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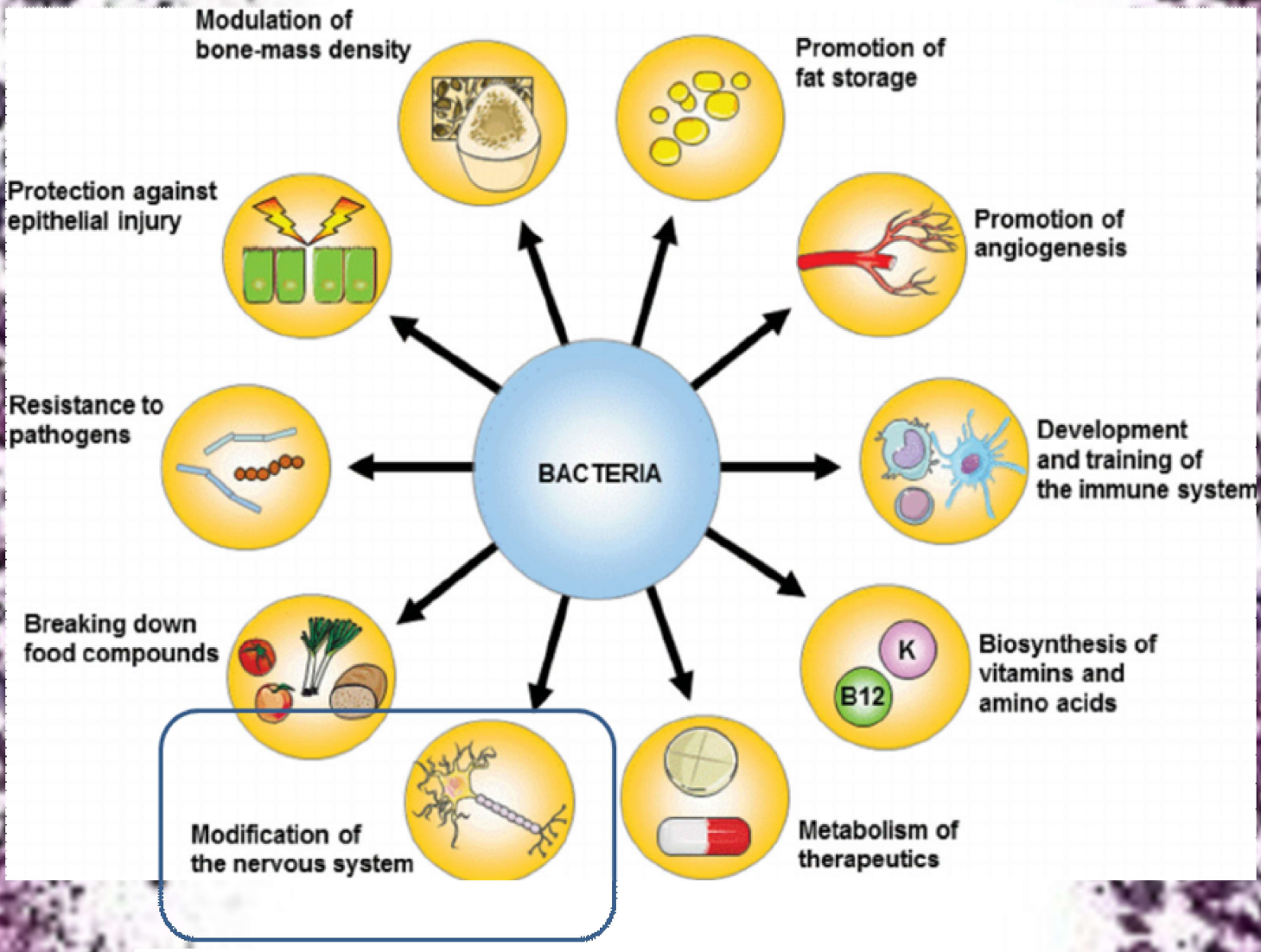
Cell Biology Newsletter



Courtesy to Professor Debby Laukens

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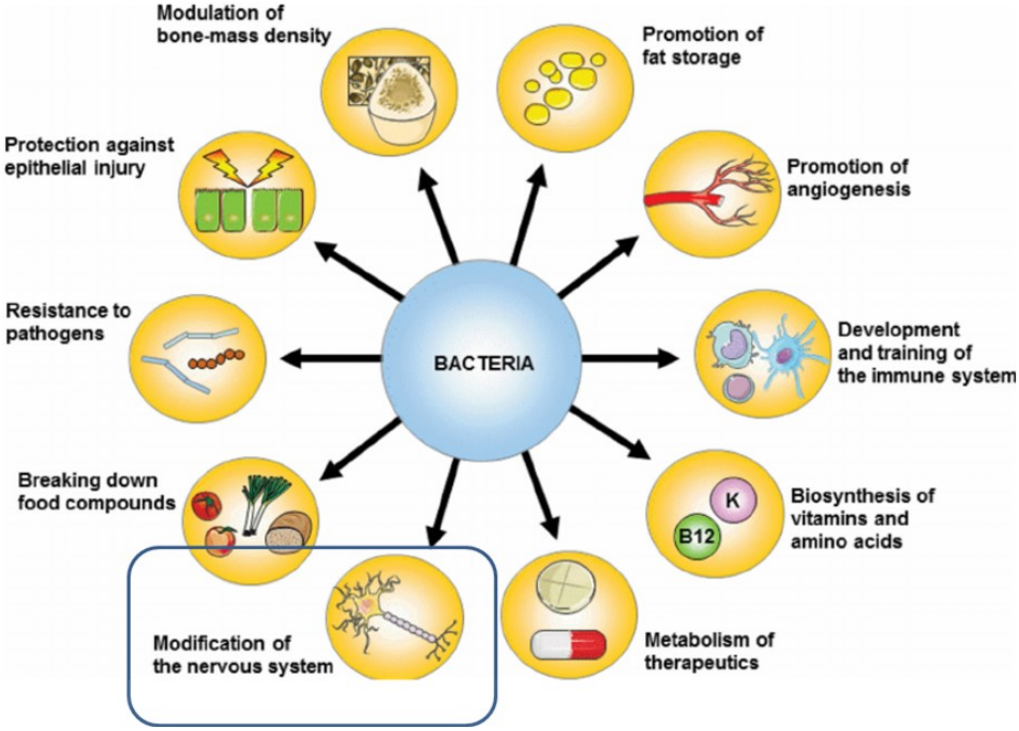

