cilia to demonstrate the importance of UbK63 polyubiquitin chains in the retrieval of activated GPCRs. Cilia-targeted AMSH blocked the exit of GPR161, SSTR3, and Smoothened (SMO) from cilia (Shinde et al., 2020) (Figure 1).

I further my studies, extending them to the photoreceptor outer segments (POS) (hyperspecialized primary cilia). The molecular mechanisms of retinal degeneration in BBS patients still need to be well studied. Over 100 non-OS proteins are accumulated in the POS of Bbs mutant mice. Immunofluorescence assays using well-characterized ubiquitin antibodies to stain retinal sections from wildtype and Bbs4-/- mice showed a significant increase in the ubiquitin levels in the POS of Bbs4-/- mice retinas. These results suggest ubiquitin tags the mislocalised non-OS proteins for their BBSome-dependent removal from OS (Shinde et al., 2020).

Next, we aimed to unravel the mechanism of ubiquitin recognition in the cilia. *In vitro* assays revealed that BBSome/retrieval machinery does not directly bind to UbK63 chains. Further, we hypothesized a possible role of UbK63 readers in

cilia to regulate the ciliary exit of activated GPCRs. We performed proximity labelling followed by mass spectrometry to identify ciliary UbK63 readers. This proteomics data revealed the presence of ESCRT-0 complex proteins in the cilia. In parallel, we performed a focused RNAi screen to test the possible role of ESCRT complexes in cilia as UbK63 readers regulating GPCR exit. These approaches led us to identify TOM1L2, the target of myb1-like 2 membrane trafficking protein, as a UbK63 reader in cilia. Further, CRISPR-mediated knockout of Tom1l2 resulted in ciliary accumulation of ubiquitin and activated GPCRs. Biochemical characterization of TOM1L2-BBSome binding revealed that the 'WLR' motif on the C-terminus of Tom1l2 is necessary for binding to the BBSome (Figure 1). We performed rescue experiments by reintroducing WT and mutant versions of TOM1L2 that cannot bind ubiquitin or BBSome in TOM1L2 knock of cells and studied GPR161 exit. The results support the role of TOM1L2 in mediating the removal of activated GPCRs from cilia (Shinde et al., 2023).

Our studies unravel a mechanism of recognition and removal of activated GPCRs and mislocalised proteins from cilia. We propose a quality control mechanism to maintain ciliary proteome.



DNA Repair Pathways: Elucidating the Role of Genes Involved in Oxidative Stress

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Aerobic respiration involving the reduction of oxygen to water generates reactive oxygen species (ROS), like superoxide radical ($\cdot O^{2-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($\bullet OH$) (Farr and Kogoma 1991). Oxidative stress arises due to an imbalance between ROS and its scavengers inside cells, leading to damage to cellular biomolecules including DNA, resulting in various diseases as well as aging. DNA Repair Pathways play an important role in correcting damaged DNA. Thus, studying the mechanism of oxidative stress and the organism's defense system to protect them against oxidative stress becomes imperative.

Escherichia coli (*E. coli*) has been used as a model microorganism for years to study oxidative stress. *E. coli,* when exposed to H_2O_2 (at concentrations of 1-3mM), leads to mode-one killing, primarily due to DNA damage, and mode-two killing (at concentrations of up to 50 mM) due to unrecognized damage (Imlay and Linn, 1986).

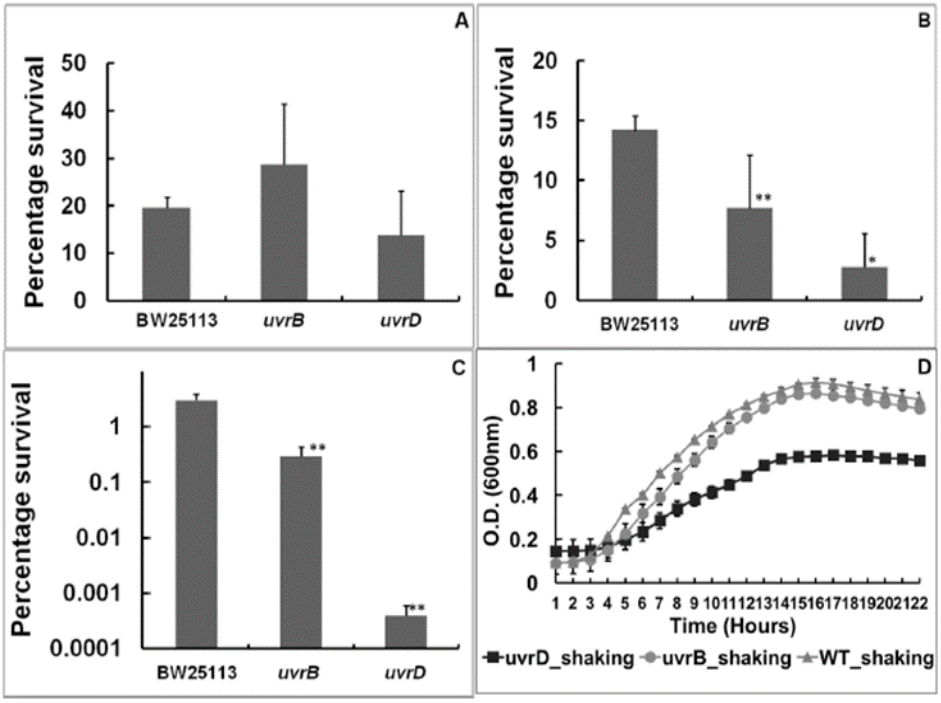

Figure 1: Survival of *E. coli* NER pathway mutants to oxidative stress in nutritionally rich medium. Strains grown in LB medium with aeration were exposed to (A) 2 mM H2O2 for 90 min (B) 3 mM H2O2 for 60 min (C) 3 mM H2O2 for 90 min. (D) Growth kinetics of the strains grown in LB medium with aeration. Key: * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$, suggesting significant difference in the percentage survival as compared to wild type. Error bars represent SD of mean. (Dhawale et al 2021).

DNA damage is achieved through the generation of single stranded (ss) and/or double stranded (ds) breaks (Demple and Harrison, 1994). These DNA damages can be repaired via various DNA Repair machineries like Base Excision Repair (BER), Nucleotide Excision Repair (NER) and Homologous Recombination Repair (HRR) comprising of RecF pathway, RecBCD, RuvABC and RecG. Various *E. coli* mutants of genes in these pathways have been used to understand the mechanism of repair. However, there were several gaps in the previously reported studies, for example, *E. coli* recF mutants were found to be sensitive to mode-one killing by H_2O_2 (Imlay and Linn, 1987), however, roles of other mutants such as recQ and recJ have not been explored. They also did not find sensitivity in uvrA, uvrB, uvrC and uvrD mutants in response to oxidative stress, however; Goerlich et al in 1989 showed increased response in uvrA mutant exposed to oxidative stress. *E. coli* ruvA mutant were found to be 10-15 fold more sensitive to H_2O_2 at concentrations of 1-3 mM (Konola et al., 2000), but other genes in Resolvase pathway were not studied in detail. Role of RecG in survival of *E. coli* to oxidative stress has not been defined. There are contradictory reports of other stress conditions, like low aeration and nutrient deprivation, in providing protection against oxidative stress (Imlay and Linn, 1986, Brandi et al., 1987 and Jenkins et al., 1998).

To understand these gaps, we studied the role of various DNA repair pathways on *E. coli* survival to H_2O_2-induced mode-one killing. The correlation of other environmental stressors in providing protection against oxidative stress was also explored. The complete single-gene deletion mutants were constructed for HRR and NER pathways. Varying concentrations and durations of H_2O_2 exposure (3 mM and 2 mM with 60 and 90 minutes for acute exposure and 0.3 mM and 0.5 mM with 15-16 hours for chronic exposure), along with different environmental stress conditions (minimal medium vs nutritionally rich LB medium and low aeration vs aeration) were studied to assess survival. A known sensitive mutant (pnp) to oxidative stress was used as control.

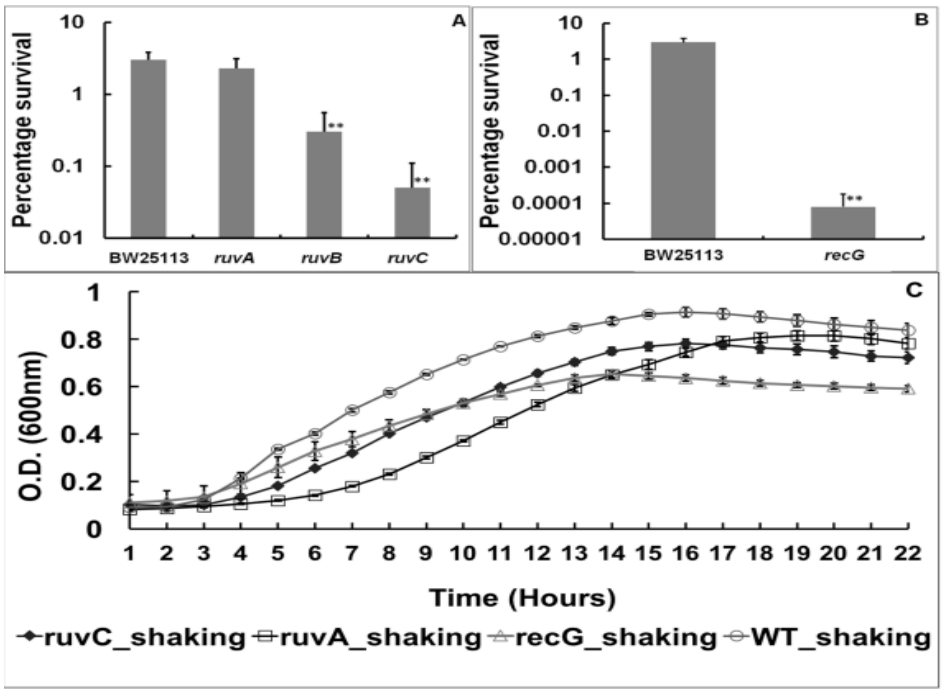

Figure 2. Survival of Holliday junction resolvase mutants to oxidative stress in rich medium. Strains grown in LB medium with aeration were exposed to 3 mM H2O2 for90 min. (A) ruvA, ruvB, ruvC (B) recG. (C) Growth kinetics of the strains grown in LB medium with aeration. Key: **- P < 0.01, suggesting significant difference in thepercentage survival as compared to wild type. Error bars represent SD of mean. (Dhawale et al 2021).

In contrast to the earlier reports, the present study revealed the crucial role of NER in response to oxidative stress; with uvrD and uvrB being most critical genes in providing protection against oxidative stress (Figure 1; Dhawale et al., 2021). Moreover, RecF pathway mutants showed substantial sensitivity to H_2O_2 exposure, highlighting its critical role in survival of *E. coli* against oxidative stress. Hypersensitivity of the recG mutant in response to all the parameters under study suggested its significant role in providing protection to oxidative stress induced DNA damage (Figure 2; Dhawale et al., 2021). Increased percentage survival in conditions like reduced aeration and minimal nutrition conditions suggest that, these parameters provide significant cross-protection against mode-one killing.

In summary, the present study emphasized the involvement of NER pathway in providing protection against oxidative stress induced mode-one killing. The study identifies the critical role of RecG in oxidative stress survival and also re-establishes the importance of RecF pathway. Further, the study highlights the influence of environmental factors in the protective mechanisms against oxidative stress, offering critical insights into natural adaptation strategies in *E. coli.*

References

1. Brandi, G., Sestili, P., Pedrini, M. A., Salvaggio, L., Cattabeni, F., Cantoni, O., 1987, "The effect of temperature or anoxia on Escherichia coli killing induced by hydrogen peroxide", Mut Res., 190(4), 237-240.
2. Demple, B., and Harrison, L., 1994, "Repair of oxidative damage to DNA: enzymology and biology", Annu Rev Biochem., 63(1), 915-948.
3. Dhawale A., Bindal G., Rath D., Rath A., 2021, "DNA repair pathways important for the survival of Escherichia coli to hydrogen peroxide mediated killing". Gene. 2021 Feb 5;768:145297. doi: 10.1016/j.gene.2020.145297. Epub 2020 Nov 9. PMID: 33181253.
4. Farr, S. B., and Kogoma, T., 1991, "Oxidative stress responses in Escherichia coli and Salmonella typhimurium", Microbiol Mol Biol Rev., 55(4), 561-585.
5. Goerlich, O., Quillardet, P., Hofnung, M., 1989, "Induction of the SOS response by hydrogen peroxide in various Escherichia coli mutants with altered protection against oxidative DNA damage", J Bacteriol., 171(11), 6141-6147.
6. Imlay, J. A., and S. Linn., 1986, "Bimodal pattern of killing of DNA-repair defective or anoxically grown Escherichia coli by hydrogen peroxide", J Bacteriol., 166:519–527.
7. Imlay, J. A., and Linn, S., 1987, "Mutagenesis and stress responses induced in Escherichia coli by hydrogen peroxide", J Bacteriol., 169(7), 2967-2976.
8. Jenkins, D. E., Schultz, J. E., Matin, A., 1988, "Starvation-induced cross protection against heat or H2O2 challenge in Escherichia coli", J Bacteriol., 170(9), 3910-3914.
9. Konola, J. T., Sargent, K. E., Gow, J. B., 2000, "Efficient repair of hydrogen peroxide-induced DNA damage by Escherichia coli requires SOS induction of RecA and RuvA proteins", Mutat Res., 459:187–194.

Deltex Facilitates the Expansion of Wingless Gradient and Antagonizes Wingless Signaling Through a Conserved Mechanism of Armadillo/β-catenin Degradation

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Morphogens are crucial in determining cell fate in a concentration-dependent fashion, playing an essential role in tissue patterning throughout development. By establishing a concentration gradient, these molecules facilitate long-range signaling, guiding cell differentiation according to positional cues. The functions of morphogens, including Wingless (Wg), Hedgehog (Hh), and Decapentaplegic (Dpp), have been extensively explored in the development of *Drosophila* (Cadigan and Nusse, 1997; Lecuit et al., 1996).