Figure 1. Main functions of bacteria in the gut. Bacteria benefit the host in many ways. Besides breaking down food compounds and synthesizing vitamins and other nutrients, they play an important role in the development and training of the immune system (Hill and Artis 2010; Renz, Brandtzaeg and Hornef 2011; Sonnenberg and Artis 2012). They provide colonization resistance (Kamada et al. 2013; Lawley and Walker 2013), protect against epithelial injury (Rakoff-Nahoum et al. 2004) and promote angiogenesis (Stappenbeck, Hooper and Gordon 2002; Reinhardt et al. 2012) and fat storage (B ackhed¨ et al. 2004). They are also able to modulate bone-mass density (Sj ogren¨ et al. 2012), modify the nervous system (Hsiao et al. 2013) and metabolize therapeutics into active compounds (Claus et al. 2011).

(Ref: Debby Laukens et al. (2016) Heterogeneity of the gut microbiome in mice: guidelines for optimizing experimental design. *FEMS Microbiology Reviews*, doi: 10.1093/femsre/fuv036).

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Chairman's message

Dear Members,

It is time to say good bye to all of you as an outgoing office bearer. Officially, we have come out of exit gate and the Society has a new team at work by the time this News Letter reaches you. However, we will continue to be part of the Society and participate in various activities.

As I have stated in on earlier occasion, the challenges in front of ISCB are in many ways similar to other organisations and different in many other aspects. Now, cell biology has come of age. Our young and vibrant group of researchers need to take up more challenging questions in biology. The revolution in cell biology we have been witnessing worldwide is phenomenal and we need to be part of this excitement. The tripartite conference of ISCB, APOCB and ICCB hosted by our Society in early 2018 is clearly a great opportunity in this direction. For all of us, young and old, this is a wonderful occasion to participate in the mega event to be held first time in India. We had such mega meetings of IUBMB, FAOBMB and other organizations earlier. With the coming of age of cell biology and its current growth in India, the holding of conference augurs well for the society. I would urge all the members to participate in this event and get enriched with the highest quality of science to be presented and deliberated. A large number of eminent cell biologists in diverse areas would be participating in the main and some of the satellite meetings being planned. The arrangements are going on by the capable convenors and it is time to wish them success and also plan your participation.

Sincerely,

V Nagaraja

Composition of the Executive Committee for 2017-2019

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16	Prof. Chandramani Pathak	Member	UIAR, Gandhinagar
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18	Dr Angshuman Sarkar	Member	BITS-Pillani, Goa

Composition of the Executive Committee for 2015-2017

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1	Prof. V Nagaraja	President	IISc, Bengaluru
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16	Dr Abhijit De	Member	TMC-ACTREC, Navi Mumbai
17	Dr Lolitika Mandal	Member	IISER, Mohali
18	Dr Anand K Tiwari	Member	UIAR, Gandhinagar

Professor Sachi Prasad Ray-Chaudhuri

Professor S P Ray-Chaudhuri

(1907-1994)

The Indian Society of Cell Biology came into being in 1976 and it elected as its first President Prof. Sachi Prasad Ray-Chaudhuri. Prof. Ray-Chaudhuri was a doyen among the chromosome biologists in the country who pioneered research and teaching of animal genetics in India. Having trained under the tutelage of Prof. H. J. Muller (N.L.) in Edinburgh, Prof. Ray-Chaudhuri initiated research in areas of radiation genetics and comparative cytogenetics in Calcutta University. In 1961 he moved to Banaras Hindu University, Varanasi as the Chair of Department of Zoology. During his tenure as Head, his inspiring leadership modernized Zoology Department of BHU, and catapulted it to the most dynamic department of animal science in the country. Besides his own research in various areas of genetics and cytogenetics, Prof. Ray-Chaudhuri actively encouraged and pursued development of other areas such as Physiology and Biochemistry, Reproduction Biology, Ecology etc. in the department. A whole generation of students who worked under him not only did commendable work but succeeded in making dynamic schools of their own. His benign and caring support was not confined to his own Ph.D. students but to any one who came in contact with him. He had an abiding influence on all those who were even remotely associated with him. After his retirement from Banaras Hindu University in 1971, Prof. Ray-Chaudhuri went back to Calcutta University and continued to work until mid nineteen eighties till his age and ill health prevented him from active lab life. He breathed his last in the year 1994 at 87 years of age.

Indian Society of Cell Biology, most appropriately, created an endowment fund on his 75th Birthday and created a lecture series in 1982 whose first lecture was delivered by the celebrated geneticist, Dr Obaid Siddiqi in the Cell Biology meeting held in Madurai Kamraj University.

To honor Professor S. P. Ray-Chaudhury, Indian Society of Cell Biology at its 40th meeting at Jiwaji University, Gwalior, has decided to deliberate a talk by an eminent scientist and renowned academician Professor Samit Chattopadhyay.

(H. S. Misra)
Secretary, ISCB (2015-17)

18th S.P. Ray-Chaudhuri 75th Birthday Endowment Lecture

ON

Regulation of alternative splicing and metabolism of cancer cells by SMAR1

BY

Samit Chattopadhyay

CSIR-Indian Institute of Chemical Biology,
Jadavpur, Kolkata

The eukaryotic interphase chromatin is a highly organized structure. An important feature of DNA packaging involves folding of the chromatin into loop domains, which are systematically attached to the nuclear matrix through binding to specialized DNA sequences called Matrix Attachment Regions or MARs. We study how MAR binding proteins (MARBPs) specifically bind to MARs, regulate genomic DNA organization and nuclear functions such as transcription, recombination, splicing, repair etc.

Past several years our lab has been engaged in understanding the role of nuclear matrix and associated proteins in pathophysiological processes. We have focused on one such novel matrix associated protein SMAR1 that is down regulated in human breast cancer (Singh et al., PLoS-One, 2007). It acts as a global repressor for many genes including Cyclin D1, IαBα, CK8, Bax and Puma by directly recruiting HDAC1-mSin3a dependent repressor complex *(Rampalli et al., MCB, 2005; Singh et al., PLoS One, 2007; Singh et al., JBC, 2009)*. Our findings reveal that SMAR1 functions in two different ways to regulate global gene expression. Thus, a change in the level of SMAR1as is seen in several cancers is inversely correlated to the oncogenic activities of these cofactors.

Alternative splicing allows a single gene to produce many mRNAs that translates into protein isoforms. More than 95% RNAs go through alternative splicing and many of these protein isoforms plays important role in cancers. We have recently delineated the role of major master regulators involved in the alternative splicing of receptor molecule CD44. The incorporation of the CD44 variable exons confers the metastatic potential to several cancers. We observed that tumour suppressor protein SMAR1 (Scaffold/Matrix

Associated Region Binding protein 1) interacts with splicing co-activator SRm160, which is known to regulate Ras dependent CD44 alternative splicing. Together, we hall discuss a recent development on the regulation of SMAR1 in cancers and its overall influence on downstream genes in cancerous cells. These rapidly proliferating cancer cells show significant increase in glycolysis known as the “Warburg effect”. Apart from genotoxic stresses, another major type of stress which any cell faces is the metabolic stress. As these rapidly proliferating cells have a much higher glucose requirement compared to normal healthy cells, it is really interesting to study the effects of glucose deprivation on these cells. Now we have observed an interesting connectivity between higher cancer cell metabolism associated with lowered SMAR1 expression.

Dr. Chattopadhyay is working on understanding the role of nuclear matrix binding proteins and their association with chromatin modifying complexes in pathophysiological and disease conditions. He is currently Director of CSIR- Indian Institute of Chemical Biology, Kolkata. He has published several research articles in peer reviewed International and National Journals.

1st Prof. Rita Mulherkar Award Lecture

on

Multi level regulation over steer-driveless misfolding of proteins: threaten proteostasis a tuning point in neurodegenerative disorders andageing

By

Dr Amit Mishra

Department of Biology, Indian Institute of Technology Jodhpur, Rajasthan- 342011

Elimination of aberrant proteins is a well conserved and highly regulated complex mechanism in cells governs by cellular protein quality control (QC) mechanism. Protein QC process engrosses to major efficient tightly regulated machineries i.e. ubiquitin proteasome system and cellular autophagy organization. Defects in these pathways effect continuous elimination of deleterious non-native proteins, which may contribute into the pathogenesis of neurodegenerative diseases. In cellular QC system, E3 ubiquitin ligases are significant employees for defence mechanism against aggregation of abnormal toxic proteins. Few findings indicate that lack of functions of E3 ubiquitin ligases can be a causative factor of neurodevelopmental disorders, neurodegeneration, cancer, and ageing. However, the detailed molecular patho-mechanism implying E3 ubiquitin ligases in cellular functions in multifactorial disease conditions are not well understood. Our findings systematically represent the unique characteristics, molecular nature, and recent developments in the knowledge of neurobiological functions of few crucial E3 ubiquitin ligases. Here, we present our recent findings on roles of E6-AP, MGRN1, and ITCH E3 ubiquitin ligases in the neuropathobiological mechanisms, with precise focus on the processes of neurodegeneration, and thereby propose new lines of potential targets for therapeutic interventions.