The Wingless (Wg) signaling pathway plays a pivotal role in embryonic development, tissue homeostasis, and various cellular processes across species (Cadigan and Nusse., 1997, Logan and Nusse., 2004). Thus, abnormal Wnt signaling is linked to a broad spectrum of human pathologies (Coombs et al., 2008). Precise control of Wnt/Wg signaling is crucial for maintaining proper cellular homeostasis. Our recent work delves into the molecular intricacies of this pathway, focusing on the contribution of E3 ubiquitin ligase Deltex (Dx) (Sharma et al., 2023). Dx is an evolutionarily conserved candidate in the Notch interactome and it regulates Notch signaling in a context-dependent manner (Matsuno et al., 1995; Mukherjee et al., 2005). Our previous reports have unveiled the involvement of Dx in different signaling cascades such as JNK, Toll, and Decapentaplegic (Sharma et al., 2021a, 2021b, 2022). Identifying Dx as a facilitator of Wingless gradient expansion and an antagonist of Wingless signaling opens a new chapter in our comprehension of this essential cellular communication network.

Our study provides novel evidence that dx genetically interacts with wg and other components of the Wg signaling cascade, particularly the transcriptional effector Armadillo (Arm). Through our loss-of-function and gain-of-function investigations, we establish involvement of Dx in regulating Wg signaling. Notably, the overexpression of Dx plays a crucial role in promoting the spread of the morphogen Wg. This, in turn, results in the erosion of the Wg gradient, leading to the loss of short-range targets, Senseless (Sens) and Cut. Importantly, our observations reveal that the reduction in Wg downstream targets upon Dx overexpression is unrelated to Notch signaling. Conversely, reducing Dx dosage narrows the Wg expression gradient, further supporting direct role of Dx in Wg spreading (Figure 1).

Additionally, when Rab5, the small GTPase crucial for early endosome formation, is knocked down in conjunction with Dx overexpression, a reduction in wing size is observed and it is accompanied by decreased Wg expression. Similarly, overexpressing Rab7, which facilitates late endosome formation,

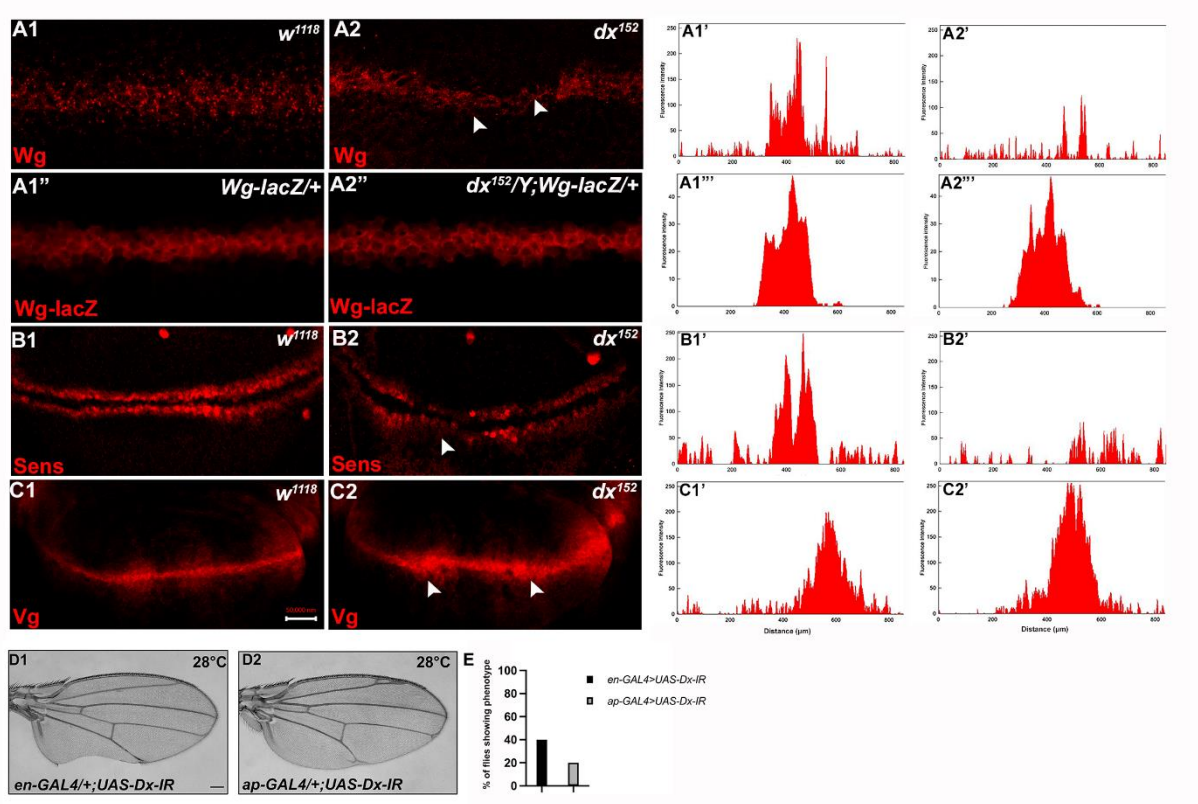

Figure 1: Loss of Dx reduces Wg Signaling gradient and target gene expression. (A2) dx152 wing disc shows a narrow Wg expression gradient compared to wild-type third instar larval wing discs (A1). (A1" and A2") show Wg-lacZ staining in the mentioned genotype. (B2) A constricted expression of Sens was observed in dx null discs (dx152) (marked by arrowhead) compared to the control wild-type wing imaginal discs (B1). (C1) The expression of Vg in the wild-type disc. (C2) dx null discs showed a broadened Vg expression, marked by arrowheads. (A1'-C2') Show the average fluorescence intensity of images in A1-C2. (D1-D2) Expression of the RNAi targeting Dx by en-GAL4 or ap-GAL4 results in morphological aberrations in the expressing regions. (E) Graph showing the percentage of flies showing respective phenotypes in the mentioned genotype (n=100). Images in A-C are representatives of 3 independent experiments (n=6). Scale Bar: A1-A2: 10µm. B1-C2: 50µm. D1-D2: 200µm.

along with Dx, leads to a comparable reduction in Wg expression. This suggests that Rab7, in collaboration with Dx, may contribute to Wg trafficking, thereby maintaining its proper gradient.

This study also investigates the regulatory role of Dx over Wg signaling and presents a model proposing the regulation of Wg through Dx via down-regulation of Arm. The *Drosophila* orthologue of mammalian β-catenin, Arm, acts as a nuclear transcriptional effector that transduces the Wg signaling. When overexpressed in wing and eye tissues, Dx demonstrates the capacity to down-regulate Arm. Given the E3 ubiquitin ligase activity of Dx, the observed down-regulation of Arm can be attributed to a ubiquitination-dependent mechanism. Our results substantiate this hypothesis, as post-MG132 treatment effectively rescues the loss of Arm in the presence of Dx overexpression (Figure 2). Additionally, we provide evidence that human Deltex (DTX1) exhibits a conserved function of proteasomal degradation of β-catenin (Sharma et al, 2023).

The Wg pathway is instrumental in embryonic development, dictating cell fate decisions and tissue patterning. The role of Dx in modulating the Wg gradient adds a layer of complexity to our understanding of how spatial and temporal cues are integrated to orchestrate precise developmental programs. Moreover, the conserved nature of the Dx-mediated regulatory mechanism suggests that targeting this pathway could have broad implications for diseases associated with dysregulated Wnt signaling. The research thus contributes not only to our fundamental understanding of cellular processes but also holds promises for translational applications in the field of therapeutic development.

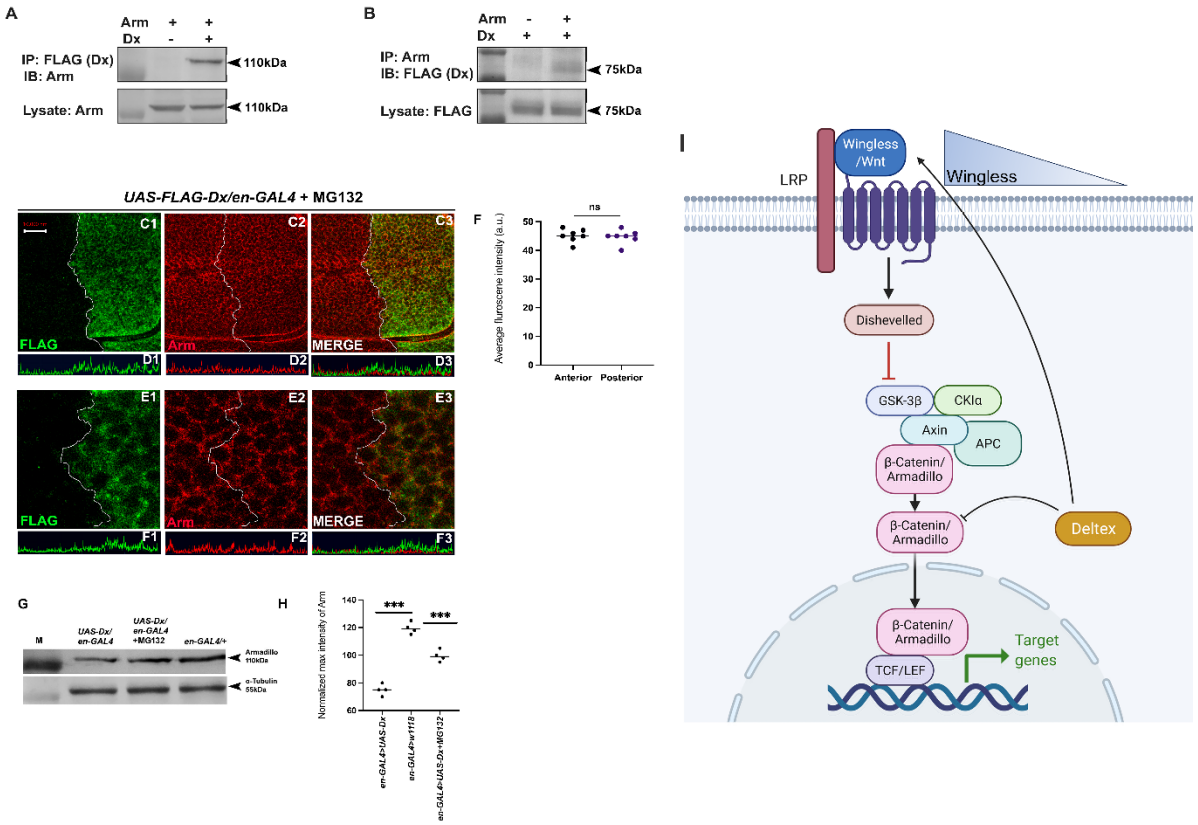

Figure 2: Dx degrades Arm by a proteasome-mediated mechanism. (A, B) Co-immunoprecipitation of FLAG-Dx and Arm. Co-immunoprecipitation was carried out with lysate over-expressing Arm and FLAG-Dx using GMAR-GAL4. The + symbol indicates the presence of lysate and the – symbol shows the absence of lysate. FLAG-Dx immunoprecipitated Arm was detected by anti-Arm antibody (A). Arm immunoprecipitated FLAG-Dx was detected by FLAG antibody. (C1-C3) Dx over-expressed discs treated with MG132 for 3hrs showed a rescue in Arm expression and a more intense Arm staining in the posterior compartment was found after proteasome inhibitor treatment. (D1-D3) Confocal Z stack Intensity profiling shows a comparable Arm level in the disc's posterior compartment. (E1-E3) A higher magnification picture shows no significant change in the expression of Arm in the posterior compartment of the disc suggesting the rescue in Arm degradation. (F) The graph shows the average Arm fluorescence intensity (a.u., arbitrary units) (n=7). ns; unpaired t-test. (G) Western blot analysis confirms the immunocytochemical studies where proteasome inhibitor MG132 increases Arm protein levels in Dx over-expressed tissue samples. (H) Graph G represents the intensity profiling of the Western blot. (I) The cartoon explains the two plausible mechanisms of Wg regulation through Dx. Dx facilitates Wg gradient formation on one hand and on the other it targets Arm for its degradation thereby regulating the signaling output. ***P<.001; unpaired t-test. Images in A-F are representatives of 3 independent experiments (n=6). Scale Bar: Scale Bar: A1-A4: 10µm. C1-C4: 2µm.

References

1. Cadigan, K.M., and R. Nusse. 1997. Wnt signaling: a common theme in animal development. Genes & development. 11:3286-3305.
2. Coombs, G.S., T.M. Covey, and D.M. Virshup. 2008. Wnt signaling in development, disease and translational medicine. Current drug targets. 9:513-531.
3. Lecuit, T., W.J. Brook, M. Ng, M. Calleja, H. Sun, and S.M. Cohen. 1996. Two distinct mechanisms for long-range patterning by Decapentaplegic in the Drosophila wing. Nature. 381:387-393.
4. Logan, C.Y., and R. Nusse. 2004. The Wnt signaling pathway in development and disease. Annu. Rev. Cell Dev. Biol. 20:781-810.
5. Matsuno, K., R.J. Diederich, M.J. Go, C.M. Blaumueller, and S. Artavanis-Tsakonas. 1995. Deltex acts as a positive regulator of Notch signaling through interactions with the Notch ankyrin repeats. Development. 121:2633-2644.
6. Mukherjee, A., A. Veraksa, A. Bauer, C. Rosse, J. Camonis, and S. Artavanis-Tsakonas. 2005. Regulation of Notch signalling by non-visual β-arrestin. Nature cell biology. 7:1191-1201.
7. Sharma, V., M. Mutsuddi, and A. Mukherjee. 2021a. Deltex cooperates with TRAF6 to promote apoptosis and cell migration through Eiger-independent JNK activation in Drosophila. Cell biology international. 45:686-700.
8. Sharma, V., M. Mutsuddi., and A. Mukherjee. 2021b. Deltex positively regulates Toll signaling in a JNK-independent manner in Drosophila. Genes to Cells. 26(4):254-263.

9. Sharma, V., B. Sarkar, M. Mutsuddi, and A. Mukherjee. 2022. Deltex modulates Dpp morphogen gradient formation and affects Dpp signaling in Drosophila. J Cell Sci. 135.Sharma, V., N. Sachan, B. Sarkar, M. Mutsuddi, and A. Mukherjee. 2023. E3 ubiquitin ligase Deltex facilitates the expansion of Wingless gradient and antagonizes Wingless signaling through a conserved mechanism of transcriptional effector Armadillo/β-catenin degradation. eLife12:RP88466. doi.org/10.7554/eLife.88466.1.
10. Sharma, V., N. Sachan, B. Sarkar, M. Mutsuddi, and A. Mukherjee. 2023. E3 ubiquitin ligase Deltex facilitates the expansion of Wingless gradient and antagonizes Wingless signaling through a conserved mechanism of transcriptional effector Armadillo/β-catenin degradation. eLife12:RP88466. doi.org/10.7554/eLife.88466.1.