Dr Amit Mishra is working on the molecular pathogenesis of various E3 ubiquitin ligases and chaperons implicated in neurodegenerative diseases, neurodevelopmental disorder and ageing. He has been recipient of various awards and fellowships including the prestigious Ramalinganswami Fellowship and Innovative Young Biotechnologist Award from the Department of Biotechnology, India

XL All India Cell Biology Conference & International Symposium on Functional Genomics & Epigenomics

November 17-19, 2016

Meeting report

The focus of the XL All India Cell Biology Conference & International Symposium was Functional Genomics and Epigenomics. The theme covered almost all the areas of Cell Biology and its offshoots, including Functional Genomics, Epigenomics, Development & Evolution, Neurobiology, Aging & Behavior, Cell Structure & Function, Infection, Immunity & Diseases, Plant Biology & Microbiology, Molecular Toxicology, Environment & Conservation Biology, Cellular & Molecular Biology of Stress, Cancer Biology & Drug Development, Chromosome Biology, Cytogenetics & Clinical Diagnosis, Stem Cell Biology & Therapeutics, etc. This conference was organized by Centre for Genomics and School of studies in Zoology & Neuroscience, Jiwaji University, Gwalior, from November 17-19, 2016.

In this three day conference, several eminent cell biologists, including Dr. Akhilesh Kumar Tyagi (Director, NIPGR, New Delhi), Dr. Subrata Sinha (Director, NBRC, Manesar), Dr. A.K. Tripathi (Director, CSIR-CIMAP, Lucknow), Dr. S.K. Apte (HNBI, Mumbai), Dr. Joyoti Basu (Bose Institute, Kolkata), Dr. Sanjeev Galande (IISER Pune), Dr. Umesh Varshney (IISc, Bengaluru) and Dr. Tej K. Pandita (The Methodist Hospital Research Institute, Houston, USA), presented their research findings. Besides, the distinguished speakers, society members, research scholars and students from different Universities and research institutes also participated and presented their work in posters and oral presentation sessions.

Before the conference, a one day Science Communication & Career Workshop was conducted by Nature India & Naturejobs in partnership with WellCome Trust/DBT India Alliance on 16th November 2016. The goal of this pre-conference workshop was to provide tools and strategies to the young researchers for efficient presentation of their ideas, experiments and scientific results, which may help them to make science career as their career choice. In this program, Dr Sarah Iqbal (Public Engagement Officer, WellCome Trust/DBT India Alliance) discussed *Ethics in research*. Dr Bela Desai (Grants Adviser, WellCome Trust/DBT India Alliance)

described the *manuscript writing*. Dr Sarah Iqbal talked about important points taken care during oral and poster presentations. In the afternoon session, Subhra Priyadarshini (Editor, Nature India) discussed Science journalism, and popular science writing. In the evening session, the speakers of the workshop discussed about Alternate Science Career, including Career in the Industry, Science Promotion/Intellectual Property and Policy/Grant organizations / Journalism. Approximately 140 participants from different Universities and research institutes participated in this workshop. Of note, PhD students, postdoctoral fellows or junior scientists was a pre-requisite for participation in this workshop.

The conference was inaugurated on 17th November by Dr. V. M. Katoch, NASI-ICMR Chair on Public Health Research at RUHS, Jaipur and Former Secretary, Department of Health Research, Govt. of India and Director General, ICMR, New Delhi and presided by Prof. Sangeeta Shukla, Vice Chancellor, Jiwaji University, Gwalior.

The scientific program was divided into several sub-areas as mentioned below.

Functional Genomics & Epigenomics-I

On the first day of the conference, Dr. S.K. Apte (HNBI, Mumbai) delivered a plenary lecture on the Molecular basis of radiation-responsive gene expression in *Deinococcus radiodurans*. He showed that only radiation desiccation response motif (RDRM)-based promoters cause radiation induction in *D. radiodurans*, the DdrO protein repressed GFP expression in heterologous host *E. coli* and the radiation-induction of RDRM-based genes was eliminated in *D. radiodurans* irrE mutant.
Dr. Tapas Kundu (JNCASR, Bangalore) discussed about the physiological and pathophysiological roles of multi-functional transcriptional co-activator and chromatin protein, PC4. Dr. Kundu revealed that PC4 is human transcriptional co-activator and histone interacting chromatin protein. It plays crucial roles in epigenetics and in the regulation of gene expression at genome level. PC4 also regulates progression of breast cancer. Moreover, conditional knockout of PC4 in the brain of mice showed spatial memory deficits due to dysregulation in the expression of many neural function-associated genes.

Deepti D. Deobagkar (Savitribai Phule Pune University, Pune) presented her work on DNA methylation as an epigenetic modulator in X aneuploidy and stress. Dr. Deobagkar investigated global DNA methylation in human cells using a novel microarray based approach for variable number of inactivated X chromosomes explaining variability and penetrance of the Turner and other disease phenotypes.
Shantanu Sengupta (CSIR-Institute of Genomics and Integrative Biology, Mathura Road, New Delhi) discussed role of epigenetic alteration in Coronary Artery Disease (CAD). Dr. Sengupta showed that decreased level of vitamin B12 is associated with altered DNA methylation at the whole genome level in CAD. Upon supplementation of vitamin B12, the effect was reversed.