Cadherins and Nectins in Action: From Embryogenesis to Tumorigenesis

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Adherens junction is a multiprotein junctional complex that establishes cell-to-cell adherence and regulates important biological events like early developmental processes, morphogenesis, cell migration, and various signaling pathways. The formation and maintenance of this junction are achieved through the dynamic assembly and organization of cadherins and the nectin family of cell adhesion molecules (Meng et al., 2009) (Figure 1a). Comprehending the role of cadherins and nectins in adherens junction formation has been one of the primary goals of our lab.

Cadherins engage in homophilic trans interactions to mediate cell-to-cell contact between interacting cells and establish adherens junction formation. Nectins, on the other hand, colocalize with cadherins and participate in heterophilic trans interactions to facilitate the cell adhesion function. Through their cytoplasmic domain, they then bind to the actin-binding adaptor molecules to link the interacting cell through the cytoskeletal framework and organize the architecture of the adherens junction. To date, extensive studies focus on the structure and function of these adhesion molecules, however, not much literature elucidates the direct interactive crosstalk among cadherins and nectins. Through biophysical studies, we have shown that N-cadherin and nectin-2 (Duraivelan et al., 2018) and N-cadherin and E-cadherin (Dash et al., 2022) directly interact with each other. Further screening and investigation revealed a novel interaction between E-cadherin and nectin-4 (unpublished data) (Figure 1a).

Discovered as uvomorulin, E-cadherin is a calcium-dependent transmembrane cell adhesion molecule that is expressed in zygotic stages to form epithelial junctions for embryo compaction and blastocyst formation (Larue et al., 1994). Following to the early developmental events, cadherins, and nectins, show tissue-specific expression and interact with a diverse group of biomolecules to regulate various physiological processes. Apart from these biological functions, the role of cadherins and nectins in host-pathogen interactions is an active area of research (Figure 1b). The cellular localization of cadherins and nectins make them an easy target by the pathogen to act as entry mediators. One noteworthy example is Listeria monocytogenes which exploits E-cadherin to enter into its host by the heterophilic interaction of internalin and host cell E-cadherin. In this regard, a comprehensive review by us discusses the role of cadherins in host-pathogen interactions (Dash et al., 2021). Nectins have also been extensively studied for their role in entry and infection of several viruses. Establishing contact between the host cells and the pathogens is the preliminary step for the pathogen to hijack the host system and thus understanding this step of pathogenesis is essential for treating any infectious disease. We are actively working on deciphering such host-pathogen interactions mediated by cadherins and nectins.

Cadherins and nectins play a critical role in normal development as they maintain tissue integrity. As a consequence, their dysregulated expression drives many developmental disorders (Figure 1c).

Moreover, most of the members of the cadherin and nectin family are implicated in cancer associated signalling pathways. While the downregulation of E-cadherin on epithelial cells, VE-cadherin on endothelial cells and cadherin-16 (Ksp-cadherin) on kidney cells triggers the disruption of cell junctions leading to tumour metastasis, the upregulation of N-cadherin, P-cadherin, R-cadherin, OB-cadherin, LI-cadherin or the desmosomal cadherin such as desmoglein 2 lists them as cancer biomarkers (Van Roy et al., 2014). Similarly, numerous studies discussing the upregulation of nectin and nectin-like molecules and their role in cancer progression are under focus (Duraivelan et al., 2021).

Several studies are being put forth that emphasize targeting cadherin and nectin molecules for designing anticancer agents. Recent literatures are in the limelight for cancer research and various curative interventions are proposed such as the idea of restoration of E-cadherin on the cell junction as a measure for treatment of gastric cancer by causing alterations in the EMT pathway, design of antagonists against N-cadherin (important mediator of EMT pathway) or P-cadherin targeted radioimmunotherapy (90 Y-FF-21101) to kill solid tumour. On a similar note, nectins are also in hotspot for cancer immunotherapy applications. Numerous therapeutic interventions targeting nectins are under research and development. One such noteworthy example is the FDA-approved enfortumab vedotin-ejfv (PADCEV®) for treating urothelial carcinoma that targets nectin-4, a new biomarker for several cancer conditions (Figure 1d). In this regard, the molecular and structural basis of nectin-4 and TIGIT (important immune checkpoint molecule) interaction was studied in our lab, which in future could be used for therapeutic purposes (Ganguly et al., 2023).

Cell adhesion molecules function to establish cellular connections and facilitate intercellular communication and signalling. Through these years, extensive research on their structure and function has provided us valuable information about their diverse physiological roles. Our study substantially contributes to the understanding of molecular crosstalk between the adherens junction proteins. In addition, we aim to comprehend the role of these molecules in their pathophysiological outcomes to design and develop novel therapeutics in the future.

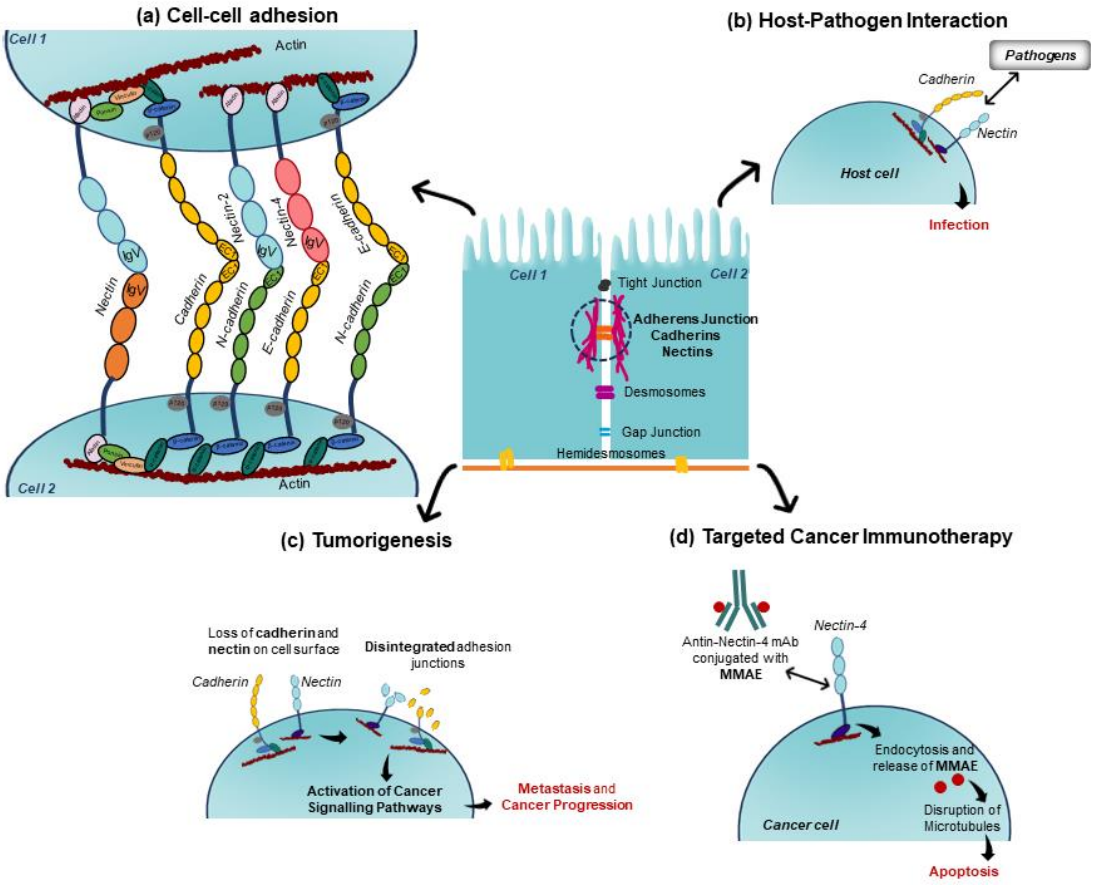

Figure 1: Cadherins and nectins at the adherens junction. (a) Primary role of cadherins and nectins in driving cell adhesion between interacting cells and governing morphogenesis and developmental process (b) Interaction of these surface localised molecules with the pathogen receptors facilitating pathogen entry and host pathogen interactions (c) Disruption of these junctional molecules triggering activation of associated cancer signalling pathways (d) Mode of action of Enfortumab Vedotin: nectin-4 targeted anticancer therapy causing disruption of microtubules thereby leading to apoptosis of cancer cell. MMAE-Monomethyl auristatin E.

References

1. W. Meng, and M. Takeichi. "Adherens junction: molecular architecture and regulation." Cold Spring Harbor perspectives in biology, 1 (6), a002899, (2009).
2. K. Duraivelan, A. J. Basak, A. Ghosh, D. Samanta, Molecular and structural bases of interaction between extracellular domains of nectin-2 and N-cadherin. Proteins, 86, 1157–1164 (2018).
3. S. Dash, et al., Heterophilic recognition between E-cadherin and N-cadherin relies on same canonical binding interface as required for E-cadherin homodimerization. Arch Biochem Biophys, 727 (2022).
4. L. Larue et al., E-cadherin null mutant embryos fail to form a trophectoderm epithelium. Proc Natl Acad Sci USA, 91, 8263–8267 (1994).
5. S. Dash, K. Duraivelan, D. Samanta, Cadherin-mediated host–pathogen interactions. Cell Microbiol, 23 (2021).
6. F. Van Roy, Beyond E-cadherin: Roles of other cadherin superfamily members in cancer. Nat Rev Cancer, 14, 121–134 (2014).
7. K. Duraivelan, D. Samanta, Emerging roles of the nectin family of cell adhesion molecules in tumour-associated pathways. Biochim Biophys Acta Rev Cancer, 1876 (2021).
8. N. Ganguli, P. Kumari, S. Dash, D. Samanta, Molecular and structural basis of TIGIT: Nectin-4 interaction, a recently discovered pathway crucial for cancer immunotherapy. Biochem Biophys Res Commun 677, 31–37 (2023).

Liver-derived S100A6 Mediates beta-Cell Dysfunction in NAFLD via an Inter-Organ Dialogue

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Non-Alcoholic Fatty Liver Disease (NAFLD) is an independent predictor of insulin resistance and type 2 diabetes mellitus (T2DM). However, how NAFLD affects the insulin-releasing pancreatic beta-cell function was not fully understood. T2DM is marked by increased blood glucose results from the interplay of insensitivity to insulin or inability to secrete enough insulin to dispose of glucose due to pancreatic beta-cell dysfunction. To fill the existing gap between the accumulation of hepatic lipids and failure of insulin secretion due to pancreatic beta-cell dysfunction in regulation, manifestation, and the development of both these pathophysiological phenotypes, our lab has designed an *in vitro* culture and *in vivo* animal model to understand the hepato-pancreatic crosstalk that contributes to the pathogenesis of NAFLD and T2DM (Dogra et al., 2022).

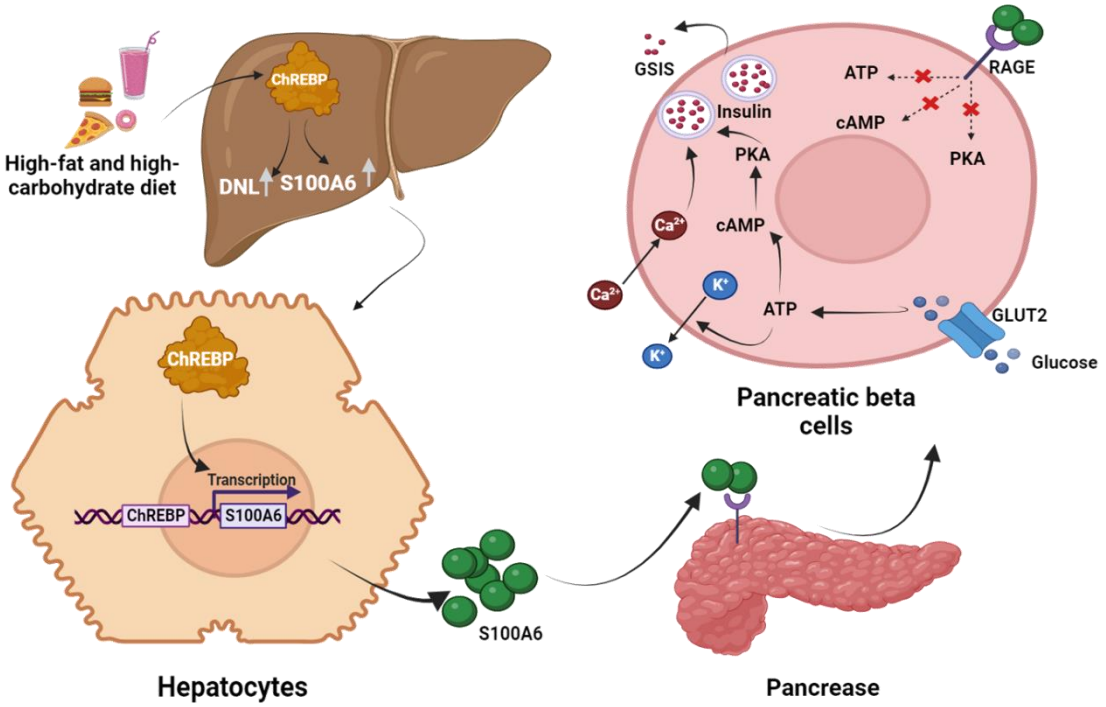

Figure 1: Proposed framework outlining hepatic ChREBP-mediated S100A6 expression and its role in regulating beta-cell function. A high-calorie diet (i.e., a high-fat and high-carbohydrate diet) leads to enhanced expression of hepatic ChREBP. ChREBP in turn leads to enhanced De novo lipogenesis and, simultaneously upregulates the transcription of S100A6 and its secretion. S100A6 engages with the RAGE receptor present on pancreatic beta-cells and impairs GSIS. S100A6-mediated RAGE receptor activation inhibits cAMP synthesis and also alters mitochondrial respiration.