Dr. A.S. Sreedhar (CSIR-Centre for Cellular and Molecular Biology, Hyderabad) presented his work on Oncogene adaptation and addiction to the cancer chaperone, Hsp90: implications in anticancer therapeutics. Dr. Sreedhar showed that a novel region on Hsp90 is involved in Raf addiction, different from the kinase-kinase domain and revealed oncogene-de- addiction through Hsp90 inhibition.
Pritha Ray (ACTREC, Navi Mumbai) presented her work on an intricate relation of IGF1R-Akt signalling with cancer stem cells and chemoresistance. Dr. Ray reported

significant role of IGF-1R-AKT signalling as a major determinant of cancer stem cell functions, chemoresistance and a potential therapeutic target in ovarian cancers.
Dr. Ashok Sharma (AIIMS, New Delhi) presented his work on epigenetic alterations and cancer germline POTE antigen activation in epithelial ovarian cancer. Dr. Sharma showed high expression levels and hypomethylation of POTE genes in LINE1 elements and pericentromeric DNA, suggesting POTE genes as biomarkers and therapeutic targets in epithelial ovarian cancers.

Sessions 2 and 3 were devoted to Poster and Oral award presentations from young scientists. There were 204 posters and 10 oral presentations.

The first day was concluded following Prof. Rita Mulherkar Award Lecture delivered by Dr. Amit Mishra, IIT Jodhpur. Dr. Amit Mishra delivered his award lecture on Multi level regulation over steer-driveless misfolding of proteins: threaten proteostasis a tuning point

Functional Genomics & Epigenomics-II

On the second day, Friday, 18th November, Dr. Tej K. Pandita (The Methodist Hospital Research Institute, Houston, USA) delivered his lecture on the role of Histone H4K16 acetylation in DNA damage response and stem cell differentiation. Dr. Pandita showed that H4K16 acetylation plays crucial role in HR related repairosome foci formation during DSB repair and in R-loop formation during stem cell differentiation.

Dr. A. K. Tripathi **(**CSIR-CIMAP, Lucknow) discussed on the Involvement of cascades of extra-cytoplasmic function sigma factors and cross-talks between non-cognate anti-sigma factors in the regulation of carotenoid biosynthesis in a plant growth promoting *Rhizobacterium*. Dr. Tripathi showed a cross-talk of ChrR proteins and non-cognate RpoE by pull-down and two-hybrid methods, indicating that both the genes are created by duplication during evolution.

Dr. Joyoti Basu (Bose Institute, Kolkata) presented her work on the role of transcriptional and post-transcriptional regulatory networks in the interaction of *Mycobacterium tuberculosis* with macrophages. Dr. Basu showed that let-7f, miR-17 and miR26a microRNAs regulate host immune response.

Sampa Das (Bose Institute, Kolkata) presented her work on regulatory modules controlling below ground *Fusarium* wilt response at early time point in chickpea. Dr. Das showed that various genes are associated with the immune response and nodal molecules might serve as hub controllers of signalling in Foc-1 infected, resistant and susceptible genotypes of chickpea.

Saravanan Matheshwaran (Indian Institute of Technology Kanpur) presented his work on rRNA binding and Actin related proteins. Dr. Matheswaran reported ribosomal RNA binding with actin related protein yArp8, which might play an important role in RNA metabolism of Ino80 complex or Arp8.

Dr. S.K. Mishra (Indian Institute of Science Education and Research, Mohali) presented his work on Ubiquitin-like processing of the conserved splicing regulator SDE2 promotes telomeric silencing and genome stability. Dr. Mishra showed that Sde2 helps splicing of a subset of pre-mRNAs in *Schizosaccharomyces pombe* in

intron-specific manner. This ubiquitin-like processing leads Sde2-C to sustain genomic integrity of *S. pombe* by specific pre-mRNA splicing.

Dr. Ankur Sharma (Genome Institute of Singapore) presented his work on “Single cell RNA-sequencing reveals divergent modes of drug resistance with convergent therapeutic susceptibilities in Head and Neck cancers”. Dr. Sharma reported that tumour cells have extraordinary phenotypic plasticity and these cells acquire different survival path in presence of chemotherapeutic drugs.

Aging and Neural Response

Dr. Subrata Sinha (National Brain Research Centre, Manesar) delivered the second Plenary Lecture on “family studies in dyslexia identify multiple pathways to a similar outcome”. Dr. Sinha showed autosomal recessive patters of dyslexia in 3 different multiplex families of different endogamous caste groups. Further, exome sequencing revealed a dinucleotide insertion in a long non-coding RNA in one family.

Dr. Preeti G. Joshi (National Institute of Mental Health and Neurosciences, Bangalore) presented her work on molecular mechanisms of glutamate induced excitotoxicity underlying degeneration of neurons in neurodegenerative diseases. Dr. Joshi showed that astrocytes around the motor neurons have less capacity to transport glutamate, which leads to increased vulnerability of motor neurons to excitotoxicity. She suggested that AMPA receptor clusters are stabilized by PSD-95 and other interacting proteins and palmitoylation regulates protein sorting and targeting to the postsynaptic membrane.

Dr. N.R. Jana (National Brain Research Centre, Manesar) presented his work on “Impairment of protein homeostasis in Huntington’s disease (HD)”. Dr. Jana focused on quality control system dysfunction in HD and effect of its rescue in delaying the disease progression. He suggested that improvement in quality control system of the cellular protein may reduce aggregated huntingtin load and induce neuroprotection.