We found a low molecular weight calcium-binding protein; S100A6 mediates β-cell dysfunction in NAFLD. Specifically, it appears that S100A6 impairs glucose-stimulated insulin secretion (GSIS) in pancreatic beta-cells which potentially explains why the development of T2DM is such a common outcome of NAFLD.We used data from human patients and *in vitro*, *ex vivo*, and *in vivo* experiments with mice and various cell lines. We found that serum expression levels of the S100A6 protein were elevated in human NASH patients and a high-fat diet-induced mouse model of NAFLD. In a series of gain- or loss-of-function experiments, we found that intrahepatic lipid accumulation increased hepatic and systemic levels of S100A6. Elevated levels of the S100A6 also appeared to be associated with increased lipogenesis and potentially adverse beta-cell function. Further, we demonstrated that carbohydrate response element-binding protein (ChREBP) can directly regulate the expression of S100A6. Our study showed endocrine communication between the liver and pancreas and discovered the cellular and molecular mechanism where hepatic messenger protein S100A6 directly mediates beta-cell dysfunction in NAFLD (Figure 1).

References

1. Surbhi Dogra, Debajyoti Das, Sujay K. Maity, Avishek Paul, Priya Rawat, P Vineeth Daniel, Kaushik Das, Souveek Mitra, Partha Chakrabarti, and Prosenjit Mondal (2022) Liver Derived S100A6 Propels β Cell Dysfunction in NAFLD. Diabetes 71 (11), 2284-2296.

CD4+ T cells, along with their Corresponding Membrane Protein CD40L, Protectively Mitigate Mouse Hepatitis Virus-Induced Neuroinflammation and Demyelination

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Multiple Sclerosis is a chronic, progressive, or relapsing-remitting demyelinating disorder that explicitly affects the central nervous system (CNS) (Filippi et al., 2018). Several infectious agents have been recognized as prospective causal factors of MS, owing to the occurrence of oligoclonal bands in the cerebrospinal fluid of more than 90% of MS patients (Gilden DH., 2005). Various experimental viral models have been employed to understand this infectious trigger of MS; among them, the Mouse hepatitis virus (MHV)-induced demyelinating model is noteworthy, where direct neural cell dystrophy could contribute to the pathology of myelin loss (Libbey and Fujinami, 2021; Das Sarma J, 2010). MHV is a positive sense, non-segmented, single-stranded RNA virus, having one of the largest RNA virus genomes at 31-32kb.

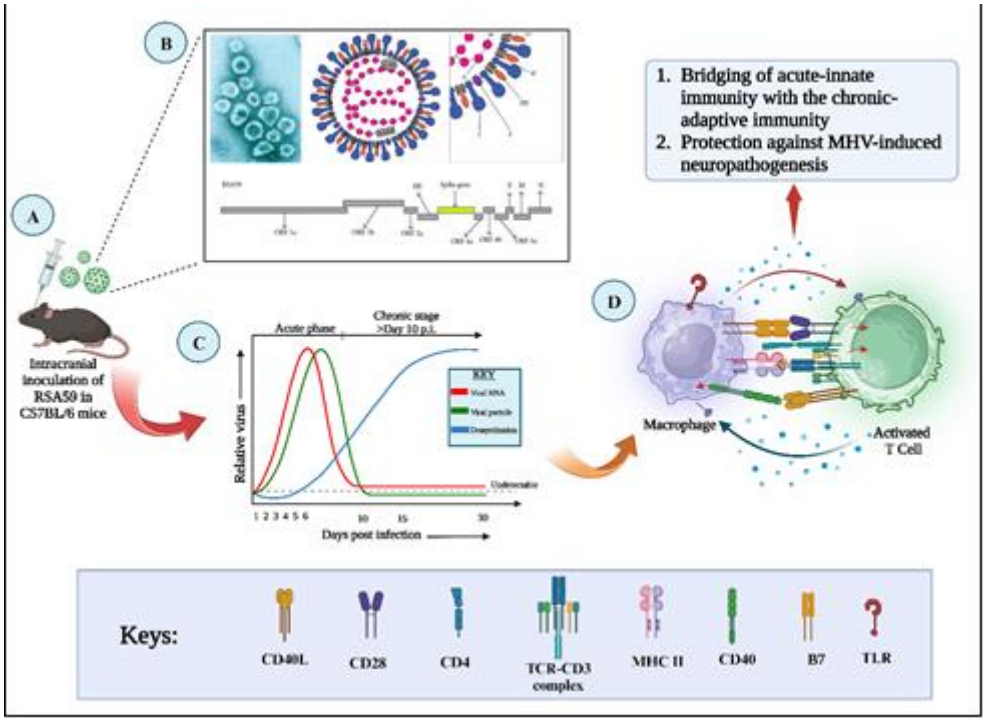

Figure 1. Intracranial inoculation of RSA59 in C57BL/6 mice (A). The genome of the virus MHV-RSA59 (B). Post-infection a biphasic disease course with acute-innate and chronic adaptive phases is seen (C). Interaction between CD4+ T cells and microglia/macrophages via the CD40/CD40L dyad bridges these two arms of immunity and mounts host immunity against MHV infection (D). [Created with BioRender.Com]

Targeted RNA recombination was used to generate various isogenic recombinant strains of MHV with structural protein variations; among them, MHV-RSA59 has been commonly employed to study the mechanisms of acute meningoencephalitis and chronic progressive demyelination (Das Sarma J., 2010) (Figure 1B). Upon intracranial inoculation of RSA59 (Figure 1A), a biphasic disease course is observed, beginning with acute-innate phase followed by a bridging acute-adaptive phase and finally a chronic-adaptive phase (Figure 1C). CD4+ T cells are multifaceted in coordinating and regulating the immune response to virus infections during the innate-adaptive bridging phase. Their functions contribute to the effective elimination of the virus, and the prevention of immune-mediated pathology. In our study, using the virus-induced neuroinflammatory demyelination model we found a protective role of CD4+ T in contrast to the pathogenic role of CD4+ T cells in MS and its experimental autoimmune encephalitis model, EAE (Zhang et al., 1992). Additionally, we also investigated CD40 ligand (CD40L), also known as CD154, a cell surface protein primarily expressed on activated T cells. It plays a crucial role in the immune system by interacting with its cognate receptor, CD40, which is present in various antigen presenting cells, including B cells, dendritic cells, and macrophages. The CD40-CD40L interaction is involved in several essential immune processes, like B cell activation, class switching of antibodies, macrophage activation (Chakravarty D. et al., 2020) (Figure 1D). Our research revealed that RSA59 infection triggers a substantial increase in the activation marker of CD4+ T cells namely CD40L, in wild-type (WT) mice. Subsequent experiments involving CD40L-deficient (CD40L-/-) mice emphasized the crucial protective function of CD40L in RSA59-induced neuroinflammatory demyelination and axonal pathology (Saadi et al., 2021). In summary, our findings indicate the essential role of CD4 and CD40L in orchestrating a host immune response crucial for activating microglia/macrophages, eradicating the virus from the central nervous system (CNS), and maintaining indispensable homeostasis.

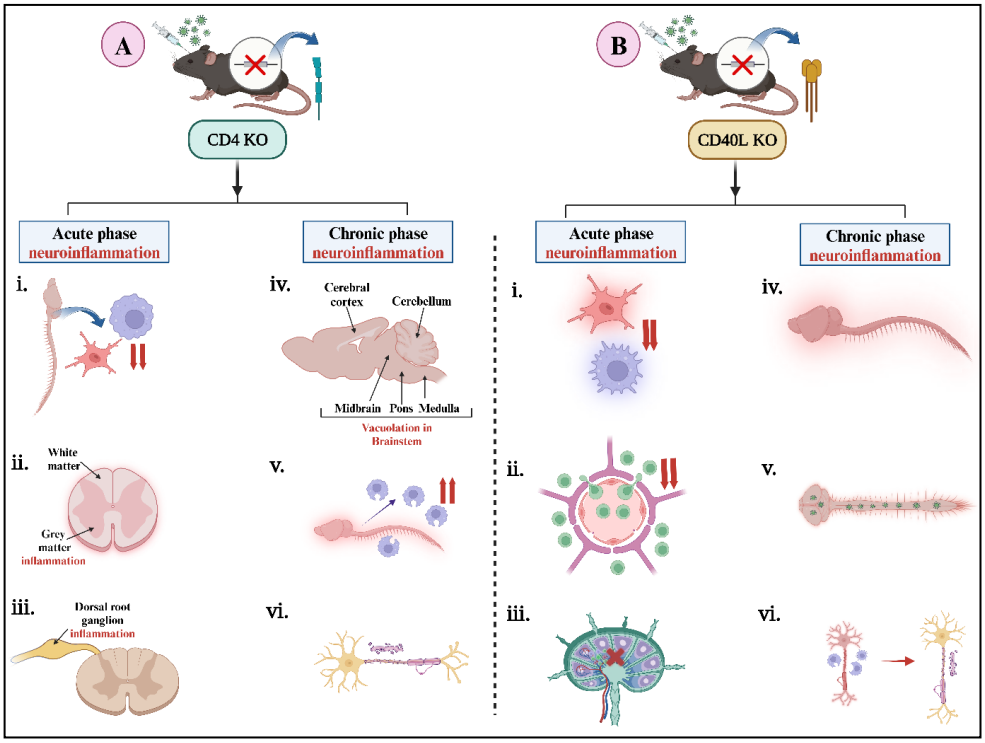

Figure 2. Consequences of RSA59 infection in CD4$^{-/-}$ (A) and CD40L$^{-/-}$ mice (B). (A) In CD4$^{-/-}$ mice, (i) a significant decrease in CD11b^{+} microglia/macrophages, (ii) poliomyelitis, (iii) dorsal root ganglionic infection was observed during the acute phase; whereas (iv) bulbar vacuolation, (v) presence of phagocytic macrophages in the inflamed regions and (vi) severe demyelination were observed during chronic phase. (B) In CD40L$^{-/-}$ mice, (i) microglia/macrophage activation got dampened, (ii) Decreased infiltration of CD4^{+} T cells, and (iii) Impaired CD4^{+} T cell activation and priming in CLN during the acute phase of the disease; whereas (iv) heightened inflammation of CNS, (v) viral persistence and (vi) myelin stripping by phagocytic macrophages were observed during chronic phase. [Created with BioRender.Com].

Age-matched C57BL/6 WT, CD4 -/-, and CD40L -/- mice were intracranially inoculated with RSA59 to study the acute phase neuroinflammation and chronic phase demyelination. Histopathology to study the neuroinflammation status, flow cytometry to study the immune cell infiltration kinetics and activation status, gene expression analysis to study the cytokine and chemokine profiles, and viral titre to check the presence of the virus were performed. We found RSA59 infected functional CD4-deficient (CD4-/-) mice displayed a marked decrease in CD11b+ microglia/macrophages during the acute phase. Concurrently, there was heightened viral replication, with persistent viral antigen still evident at day 30 post-inoculation. We reported for the first time, grey matter inflammation-poliomyelitis and dorsal root ganglionic inflammation in the CD4-/- mice. Additionally, CD4-/- mice also showed an increased susceptibility to chronic phase encephalitis and demyelination. However, during the chronic phase, CD11b+ microglia/macrophages were present in the characteristic phagocytic phenotype in both white and grey matter of the brain and spinal cord in CD4-/- mice. Thus, establishing that CD4+ T cells not only contribute to alleviating immunopathological disease, but also potentially play a role in clearing the pathogen (Chakravarty D. et al., 2020) (Figure 2A). Investigating further, the molecular pathway, we also determined that the expression of CD40L on CD4+ T cells is pivotal in initiating the host's immune response against MHV. In the absence of CD40L, mice exhibited increased mortality, heightened disease severity characterized by a reduced neuroinflammatory response by microglia/macrophage during the acute phase. This was accompanied by impaired priming and expansion of CD4+T cells in the cervical lymph node and their diminished infiltration into the CNS following RSA59 inoculation. Lack of a protective immune response from the CD4+ T resulted in extensive virus replication in the CNS which led to the persistence of the characteristic amoeboid phagocytic phenotype carrying microglia/macrophages together culminating in severe demyelination and axonopathy (Saadi et al., 2021) (Figure 2B).

Our findings indicate the essential role of CD4+ T cells and CD4+ T cell-expressed CD40L in eliminating viral particles, inducing anti-inflammatory microglial polarization, and regulating chronic progressive axonal degeneration. Our findings have clearly indicated that CD4+ T cells and their interaction with the microglia/macrophages via the CD40L-CD40 dyad plays an indispensable role in maintaining protective immune homeostasis in the CNS.

References

1.Filippi M, Bar-Or A, Piehl F, Preziosa P, Solari A, Vukusic S, Rocca MA. 2018. Multiple sclerosis. Nature Reviews Disease Primers 4:43.
2. Gilden DH. 2005. Infectious causes of multiple sclerosis. Lancet Neurol 4:195-202.
3.Libbey JE, Fujinami RS. 2021. Viral mouse models used to study multiple sclerosis: past and present. Arch Virol 166:1015-1033.
4.Das Sarma J. 2010. A mechanism of virus-induced demyelination. Interdiscip Perspect Infect Dis 2010:109239.
5.Zhang J, Weiner HL, Hafler DA. 1992. Autoreactive T cells in multiple sclerosis. Int Rev Immunol 9:183-201.
6.Chakravarty D, Saadi F, Kundu S, Bose A, Khan R, Dine K, Kenyon LC, Shindler KS, Das Sarma J. 2020. CD4 Deficiency Causes Poliomyelitis and Axonal Blebbing in Murine Coronavirus-Induced Neuroinflammation. J Virol 94.
7.Saadi F, Chakravarty D, Kumar S, Kamble M, Saha B, Shindler KS, Das Sarma J. 2021. CD40L protects against mouse hepatitis virus-induced neuroinflammatory demyelination. PLoS Pathog 17:e1010059.

LncRNA JINR1 Interacts with RBM10 and NF-κB to Promote Flavivirus-Induced Neuronal Cell Death and Virus Replication

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Long-noncoding RNAs (LncRNAs) have emerged as regulators of cellular processes in health and disease (Rinn and Chang, 2020, 2012). Viral infections are complex processes based on an intricate network of molecular interactions with host cells. Viruses hijack the host cellular machinery for their replication, circumventing the natural defense mechanisms triggered by the infected cell. Most of the knowledge about cellular and viral molecular players during infection is in the protein realm; however, the relevance of cellular lncRNAs as relevant players within the context of viral infection remains poorly understood. Central nervous system infection by flaviviruses such as Japanese encephalitis, Dengue, and West Nile viruses results in extensive neuroinflammation and neuronal cell death (Ashraf et al., 2021; Constant et al., 2022; Jan et al., 2000; Niranjan et al., 2019; Wongchitrat et al., 2019). The role of lncRNAs in regulating neuroinflammation and neuronal cell death during flavivirus infection remains poorly understood (Ashraf et al., 2021).