Dr. Neeraj Jain (National Brain Research Centre, Manesar) presented his work on aging related sensorimotor behavior. Dr. Jain studied sensorimotor changes in aging to understand the recovery process of injured spinal cord and the brain plasticity. He reported that behaviour of rats remained unaltered until 70 weeks of age and thereafter, showed rapid decline in performance. However, after 70 weeks of age, sensorimotor deficits appear and gradually increase with advancing age.

The invited talks followed Poster session on the second day also. The second day scientific deliberations were concluded by the prestigious S P Ray-Chaudhuri 75th Birthday Endowment Lecture delivered by Dr. Samit Cahttopadhyay, CSIR-IICB, Kolkata. Dr. Samit Chattopadhyay delivered his award lecture on Regulation of alternative splicing and metabolism of cancer cells by SMAR1.

Genomic Response to infections and diseases

Dr. Sreelaja Nair (TIFR, Mumbai) presented her work on Cell biological adaptations in the vertebrate embryo in response to altered ploidy. Dr. Nair showed alteration in cellular parameters of mitotic spindle in haploid and tetraploid zebrafish embryos during early development, indicating the potential of these dimensions of the genome to dictate for normal embryonic patterning and survival.

Dr. B.N. Singh (CSIR-CDRI, Lucknow) presented his work on sigma Factor and Biofilm formation in Mycobacteria. Dr. Singh showed new morphotypes of sigF deletion in different mycobacterial species which may pave path to understand the role of SigF in mycobacteria.

Dr. Jagan Pongubala (University of Hyderabad) presented his work on “Programmed changes in higher-order chromatin interactions dictate B cell fate commitment”. Dr. Pongubala showed that chromatin modifications play a crucial role in lineage-specific gene regulation during B cell fate commitment.

Braj Raj Shrivastav (Cancer Hospital and Research Institute, Gwalior) presented his work on genome-wide molecular analysis of Gallbladder Cancer. Dr. Shrivastava showed whole proteome and methylome data, which may help in identification of early diagnostic and prognostic biomarkers of GBC.

Dr. Prithvi Raj (University of Texas Southwestern Medical Centre, Dallas, USA) presented his work on the role of non-coding regulatory polymorphisms in susceptibility to Systemic Lupus Erythematosus (SLE). Dr. Sharma reported that risk loci of SLE - HLA, STAT4, TNFAIP3, BLK, BANK1 and NCF2 might have strong association with development of ANA, while other risk loci, such as ITGAM, TNFSF4, IRF5 and UBE2L3, might have role in development of disease pathology.

Rhitoban Ray Choudhury (IISER, Mohali) presented his work on dynamics of multiple *Wolbachia* infections in the host *Nasonia vitripennis*. Dr. Ray Choudhury described importance of qRT-PCR in delineating bacterial density in different developmental stages of *N. vitripennis* suggesting competition between the two infecting strains of *Wolbachia*.

Response of plants to environmental factors

Dr. Akhilesh Kumar Tyagi (University of Delhi) delivered the third Plenary Lecture of the conference on “My encounters with stress in rice”. Dr Tyagi showed that members of A20/AN1 zinc-finger containing stress-associated protein (SAP) gene

family, OsSAP11 and OSRLCK253, interacts with self. Overexpression of these genes confers tolerance in homologous and heterologous transgenic systems during water-deficit.

Baishnab C. Tripathy (JNU, New Delhi) presented his work on “Towards Development of C4 Rice: Overexpression of C4 phosphoenolpyruvate carboxylase and phosphoenolpyruvate carboxykinase in C3 *Arabidopsis thaliana* increases its photosynthetic efficiency and confers tolerance to salt stress”. Dr. Tripathy showed that C4 photosynthesis enzyme(s) overexpression may increase its photosynthetic capacity with enhanced tolerance to salinity stress in a C3 plant *Arabidopsis thaliana*.

Dr. A.K. Banerjee (IISER, Pune) presented his work on StBEL11 and StBEL29- the new mobile RNAs that control potato development. Dr. Banerjee showed that StBEL11 and StBEL29 mobile RNAs and some of the microRNAs plays critical role during tuberization process in potato.

Development, evolution and environment

Dr. Sanjeev Galande (IISER, Pune) presented his work on “Evolutionary adaptation of transcription factors into Wnt signalling network: Insights into the head organizer in hydra”. Dr. Galande showed that evolution and adaption of transcription factors of Wnt signalling network regulate the primary body axis.

Dr. Madhu G Tapadia (Banaras Hindu University, Varanasi) presented her work on Functional analysis of Yorkie in linking neurodegeneration and innate immune response. Dr. Tapadia showed crosstalk between Yorkie mediated cell proliferation and innate immune response in PolyQ expressing cells.

Dr. Umesh Varshney (Indian Institute of Science, Bangalore) presented his work on “An evolutionarily conserved element in initiator tRNAs prompts ultimate steps in ribosome maturation”. Dr. Varshney showed that the evolutionarily conserved three consecutive GC base pairs in initiator tRNA (i-tRNA) anticodon stem play an important role in ribosome maturation.