To identify differentially regulated lncRNAs in response to flavivirus infection in the human neuronal cell line, we used an unbiased approach to profile the whole transcriptome of the JEV-infected neuronal cell line (Tripathi et al., 2023). We identified several lncRNAs whose expression is altered during flavivirus infection (Tripathi et al., 2023). We functionally characterized a lncRNA named JINR1 (JEV-induced noncoding RNA 1) from this whole transcriptome screen. JINR1 is induced in a time and dose-dependent manner upon flavivirus infection (Tripathi et al., 2023). Genomic region upstream of the JINR1 locus contains a putative NF-κB consensus motif, and treating cells with an inhibitor of NF-κB suppressed the induction of JINR1 (Tripathi et al., 2023). Furthermore, the ChIP qRT-PCR assay confirmed increased occupancy of the p65 subunit of NF-κB on the JINR1 promoter during JEV infection. This suggests that JEV, via NF-κB activation, drives the transcription of JINR1 (Tripathi et al., 2023).

JINR1 knockdown and overexpression revealed that JINR1 positively regulates JEV replication and neuronal apoptosis (Tripathi et al., 2023). Since lncRNAs are known regulators of gene expression, we performed knockdown and overexpression of JINR1 during flavivirus infection, and we observed deregulation of a subset of NF-κB target genes involved in ER stress and neuroinflammation (Tripathi et al., 2023).

But how exactly does JINR1 regulate viral replication and neuronal apoptosis? To address this question, we analyzed protein interaction partners of JINR1; we used the POSTAR database and identified several proteins; among them, we focussed on RBM10. RBM10 was previously known to regulate inflammatory gene expression (Atsumi et al., 2017) and immune responses during dengue infection (Pozzi et al., 2020). Hence, we study the role of RBM10 during JEV pathogenesis; first, we confirmed the association between RBM10 and lncRNA JINR1 during JEV infection in SH-SY5Y cells using formaldehyde crosslinked RNA immunoprecipitation (RIP). Furthermore, we tested whether RBM10 plays any role in flavivirus

pathogenesis. Similar to JINR1, RBM10 also promotes viral replication and neuronal apoptosis during flavivirus infection. Moreover, like JINR1, RBM10 regulates JEV-induced ER stress and inflammatory gene expression upon flavivirus infection in SH-SY5Y cells.

Next, we explored the mechanism behind JINR1/RBM10-mediated increase in flavivirus replication. Since JINR1 and RBM10 promote the expression of NF-kB target gene GRP78, which is known to promote flavivirus replication and entry in host cells, we further evaluated the impact of GRP78 overexpression on flavivirus replication in SH-SY5Y cells during JINR1/RBM10 depletion. Interestingly, GRP78 overexpression completely prevents the decrease in flavivirus replication due to JINR1 or RBM10 depletion.

We further explored how RBM10 and JINR1 regulate gene expression during flavivirus infection. Since RBM10 regulates gene expression by regulating NF-κB transcriptional activity (Atsumi et al., 2017), and many lncRNAs are known to interact with NF-κB to regulate its transcriptional activity (Rapicavoli et al., 2013; Xue et al., 2019), we hypothesized that JINR1 directly interacts with p65 and regulate its recruitment to the promoters of the ER stress and neuroinflammatory gene (Tripathi et al., 2023). RIP analysis confirmed the interaction between JINR1 and the p65 subunit of NF-κB. Moreover, ChIP-qRT-PCR upon JINR1 and RBM10 depletion revealed that JINR1 and RBM10 regulate p65 recruitment to the promoter of genes involved in ER stress and neuroinflammation during flavivirus infection (Tripathi et al., 2023). Interestingly, we also found that JINR1 and RBM10 reciprocally regulate each other's expression through NF-κB. Our results suggest that JINR1 works as a scaffold for RBM10 and NF-κB to regulate the recruitment of NF-κB to its target gene during flavivirus infection (Tripathi et al., 2023).

The identification of the involvement of lncRNA JINR1 in regulating viral replication and neuronal cell death opens new avenues for developing novel treatment approaches for flavivirus infection (Tripathi et al., 2023). Much previous work has focused on the roles of mRNAs, even though they comprise only about 2% of the human genome. Further studies and efforts will help to grasp the complexity and dynamics of lncRNAs during virus infection and will allow additional insights into the function of lncRNAs in the cellular response to virus infection.

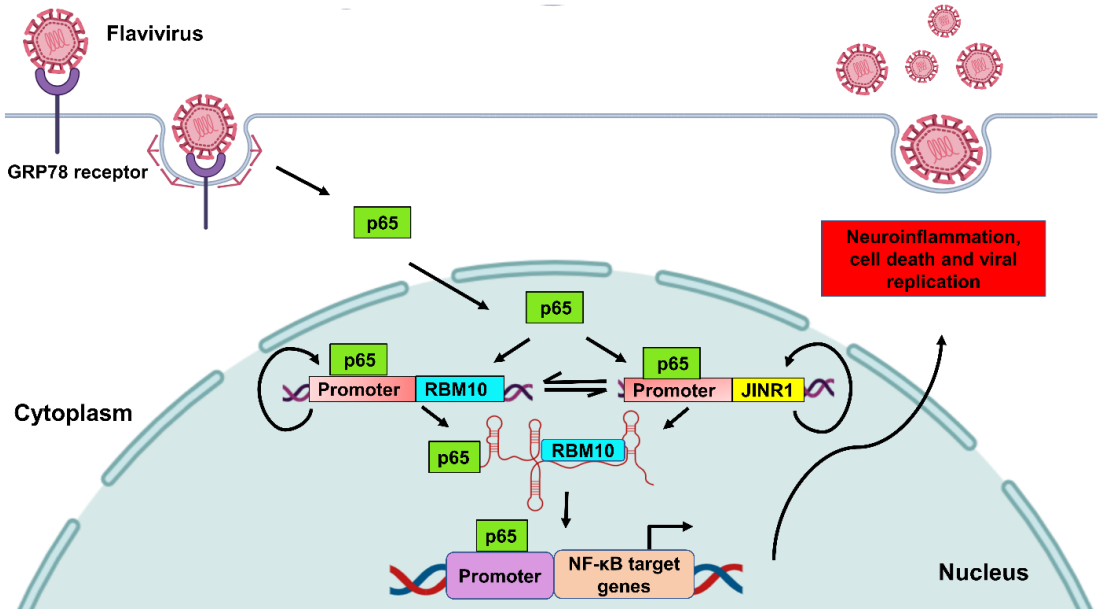

Figure 1. Flavivirus infection results in a p65-mediated increase in lncRNA JINR1 expression. It interacts with RBM10 and p65 to promote p65 binding to its target gene promoters. JINR1 regulates flavivirus replication, neuroinflammation, and neuronal cell death by promoting the expression of NF-κB target genes. (Adapted from DOI: https://doi.org/10.1128/jvi.01183-23).

References

1. Ashraf U, Ding Z, Deng S, Ye J, Cao S, Chen Z. Pathogenicity and virulence of Japanese encephalitis virus: Neuroinflammation and neuronal cell damage. Virulence 2021;12:968–80. https://doi.org/10.1080/21505594.2021.1899674.
2. Atsumi T, Suzuki H, Jiang J-J, Okuyama Y, Nakagawa I, Ota M, et al. Rbm10 regulates inflammation development via alternative splicing of Dnmt3b. International Immunology 2017;29:581–91. https://doi.org/10.1093/intimm/dxx067.
3. Constant O, Barthelemy J, Nagy A, Salinas S, Simonin Y. West Nile Virus Neuroinfection in Humans: Peripheral Biomarkers of Neuroinflammation and Neuronal Damage. Viruses 2022;14:756. https://doi.org/10.3390/v14040756.
4. Jan JT, Chen BH, Ma SH, Liu CI, Tsai HP, Wu HC, et al. Potential dengue virus-triggered apoptotic pathway in human neuroblastoma cells: arachidonic acid, superoxide anion, and NF-kappaB are sequentially involved. J Virol 2000;74:8680–91. https://doi.org/10.1128/jvi.74.18.8680-8691.2000.
5. Niranjan R, Muthukumaravel S, Jambulingam P. The Involvement of Neuroinflammation in Dengue Viral Disease: Importance of Innate and Adaptive Immunity. Neuroimmunomodulation 2019;26:111–8. https://doi.org/10.1159/000501209.
6. Pozzi B, Bragado L, Mammi P, Torti MF, Gaioli N, Gebhard LG, et al. Dengue virus targets RBM10 deregulating host cell splicing and innate immune response. Nucleic Acids Res 2020;48:6824–38. https://doi.org/10.1093/nar/gkaa340.
7. Rapicavoli NA, Qu K, Zhang J, Mikhail M, Laberge R-M, Chang HY. A mammalian pseudogene lncRNA at the interface of inflammation and anti-inflammatory therapeutics. Elife 2013;2:e00762. https://doi.org/10.7554/eLife.00762.
8. Rinn JL, Chang HY. Long Noncoding RNAs: Molecular Modalities to Organismal Functions. Annu Rev Biochem 2020;89:283–308. https://doi.org/10.1146/annurev-biochem-062917-012708.
9. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. Annu Rev Biochem 2012;81:145–66. https://doi.org/10.1146/annurev-biochem-051410-092902.
10. Tripathi S, Sengar S, Shree B, Mohapatra S, Basu A, Sharma V. An RBM10 and NF-κB interacting host lncRNA promotes JEV replication and neuronal cell death. J Virol 2023:e0118323. https://doi.org/10.1128/jvi.01183-23.
11. Wongchitrat P, Samutpong A, Lerdsamran H, Prasertsopon J, Yasawong M, Govitrapong P, et al. Elevation of Cleaved p18 Bax Levels Associated with the Kinetics of Neuronal Cell Death during Japanese Encephalitis Virus Infection. Int J Mol Sci 2019;20:E5016. https://doi.org/10.3390/ijms20205016.
12. .Xue Z, Zhang Zimu, Liu H, Li W, Guo X, Zhang Zhihui, et al. lincRNA-Cox2 regulates NLRP3 inflammasome and autophagy mediated neuroinflammation. Cell Death Differ 2019;26:130–45. https://doi.org/10.1038/s41418-018-0105-8.

Decoding Signaling Pathways Crucial for Acquiring Chemoresistance and Metastasis

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The acquirement of chemoresistance in cancer is one of the most disconcerting issues plaguing the scientific community. Perturbations/rewiring of key signaling pathways have often been attributed to this chemoresistant phenotype. However, we have only scratched the surface, as newer studies are still furthering our understanding of the signaling cascades associated with resistance. Our lab has been interested in identifying the dichotomous role of many such key signaling hubs linked with acquired therapy resistance of Epithelial Ovarian Cancer (EOC).

Considered to be the deadliest gynecological malignancy, EOC has an abysmal 5-year survival rate of <30%. In 2020, 0.3 million cases were reported worldwide which is estimated to rise to 0.4 million by 2040. In India, EOC is the 3rd most common cancer amongst women and the deadliest amongst the gynecological malignancies with a dismal 5-year survival rate (<40%) (Ferlay et al., 2008). Two major challenges associated with EOC are late detection and development drug resistance and relapse within short duration of the end of the last treatment cycle. The standard treatment of care involves platinum-taxol treatment either pre- or post-debulking surgery. The platinum free-interval (PFI) forms the basis of devising a chemotherapeutic regimen upon recurrence. Resistant relapse (disease relapsing within 6 months of the last treatment dose) cases are the most critical as they include a heterogenous group of tumors and they fail to respond when re-challenged with platinum (Luvero et al., 2014). Second-line chemotherapeutics like topotecan, irinotecan, gemcitabine, etc. do exist but they have a poor response rate of only 15-30% (Tomao et al., 2017; Kucukoner et al., 2012). Therefore, we believe delineating the underlying signaling pathways during different stages in the acquirement of chemoresistance would allow targeting and thereby reduce the tumor burden.

Towards this, we developed clinically relevant A2780 and OAW42 ovarian cancer cell lines for cisplatin, paclitaxel and cisplatin-paclitaxel. To identify the dynamic molecular changes, the cells were categorized into early-resistant (ER) and late-resistant (LR) cells (Shenoy at al., 2022). Reports from the lab highlight the importance of insulin-like growth factor-1 receptor (IGF-1R) during acquirement of chemoresistance. Under physiological conditions, IGF-1R plays a crucial role in organ growth and development, disruption of which causes defects like hypoplasia, delayed bone development, defective skin formation, etc (Werner et al., 2023). We found that IGF-1R signaling played an indispensable role during the onset of chemoresistance which eventually attenuates at the late stage. Interestingly, hyper-activation of AKT was observed in late resistance, which triggers a feedback repression of IGF-1R, explaining the redundant role of IGF-1R in later stages of developing resistance (Singh at al., 2014). A small cohort of neo-adjuvant paired cases of high-grade serous ovarian cancer (HGSOC) patients reflected a similar transcriptional surge in IGF-1R expression. The undulating IGF-1R expression across stages of acquired resistance, is primarily due to the involvement of runt-related transcription factor 1 (RUNX1), which significantly upregulates IGF-1R expression in the early stages of chemoresistance.

RUNX1, otherwise instrumental in hematopoiesis, along with Forkhead box O3a (FOXO3a), transcriptionally regulates IGF-1R in the ER cells. However, FOXO3a falls off from the IGF-1R promoter in the LR cells, even though the promoter occupancy of RUNX1 remains unchanged. This low occupancy is attributed to the AKT-mediated degradation of FOXO3a in the LR cells (Dhadve et al., 2020). The early resistant cells employ ERK1/2-mediated induction and completion of the autophagic flux indicating that enhanced IGF-1R signaling culminates in controlling the autophagic flux during the onset of chemoresistance and fosters cellular viability by evading chemotherapeutic stress (Bishnu et al., 2021) (Figure 1). An autophagy sensor (mtFL-p62), designed in the lab, suggests that blocking this autophagy flux using trametinib (ERK1 inhibitor) and/or chloroquine (autophagy blocker) led to higher therapeutic efficacy. This stage-specific reliance on ERK1/2-mediated autophagy further opens up a therapeutic window in combating chemoresistance (Bishnu et al., 2021).