Dr. Deepa Agashe (National Centre for Biological Sciences, Bangalore) presented her work on “Host-microbial associations and the changing paradigm of eukaryote fitness”. Dr. Agashe showed the ubiquity of host-microbiome associations, suggesting that eukaryotic fitness is more complicated than we currently understand.

The last session of day-III, was organized on face-to-face interaction of school children with senior reputed Indian scientists and teachers. In this event, Prof. S.C. Lakhotia (Banaras Hindu University, Varanasi) interacted with the students from different schools of Gwalior city and answered the queries of young minds. Dr. M.C. Arunan from HBNI, Mumbai, also interacted with the students.

For young researchers, 10 abstracts were selected for oral presentation on day-I and 204 posters were presented in two days (day I and day 2). The best oral (1) and posters presentations were awarded by the society (3) and the organizers (4).

A PARTNERSHIP STORY: INSECT-BACTERIAL ASSOCIATIONS

Aparna Agarwal, Kruttika Phalnikar and Deepa Agashe*

National Centre for Biological Sciences (NCBS), Bangalore

The world around us is full of microbes that influence both biotic and abiotic components of ecosystems. For instance, nitrogen-fixing bacteria enrich the soil[1], and algae in marine ecosystems provide sustenance to a variety of organisms[2,3]. On the other hand, pathogenic bacteria cause diseases across trophic levels, changing the environment around them dramatically. Such interactions have been extensively studied for a long period of time. However, non-pathogenic host-bacterial associations also influence host physiology and even host behaviour[4,5]. For example, in mice, differences in gut bacterial communities determine utilization of specific dietary components and the propensity for diseases like obesity and diabetes[6,7]. Gut bacteria are also linked to several neurological disorders such as depression and anxiety[8]. Such dependence of animal hosts on their gut microbes is not limited to humans, but extends across the tree of life.

Host-microbial interactions are especially well studied in insects, which are one of the most abundant and diverse class of animals and occupy several ecological niches, with different diets, life-history traits and behaviours. Thus, studying host-microbe interactions in insects provides us with many general insights (Figure 1A). For instance, wood-eating termites rely on their gut bacteria to digest lignocellulose, a major part of their diet[9]. In fact, microbial nitrogen fixation accounts for 60% of the nitrogen content of some termite colonies[4,10]. In pea aphids (plant sap sucking insects), gut bacteria supplement their host's diet with essential amino acids[11]. Similarly, in mosquitos, midgut bacteria help lyse RBCs and aid efficient protein utilization[12]. Insects often feed on plants that contain toxins such as tannins and cardiac glycosides[13] as well as artificially sprayed pesticides. Gut bacteria aid their hosts in occupying these niches by detoxification of such compounds. For instance, coffee borer gut bacteria detoxify caffeine in coffee seeds, allowing the host to feed on coffee beans despite very high concentrations of caffeine[14]. Similarly, bacterial symbionts of insects such as bean bugs and stink bugs allow them to detoxify insecticides[15]. These examples show that gut bacteria can aid host survival on a suboptimal diet. Thus, host-bacterial associations can allow hosts to occupy niches that were not previously available to them. In this manner, such associations could dramatically expand the ecological niche of the host.

These results in turn raise several questions. How important are these bacteria across different life stages? How do hosts maintain their respective gut bacteria, and how do the bacteria interact with the host's immune system? In this review, we summarize recent studies that answer some of these questions.

What is the effect of gut flora on development?

As mentioned above, gut bacteria can influence nutrient acquisition and detoxification, and may thus impact host development[5]. In fact, in many insects, gut bacteria are essential for proper host development. For instance, in stinkbugs, removing the bacterial symbiont *Ishikawella capsulata* from nymphs (juvenile stages) causes retarded development, arrested growth, and abnormal body coloration[16]. In weevils, removing endosymbionts via antibiotics prevents the hardening and maturation of the exoskeleton[17]. Larvae of the mosquito *Anopheles stephensi* also show delayed growth when treated with antibiotics. Interestingly, mosquitos carrying an antibiotic-resistant strain of the bacterium *Asaia* are not affected by antibiotic treatment[18]. Such host-bacterial interactions become even more important under food deprivation. When germ-free *Drosophila melanogaster* larvae face nutrient scarcity, their development is severely affected and mortality increases. However, larvae with an intact gut bacterial community can complete development even under nutrient limitation and their mortality is comparable to flies reared in nutrient rich media. Additionally, this developmental defect can be rescued by providing germ free flies with the bacterium *Acetobacter pomorum*[19]. Thus, in some cases even a single bacterium can have large effects on the host's development. In some insects like fruit flies, termites and mosquitoes, gut bacteria can also influence the expression of genes involved in longevity and oviposition rate, influencing host fitness[12,20,21]. Thus, the maintenance of gut bacterial partners across life stages and across generations is critical for these insects.