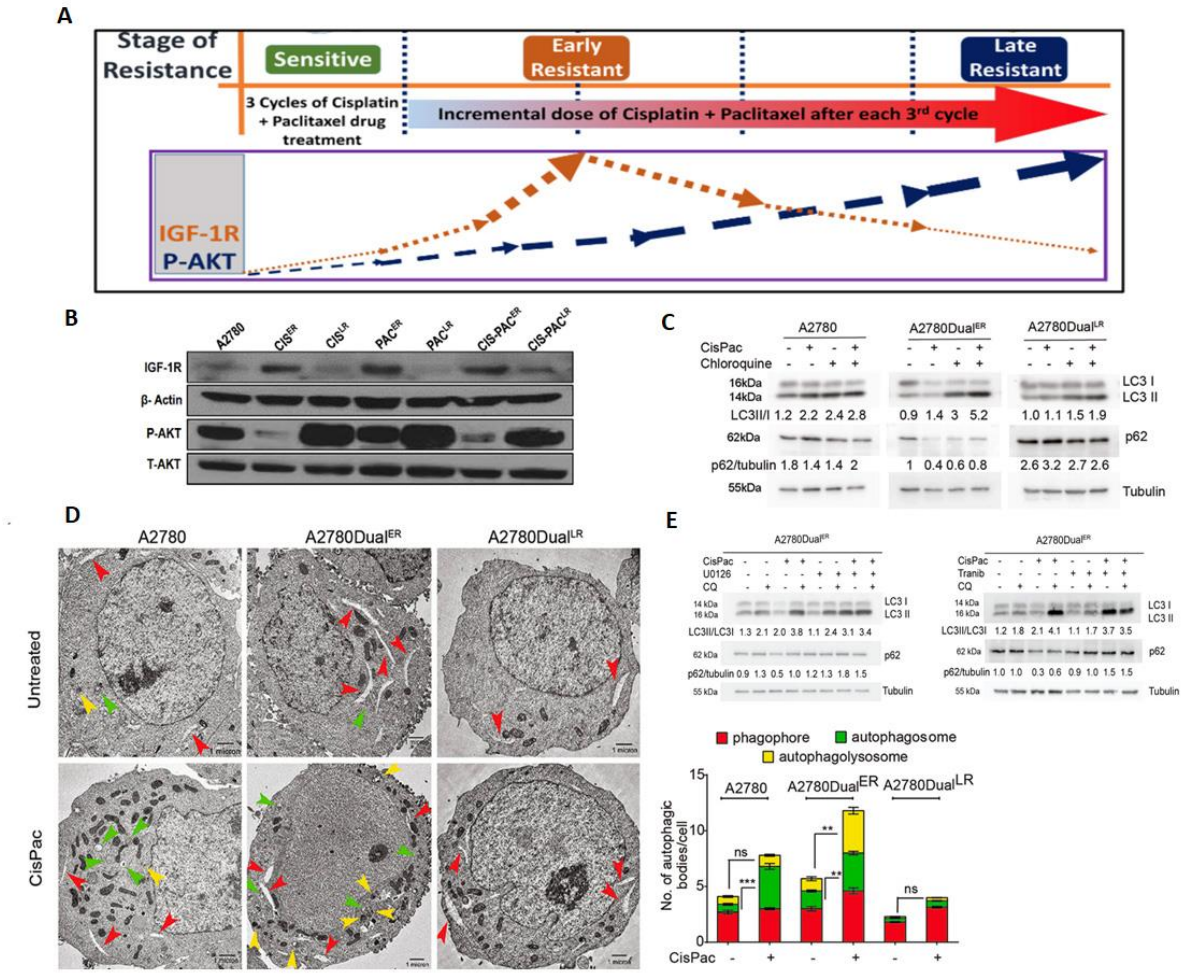

Figure 1: Pulsatile expression and role of IGF-1R during different stages of acquired chemoresistance: A) Schematic representing the pulsatile expression of IGF-1R during the acquirement of resistance. B) IGF-1R and P-AKT protein expression in A2780 resistant models (adapted from [7]). C) Immunoblot depicting LC3I–II conversion and p62 level post 24 h of CQ treatment either alone or in combination with CisPac. Highest differences in LC3I–II conversion and p62 accumulation were observed in A2780-dualER cells treated with CisPac in presence and absence of CQ compared to sensitive and A2780-dualLR cells. D) Representative electron microscopy images and quantification of sensitive, early and late resistant A2780 with or without CisPac treatment depicting individual autophagic structure phagophore (red arrow), autophagosome (green arrow), and autophagolysosome (yellow arrow). E) Immunoblot depicting increased LC3 conversion and p62 accumulation in CisPac + CQ-treated A2780-dualER cells in comparison to cells treated with only CisPac, while application of CQ along with CisPac + U0126 or CisPac + Trametinib (Tranib) did not alter LC3 conversion or p62 level in comparison to cells treated with CisPac + U0126 or CisPac + Trametinib. G (adapted from (Bishnu et al., 2021).

Cancer stem cell (CSC) population plays a pivotal role in resistance and is also attributed to tumor recurrence. Our studies suggest that the CSC population increase with an incremental acquirement of resistance (irrespective of drugs). However, this CSC population show a characteristically distinct tumor growth kinetics at different stages. The CSC population during the early stages of resistance show rapid tumor initiation whereas at the later stages there is a severe delay in tumor formation (Singh at al., 2016). This higher tumorigenic potential is attributed to the IGF-1R-AKT signaling critical in maintaining this subpopulation of cancer cells. Further studies also reveal that the increase in the CSC properties is due to RUNX1-mediated upregulation of ID1/ID3 proteins thereby preventing differentiation. This signaling axis is paramount in the maintenance of CSCs, which upon RUNX1 inhibition gets significantly affected and results in chemo-sensitization of the CSCs to chemotherapeutics (Dhadve et al., 2022).

In order to study the tumor dissemination pattern of EOC, we have also developed the A2780 orthotopic mouse model. This model is primarily characterized by an absence of ascites (the preferred route of tumor dissemination) and lung metastasis (a rarity in EOC metastases). This particular lung homing is observed as the result of RUNX1-mediated upregulation of $\alpha_6\beta_4$ integrin. Cancer cells at the late stage of resistance display the secretion of increased IGF-1, when co-cultured with lung primary fibroblasts. Even though these cells express relatively lower levels of IGF-1R, the secreted IGF1 and $\alpha_6\beta_4$ integrin successfully forms a tripartite complex. This complex relays the signal to upregulate S100A4 expression, instrumental in priming the pre-metastatic niche (activated lung fibroblasts) in the lungs. These activated lung fibroblasts allow the re-modelling of the organ-microenvironment to provide a conducive niche for the tumor cells to home and eventually form secondary tumors (Deo et al., 2022) (Figure 2).

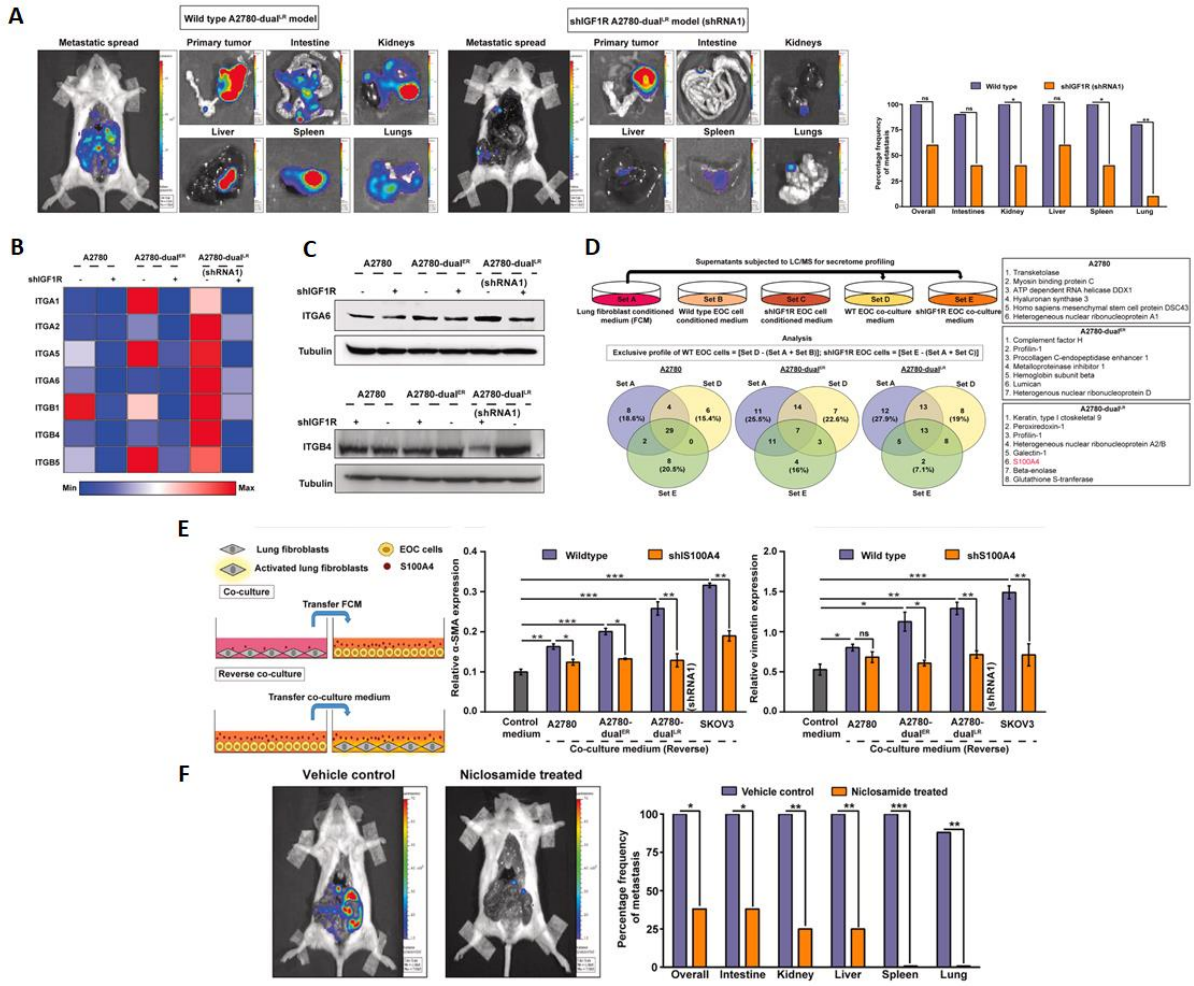

Figure 2: Role of IGF-1R signaling in metastasis and organ homing: A) IGF1R inhibition attenuates lung-tropism of A2780-dualLR cells through impairment of their interaction with resident fibroblasts. Post-mortem bioluminescence imaging of the whole body and individual organs of wild type and IGF1R-KD (shRNA1) A2780-dualLR orthotopic xenograft mouse models. B) Heat map showing the relative expression profile (transcript level) of various integrins (α1, α2, α5, α6, β1, β4, and β5) mapped in the A2780 chemoresistant model along with the corresponding IGF1R knock-downs. C) After IGF1R knock-down, the expression of α6 and β4 integrins was downregulated across the A2780 chemoresistant model. D) Graphical representation of various conditioned media subjected to LC/MS for secretome profiling. The Venn diagram depicts the number/percentage of overlapping and exclusive candidates between lung fibroblast conditioned medium (FCM), wildtype EOC co-culture media, and shIGF1R EOC-co-culture media for A2780, A2780-dualER, and A2780-dualLR cells. E) Schematic representation of the reverse co-culture strategy designed to evaluate the reciprocal effect of secretory S100A4 on primary lung fibroblast cells and assessment of relative expression levels of α-SMA and vimentin in primary lung fibroblast cells under reverse co-culture conditions indicated S100A4-mediated activation of lung fibroblast cells that correlated with the S100A4 levels in the co-culture medium. F) Post mortem whole-body bioluminescence imaging of metastatic dissemination clearly showed significantly reduced tumor burden in the niclosamide (S100A4 inhibitor)-administered group as compared to the vehicle-treated control. Pharmacologic inhibition of S100A4 effectively restricted the metastatic dispersal of A2780-dualLR cells and completely abolished the colonization of A2780-dualLR cells to spleen and lungs (adapted from (Deo et al., 2022)).

Until now, our studies have been successful in punctuating a universal role of IGF-1R signaling axes which keeps switching the activation of its downstream targets from ERK1/2 during the early stages of acquiring chemoresistance to AKT1/2 at the late stage. Current work in our lab involves further exploration of the role of RUNX1/ID axis in CSC maintenance and chemoresistance. The involvement of multiple signaling pathways and consistent signal switching during different stages of resistance highlights the dynamicity and malleability of the cancer cells to adapt to its ever-changing environment.

References

1. Ferlay, J., Shin, H.R., Bray, F., Forman, D., Mathers, C., and Parkin, D.M.: 'Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008', Int J Cancer, 2010, 127, (12), pp. 2893-2917
2. Luvero, D., Milani, A., and Ledermann, J.A.: 'Treatment options in recurrent ovarian cancer: latest evidence and clinical potential', Ther Adv Med Oncol, 2014, 6, (5), pp. 229-239
3. Tomao, F., Marchetti, C., Romito, A., Di Pinto, A., Di Donato, V., Capri, O., Palaia, I., Monti, M., Muzii, L., and Benedetti Panici, P.: 'Overcoming platinum resistance in ovarian cancer treatment: from clinical practice to emerging chemical therapies', Expert Opin Pharmacother, 2017, 18, (14), pp. 1443-1455
4. Kucukoner, M., Isikdogan, A., Yaman, S., Gumusay, O., Unal, O., Ulas, A., Elkiran, E.T., Kaplan, M.A., Ozdemir, N., Inal, A., Urakci, Z., and Buyukberber, S.: 'Oral etoposide for platinum-resistant and recurrent epithelial ovarian cancer: a study by the Anatolian Society of Medical Oncology', Asian Pac J Cancer Prev, 2012, 13, (8), pp. 3973-3976
5. Shenoy, P.S., Chakraborty, S., Gaikwad, S.M., Sakpal, A., and Ray, P.: 'Developing Clinically Relevant Acquired Chemoresistance Models in Epithelial Ovarian Cancer Cell Lines', Bio Protoc, 2022, 12, (3), pp. e4310
6. Werner, H.: 'The IGF1 Signaling Pathway: From Basic Concepts to Therapeutic Opportunities', International Journal of Molecular Sciences, 2023, 24, (19), pp. 14882
7. Singh, R.K., Gaikwad, S.M., Jinager, A., Chaudhury, S., Maheshwari, A., and Ray, P.: 'IGF-1R inhibition potentiates cytotoxic effects of chemotherapeutic agents in early stages of chemoresistant ovarian cancer cells', Cancer Lett, 2014, 354, (2), pp. 254-262
8. Dhadve, A.C., Hari, K., Rekhi, B., Jolly, M.K., De, A., and Ray, P.: 'Decoding molecular interplay between RUNX1 and FOXO3a underlying the pulsatile IGF1R expression during acquirement of chemoresistance', Biochim Biophys Acta Mol Basis Dis, 2020, 1866, (6), pp. 165754
9. Bishnu, A., Phadte, P., Dhadve, A., Sakpal, A., Rekhi, B., and Ray, P.: 'Molecular imaging of the kinetics of hyperactivated ERK1/2-mediated autophagy during acquirement of chemoresistance', Cell Death Dis, 2021, 12, (2), pp. 161
10. Singh, R.K., Dhadve, A., Sakpal, A., De, A., and Ray, P.: 'An active IGF-1R-AKT signaling imparts functional heterogeneity in ovarian CSC population', Scientific Reports, 2016, 6, (1), pp. 36612
11. DHADVE, A.-C., and RAY, P.: 'An active RUNX1-ID1/ID3 axis governs differentiation and chemoresistance of cancer stem cell population in epithelial ovarian cancer cells', BIOCELL, 2022, 46, (1), pp. 75—86
12. Deo, A.N., Thorat, R., Dhadve, A.C., De, A., Rekhi, B., and Ray, P.: 'IGF1R-α6 integrin-S100A4 network governs the organ-specific metastasis of chemoresistant epithelial ovarian cancer cells', Biochim Biophys Acta Mol Basis Dis, 2022, 1868, (1), pp. 166282

Triumvirate's of HPIP, RUFY3, and RAB5: a Cellular Ballet in Cancer Cell Migration

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In the complex rhythm of life, living organisms possess the incredible capacity to reproduce and migrate while being finely attuned to the nuances of their surrounding environment (Nathan et al., 2013). Similarly, cancer cells are endowed with remarkable adaptability; they sense environmental stress, such as lack of oxygen (hypoxia) and nutrient levels, and trigger cellular reorganization to facilitate migration (Wu et al., 2021). The migration and invasion are the main culprits underlying patient mortality (Zijl et al., 2011) and involve a sophisticated interplay of integrins, focal adhesion kinase (FAK), and Src kinases (Serrels et al., 2021). Within this complex landscape of cancer cell dynamics, our laboratory focuses on unraveling the pivotal role of a scaffold protein, Hematopoietic PBX-interacting protein (HPIP), in cancer progression and development. Over the decades, we revealed that HPIP plays a crucial role in forming the FAK and Src kinase complex, ultimately promoting cancer cell migration (Bugide et al., 2015). It enhances cancer cell proliferation in normoxia (Khumukcham et al., 2019, 2022); however, when faced with a scarcity of resources, such as glucose or hypoxia, it orchestrates a cellular response, driving the migration of cells to other organs (Khumukcham et al., 2022; Penugurti et al., 2021). The pivotal role of HPIP in cancer cell migration is highlighted by its capacity to switch between proliferative and invasive (mesenchymal) phenotypes (Khumukcham et al., 2022). However, the detailed mechanisms underlying its role in cancer cell migration remain elusive.

As cancer cells move forward, they establish integrin-mediated focal adhesion contacts at the leading cellular edge, generating pulling forces towards the direction of movement while efficiently retracting rear focal adhesion contacts, facilitating a cooperative forward movement (Helvert et al., 2016) (Figure 1). They demonstrate a remarkable strategy of recycling (turnover) focal adhesion complexes instead of synthesizing them anew, conserving energy and promoting faster cell movement. Endocytosis, a fundamental cellular mechanism that regulates various signaling pathways, plays a central role in this process. Clathrin-mediated endocytosis, in particular, is involved in the internalization of integrins, thereby regulating focal adhesion turnover (Ezratty et al., 2009; Webb et al., 2002). Our study unveils a fascinating connection involving HPIP, which localizes to focal adhesions and endosomal compartments. We identified two essential coiled-coil domains (CC1 and CC2) in HPIP, required for its interaction with Rab5 and RUFY3. Upon the establishment of this complex, HPIP and RUFY3 activate Rab5 through noncanonical guanine nucleotide exchange factors. This triggers a sequence of events essential for initiating endocytosis and facilitating the recycling and turnover of focal adhesions (Figure 1). Deleting coiled-coil domains or silencing HPIP or RUFY3 disrupts Rab5 activation, leading to defects in Rab5-mediated focal adhesion disassembly, focal adhesion kinase (FAK) activation, and fibronectin-associated-β1 integrin trafficking, ultimately compromising Rab5-dependent cell migration. The focal adhesion junction acts as a central hub for various signaling pathways, integrating numerous cellular signals and scaffold proteins like HPIP,

creating connections between different cellular pathways (Serrels et al., 2012; Tan et al., 2023). Elevated expression levels of HPIP, observed across diverse cancer types, indicate its potential impact on cellular motility (Khumukcham et al., 2021). Our discovery of the collaboration between HPIP, Rab5, and RUFY3 emerges as a pivotal factor influencing focal adhesion turnover and endocytosis. This revelation not only sheds light on the intricate processes of cell migration but also underscores the broader significance of understanding this triumvirate in unraveling the complexities of cellular behavior. These findings hold promising implications for advancing our understanding of cancer progression and its therapeutic strategies. The results of this study were recently published in the esteemed Journal of Biological Chemistry (JBC) on September 1, 2023, (Khumukcham et al., 2023).

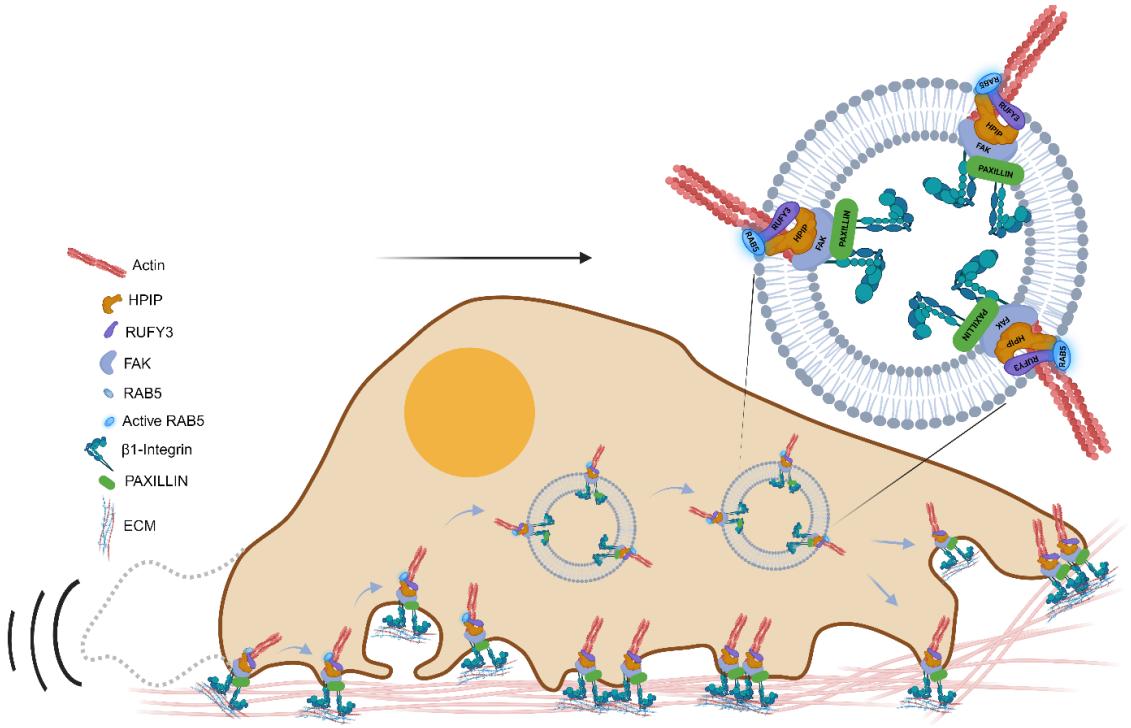

Figure 1: HPIP and RUFY3 mediated Rab5 activation and migration. The diagram illustrates the pivotal role of HPIP and RUFY3 as noncanonical guanine nucleotide exchange factors, orchestrating the activation of Rab5. This activation initiates a cascade of events critical to initiating endocytosis, ultimately driving focal adhesions' recycling and turnover processes.

References

1. Nathan, R.; Giuggioli, L. A milestone for movement ecology research. Mov Ecol 2013, 1 (1), 1. DOI: 10.1186/2051-3933-1-1 From NLM PubMed-not-MEDLINE.
2. Wu, J. S.; Jiang, J.; Chen, B. J.; Wang, K.; Tang, Y. L.; Liang, X. H. Plasticity of cancer cell invasion: Patterns and mechanisms. Transl Oncol 2021, 14 (1), 100899. DOI: 10.1016/j.tranon.2020.100899 From NLM PubMed-not-MEDLINE.
3. van Zijl, F.; Krupitza, G.; Mikulits, W. Initial steps of metastasis: cell invasion and endothelial transmigration. Mutat Res 2011, 728 (1-2), 23-34. DOI: 10.1016/j.mrrev.2011.05.002 From NLM Medline.
4. Serrels, B.; Frame, M. C. FAK and talin: who is taking whom to the integrin engagement party? J Cell Biol 2012, 196 (2), 185-187. DOI: 10.1083/jcb.201112128 From NLM Medline.
5. Bugide, S.; David, D.; Nair, A.; Kannan, N.; Samanthapudi, V. S.; Prabhakar, J.; Manavathi, B. Hematopoietic PBX-interacting protein (HPIP) is over expressed in breast infiltrative ductal carcinoma and regulates cell adhesion and migration through modulation of focal adhesion dynamics. Oncogene 2015, 34 (35), 4601-4612. DOI: 10.1038/onc.2014.389 From NLM Medline.
6. Khumukcham, S. S.; Samanthapudi, V. S. K.; Penugurti, V.; Kumari, A.; Kesavan, P. S.; Velatooru, L. R.; Kotla, S. R.; Mazumder, A.; Manavathi, B. Hematopoietic PBX-interacting protein is a substrate and an inhibitor of the APC/C-Cdc20 complex and regulates mitosis by stabilizing cyclin B1. J Biol Chem 2019, 294 (26), 10236-10252. DOI: 10.1074/jbc.RA118.006733 From NLM Medline.
7. Khumukcham, S. S.; Penugurti, V.; Soni, A.; Uppala, V.; Hari, K.; Jolly, M. K.; Dwivedi, A.; Salam Pk, A.; Padala, C.; Mukta, S.; et al. A reciprocal feedback loop between HIF-1alpha and HPIP controls phenotypic plasticity in breast cancer cells. Cancer Lett 2022, 526, 12-28. DOI: 10.1016/j.canlet.2021.11.002 From NLM Medline.

8. Penugurti, V.; Khumukcham, S. S.; Padala, C.; Dwivedi, A.; Kamireddy, K. R.; Mukta, S.; Bhopal, T.; Manavathi, B. HPIP protooncogene differentially regulates metabolic adaptation and cell fate in breast cancer cells under glucose stress via AMPK and RNF2 dependent pathways. Cancer Lett 2021, 518, 243-255. DOI: 10.1016/j.canlet.2021.07.027 From NLM Medline.
9. van Helvert, S.; Friedl, P. Strain Stiffening of Fibrillar Collagen during Individual and Collective Cell Migration Identified by AFM Nanoindentation. ACS Appl Mater Interfaces 2016, 8 (34), 21946-21955. DOI: 10.1021/acsami.6b01755 From NLM Medline.
10. Ezratty, E. J.; Bertaux, C.; Marcantonio, E. E.; Gundersen, G. G. Clathrin mediates integrin endocytosis for focal adhesion disassembly in migrating cells. J Cell Biol 2009, 187 (5), 733-747. DOI: 10.1083/jcb.200904054 From NLM Medline.
11. Webb, D. J.; Parsons, J. T.; Horwitz, A. F. Adhesion assembly, disassembly and turnover in migrating cells -- over and over and over again. Nat Cell Biol 2002, 4 (4), E97-100. DOI: 10.1038/ncb0402-e97 From NLM Medline.
12. Tan, X.; Yan, Y.; Song, B.; Zhu, S.; Mei, Q.; Wu, K. Focal adhesion kinase: from biological functions to therapeutic strategies. Exp Hematol Oncol 2023, 12 (1), 83. DOI: 10.1186/s40164-023-00446-7 From NLM PubMed-not-MEDLINE.
13. Khumukcham, S. S.; Manavathi, B. Two decades of a protooncogene HPIP/PBXIP1: Uncovering the tale from germ cell to cancer. Biochim Biophys Acta Rev Cancer 2021, 1876 (1), 188576. DOI: 10.1016/j.bbcan.2021.188576 From NLM Medline.
14. Khumukcham, S. S.; Penugurti, V.; Bugide, S.; Dwivedi, A.; Kumari, A.; Kesavan, P. S.; Kalali, S.; Mishra, Y. G.; Ramesh, V. A.; Nagarajaram, H. A.; et al. HPIP and RUFY3 are noncanonical guanine nucleotide exchange factors of Rab5 to regulate endocytosis-coupled focal adhesion turnover. J Biol Chem 2023, 299 (11), 105311. DOI: 10.1016/j.jbc.2023.105311 From NLM Medline.

Journey of Rab11 from Development to Tumour

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All multicellular organisms rely on proper intercellular and intracellular communication for optimal development and survival. This communication is promoted by protein transport mechanisms that control how proteins and peptides are released into their immediate surroundings. These mechanisms involve the packaging, sorting and recycling of biomolecules that are transported by vesicles. The Rab/Ypt protein family is one of the essential players that regulate vesicle trafficking. These proteins, which constitute the largest group within the Ras-GTPase superfamily, are excellent vesicular transport controllers in eukaryotic cells. Rab11, a subfamily of the Rab GTPases that is widely expressed and conserved throughout evolution, has been connected to the regulation of vesicular trafficking via endosome recycling.

Our laboratory is interested in the Rab11 gene and the transport pathway regulated by this gene, and we have demonstrated the contribution of Rab11 to the development of various organs at various developmental stages, using *Drosophila melanogaster* (fruit fly) as a model. Rab11 is well-known for its roles in several aspects of cellular physiology: Rab11 could play a number of plausible roles in determining cell polarity, organising molecules on the cell surface (such as adhesions and receptors), controlling the dynamics of the cytoskeleton, and carrying out cellular signalling in epithelial cells participating in morphogenetic events. Our reports say that Rab11 plays a crucial role in epithelial cellular characteristics, including cytoskeletal structure and cell-cell adhesions (Alone et al, 2005; Bhuin & Roy, 2011; Bhuin & Roy 2012) in *Drosophila*. Rab11 is strongly expressed in *Drosophila* embryos, larvae and adults and plays a significant role in the development of the fly embryonic epithelium (Sasikumar & Roy, 2009; Nandy & Roy, 2020, 2023). It is essential for the development of fly nervous system (Bhuin & Roy 2009; Rai & Roy, 2022), indirect flight muscles (Bhuin & Roy, 2009; Singh & Roy, 2013; Rai et al, 2023) and the Malpighian tubule during tubulogenesis (Choubey & Roy, 2017). Involvement of Rab11 in dorsal closure in fly embryos indicated that Rab11 may be used as a therapeutic protein in wound healing and the same is being explored.

Presently, we are trying to figure out how Rab11 fits into TUMOUR. Our study seeks to validate if Rab11 can also be referred to as a "tumour suppressor". In Drosophila larval midgut, adult midgut precursor cells (AMPs that behave like stem cells) are present that divide asymmetrically and generate peripheral cells at the late second instar. These peripheral cells encase all adult midgut precursors in a group, provide a niche for them, and keep them undifferentiated during the third instar stage. This niche degenerates during metamorphosis, and all adult midgut precursors emerge from their resting state, begin proliferation and differentiation, and form all types of epithelial cells in the adult midgut.

We show that suppressing Rab11 expression disrupted the niche microenvironment, resulting in decreased DPP signalling, which is associated with abnormal over-proliferation (Figure 1 B and D) and early differentiation of larval AMPs (Figure 1 Panels B and D compared to A and C), affecting larval and adult midgut morphology and homeostasis, and leading to tumour-like outgrowth in the adult midgut (Figure 1E). Our findings provide light on the intricate impact of Rab11 gene expression on stem cell dynamics and cellular microenvironment.

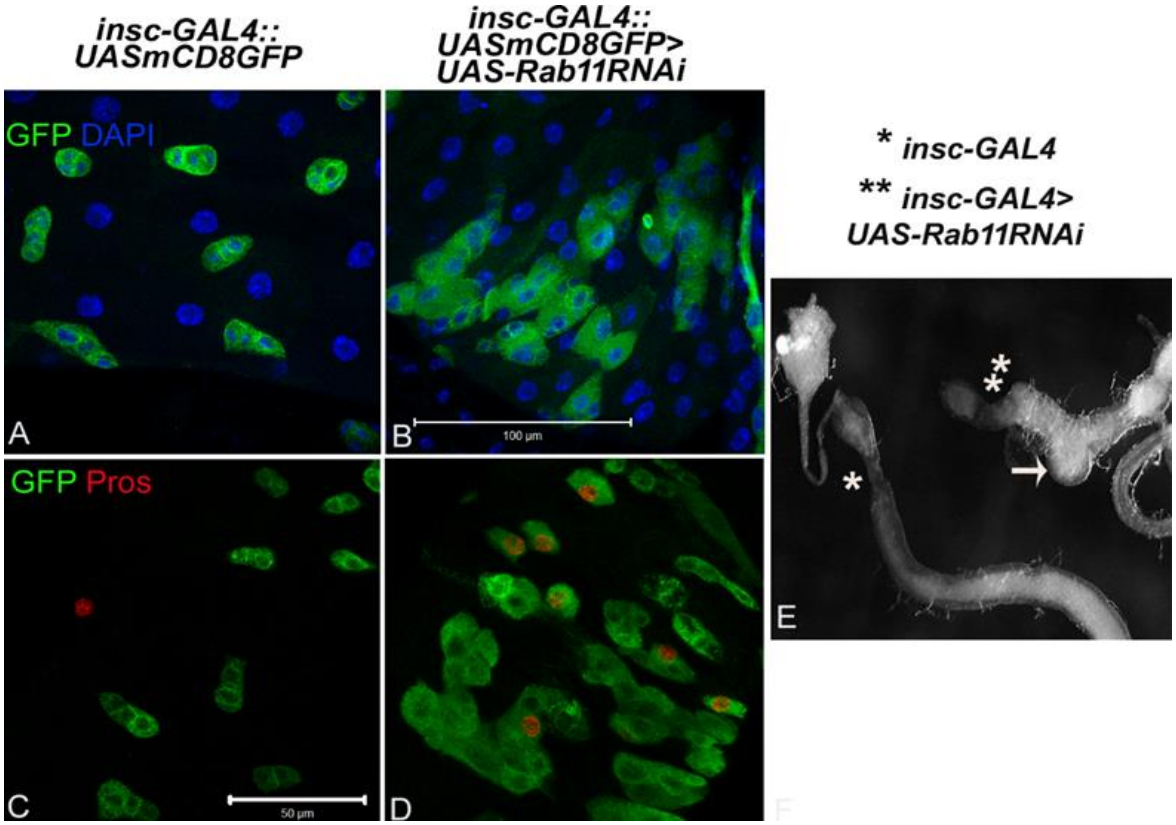

Figure 1: *Drosophila* midgut showing GFP-positive adult midgut precursors and a tumor-like outgrowth: A. *insc-GAL4::UASmCD8GFP* and B. *insc-GAL4::UASmCD8GFP>UAS-Rab11RNAi*-driven third instar larval midgut show GFP-positive adult midgut precursors (green) and DAPI is used to label the nuclei (blue). C and D represent Prospero (Pros) positive nuclei (red) and mark the enteroendocrine cells (differentiated cells) in control and Rab11RNAi backgrounds. E represents a tumour-like outgrowth in the adult midgut (indicated by the arrow) when *Rab11* is inactive.

References

1. Alone D P, Tiwari A K, Mandal L, Li M, Mechler B M, Roy J K (2005) Int J Dev Biol 49, 873-879.
2. Tiwari A K, Alone D P, Roy J K (2008) Cell Biol Int 32, 1158-1168.
3. Sasikumar S, Roy J K (2009) Genesis 47:32-39.
4. Bhuin T, Roy J K (2009) Cell Tissue Res 335, 349-356.
5. Bhuin T, Roy J K (2009) Cell Tissue Res 336, 489-499.
6. Bhuin T, Roy J K (2011) Int J Dev Biol 55, 269-279.
7. Bhuin T and Roy J K (2012) Int J Mol Cellul Med 1, 185-190.
8. Singh D, Roy J K (2013) Plos One 8, e73305.
9. Choubey P K, Roy J K (2017) Cell Tissue Res 367, 269-282.
10. Choubey P K, Roy J K (2017) Genesis 55, e23045.
11. Choubey P K, Nandy N, Pandey A, Roy J K (2020) Dev Bio 461, 19-30.
12. Nandy N, Roy J K (2020) Dev Bio 464, 188-201.
13. Rai P, Roy J K (2022) Biochem Biophys Res Commun 626, 175-186.
14. Nandy N, Roy J K (2023) Cell Tissue Res 391, 485-504.
15. Rai P, Ratnaparkhi A, Roy J K (2023) Brain Res (in press).

Indian Society of Cell Biology Sponsored Lecture at Debra Thana Sahid Kshudiram Smriti Mahavidyalaya, Debra, East Medinipur

Dr. J K Roy

A one-day Indian Society of Cell Biology sponsored visit to Debra Thana Sahid Kshudiram Smriti Mahavidyalaya, Debra, East Medinipur, was organized on 3rd October 2023 in the auditorium of the College. In spite of non-stop heavy rains throughout the day, nearly 300 students and teachers from the college and nearby places participated. J K Roy of Banaras Hindu University delivered a lecture on 'Cell Division and Cancer with special emphasis on the awareness of HPV-induced Cervix Cancer.' Later, he visited the different laboratories of the College and interacted with the teachers and different groups of students, having stimulating discussions. The enthusiasm of the principal, the teachers, and the students was praiseworthy.

Debra Thana Sahid Kshudiram Smriti Mahavidyalaya campus view

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INDIAN SICIETY OF CELL BIOLOGY
RECEIPTS & PAYMENTS A/C FOR THE PERIOD 01-04-2022 TO 31-03-2023

RECEIPTS	AMOUNT	AMOUNT	PAYMENTS	AMOUNT	AMOUNT
Opening Balances:			Medical Expenses		33,900.00
Cash	-		Travelling at Srinagar		27,553.00
State Bank of India A/c no. 7878	1,56,837.06		Award Lecture Memento		22,000.00
State Bank of India A/c no. 7770	4,06,730.42	5,63,567.48	Student Prizes		46,000.00
			Senior Faculty Travel		23,000.00
:dit Interest		11,836.00	Travelling at BHU		29,681.00
Lifetime Membership		1,46,300.00	Newsletter		50,000.00
Donation		60,000.00	Office Exp.		11,000.00
Membership Fees		30,450.00	Misc. exp.		4,972.00
Students Membership Fees		1,750.00	Bank Charges		4.72
Housing Devolopment (Interest)		3,25,730.00	Workshop		50,000.00
			Confrence		2,10,000.00
			Awards		50,000.00
			Printing		30,000.00
			Closing Balances:		
			Cash	-	
			State Bank of India A/c no. 7878	1,59,092.06	
			State Bank of India A/c no. 7770	3,92,430.70	5,51,522.76
		11,39,633.48			11,39,633.48

Place :- Varanasi
Date :- 30-10-2023

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Madhu Gwaldas Tapadia
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Executive Secretary
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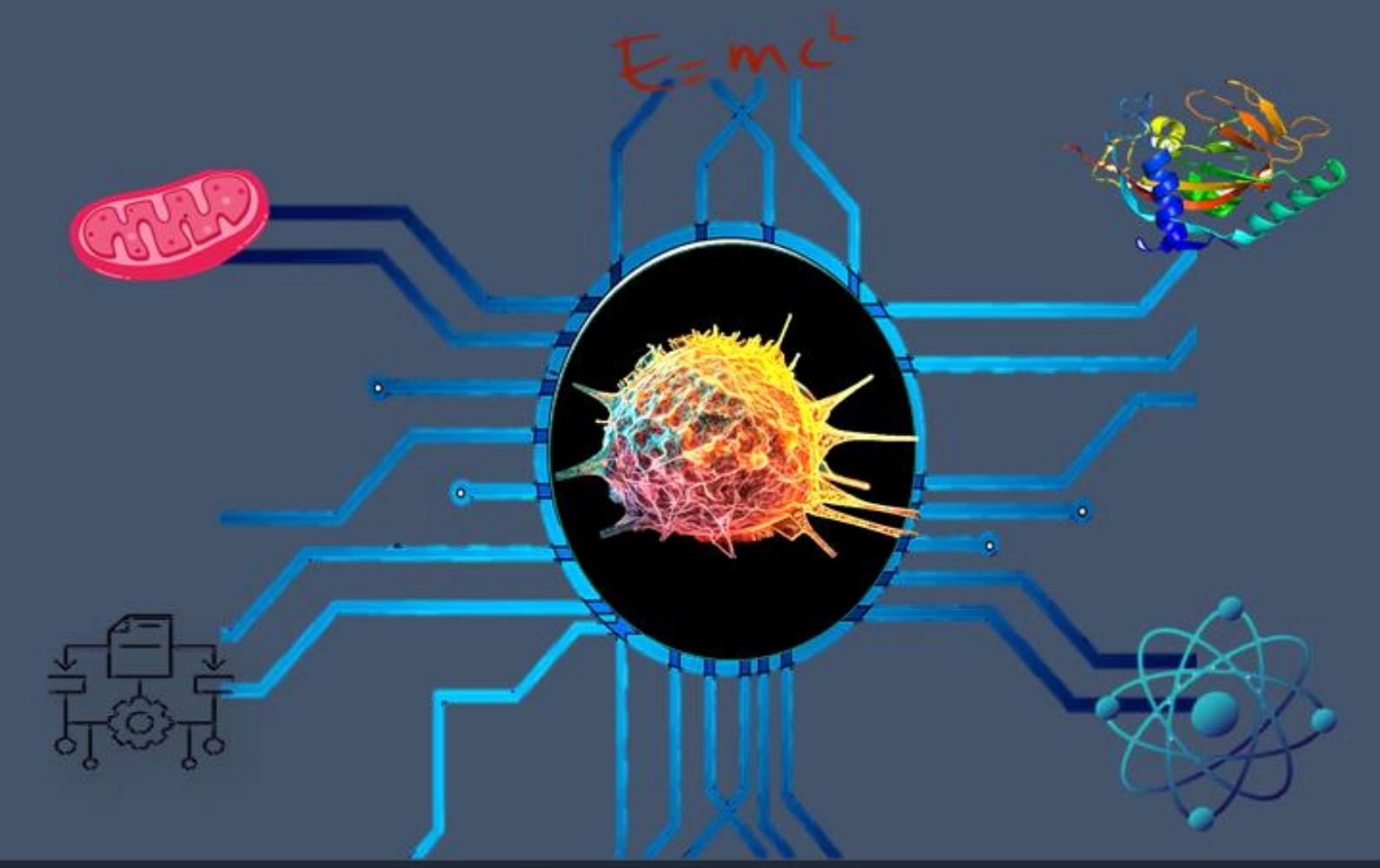
46th All India Cell Biology Conference

Theme - Cell Biology at the Crossroad of Interdisciplinary Sciences

Open to Students, Post-Docs, Faculties from Academia and Industry

With great pleasure we invite you to join the 46th edition of the All India Cell Biology Conference (AICBC2024), hosted by Advanced Centre of Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Centre, Navi Mumbai, INDIA during January 10-12, 2024. We look forward to welcome you at the 46th annual conference of ISCB



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