Acquisition, maintenance and transfer of gut bacteria

Beneficial bacteria can be acquired through the environment, horizontally from conspecifics, or transmitted to the offspring vertically by their parents (Figure 1B). Different mechanisms of vertical transmission have evolved across insects [22,23]. In pea aphids, weevils, cockroaches and whiteflies, bacterial symbionts live inside specialised host cells called bacteriocytes that are transmitted inside the egg[17,24–26]. In other insects, extracellular bacteria reside in the lumen of host tissue and are transmitted via many different mechanisms (Figure 1C). In the

European beewolf, females cultivate the beneficial bacterium *Streptomyces philanthi* in specialised glands located near their antennae, and then spread it on the ceiling of the brood cell before oviposition[27]. Similarly, in insects like reed beetles, kissing bugs, and stinkbugs, the mother smears the eggs with excreta that contains beneficial gut bacteria[28,29]. In the Japanese plataspid stinkbug (*Megacopta punctatissima)*, gut bacteria are transmitted in special capsules that are deposited on the underside of the eggs. These capsules are made in an enlarged section of the female midgut, and are packed with the symbiont *Ishakawella capsulata*[16]. Other insects like leaf-footed bugs lay their eggs in close proximity to their fecal matter[23]. In all these examples, the newly hatched larva ingests the egg shell and surrounding material, acquiring the bacteria. Other interesting cases include blood sucking parasites like tsetse flies, bat flies and louse flies. In these insects, while the adult form feeds on blood, the larval form develops inside the female body viviparously, fed by milk glands present inside the female. The milk glands provide nutrition as well as beneficial bacteria to the developing larva[30]. On the other hand, in social insects, gut bacteria are usually transmitted via social interactions. In honey bees and termites, adults tend to their larvae and feed them through trophallaxis (feeding larvae with regurgitated food), thus exposing them to the beneficial bacteria[5]. In bumble bees, social contact between young and adult bumble bees also transmits gut bacteria[31] (Figure 1C).

Many non-social holometabolous insects have discrete generations, with little or no contact between parents and offspring. In such a scenario, maintaining a stable host-bacterial association across generations becomes difficult without strict vertical transmission. In these cases, insects often acquire bacteria from the environment. However, the environment can also contain other microorganisms that may not be beneficial, or may be pathogenic. Therefore, the host needs to selectively acquire only the beneficial bacteria. An insect's gut morphology and physiology (e.g. pH, oxygen levels) creates selective niches that only some bacteria can occupy. For example, in the bean bug *Riptortus pedestris*, the insect gut has a sac-like organ that houses the symbiont. Food particles and other bacteria cannot enter the organ because the gastrointestinal tract is very narrow just before this organ. However, the symbiotic bacteria *Burkholderia* can pass through the constriction and reach the sacs[32]. Similarly, termites have specialized gut compartments that are highly alkaline, and thus allow the growth of only alkali-tolerant bacteria[5].

The insect immune system also plays a critical role in the acquisition and regulation of beneficial bacteria. Studies with *Drosophila melanogaster* show that the transcription factor *caudal* suppresses antimicrobial peptide (AMP) production in the presence of commensal

bacteria. However, AMP production is upregulated in the presence of pathogenic bacteria[19]. In fact, regardless of the mode of transmission, the maintenance of bacteria in their respective niches inside the host tissue is strongly governed by the immune system. For example, in flour weevils, the bacterial endosymbiont resides in a group of host cells called a bacteriome[17]. Weevils selectively up-regulate the production of the AMP *colA* in the bacteriome, but not in other tissues. On the other hand, another AMP *colB* is expressed in other tissues when pathogens invade the weevils, but not in the bacteriome. Moreover, the density of the endosymbiont in the bacteriome correlates with *colA* expression level. Thus, the weevils' immune system restricts the endosymbionts to the bacteriome[33,19].

Changes in bacterial genomes due to association with host

So far, we have discussed how bacteria can alter host phenotype. However such associations also affect the bacteria. For example, endosymbionts such as *Wolbachia* and *Burkholderia* tend to have very small genomes compared to their free-living relatives[34]. In fact, endosymbionts have amongst the smallest genomes of all known bacteria[35]. Since these bacteria live inside the host cells, selection for maintenance of several genes is thought to be reduced. Over time, such genes either accumulate mutations or are eliminated from the genome, thus leading to the small genomes of these bacteria[36]. The genes that remain conserved vary between different endosymbionts. These genes are often associated with the benefit these endosymbionts provide to their host. For example, *Sodalis pierantonius* is an endosymbiont of cereal weevils that feed on flour deficient in aromatic amino acids like tyrosine (Tyr) and phenylalanine (Phe). *S. pierantonius* has lost genes for many metabolic functions due to pseudogenization and rearrangements; however, the Phe and Tyr pathways have been retained[37]. Similarly, the pea aphid endosymbiont *Buchnera aphidicola* primarily retains genes for synthesis of amino acids that are required by its host[26,35]. A similar trend seems to occur with vertically transmitted gut bacteria, whose genomes are larger than those of endosymbionts but smaller than their free living relatives. However, genome reduction is not observed for bacteria that are horizontally acquired, presumably because they also have to survive outside the host and face selection to maintain metabolic pathways that are not essential within the host[5].

In conclusion

Insect bacterial associations are complex, with both partners interacting with and influencing each other. For example, insect guts present niches that only certain bacteria can occupy. In

turn, the presence of bacteria in the gut changes aspects of the gut physiology. In scarab beetle, microbial fermentation produces acetate, formate and lactate, changing the pH of the different gut compartments[38]. Similarly, the central region of termite guts is highly anoxic because the bacteria that reside there act as very strong oxygen sinks[39]. Thus, while host

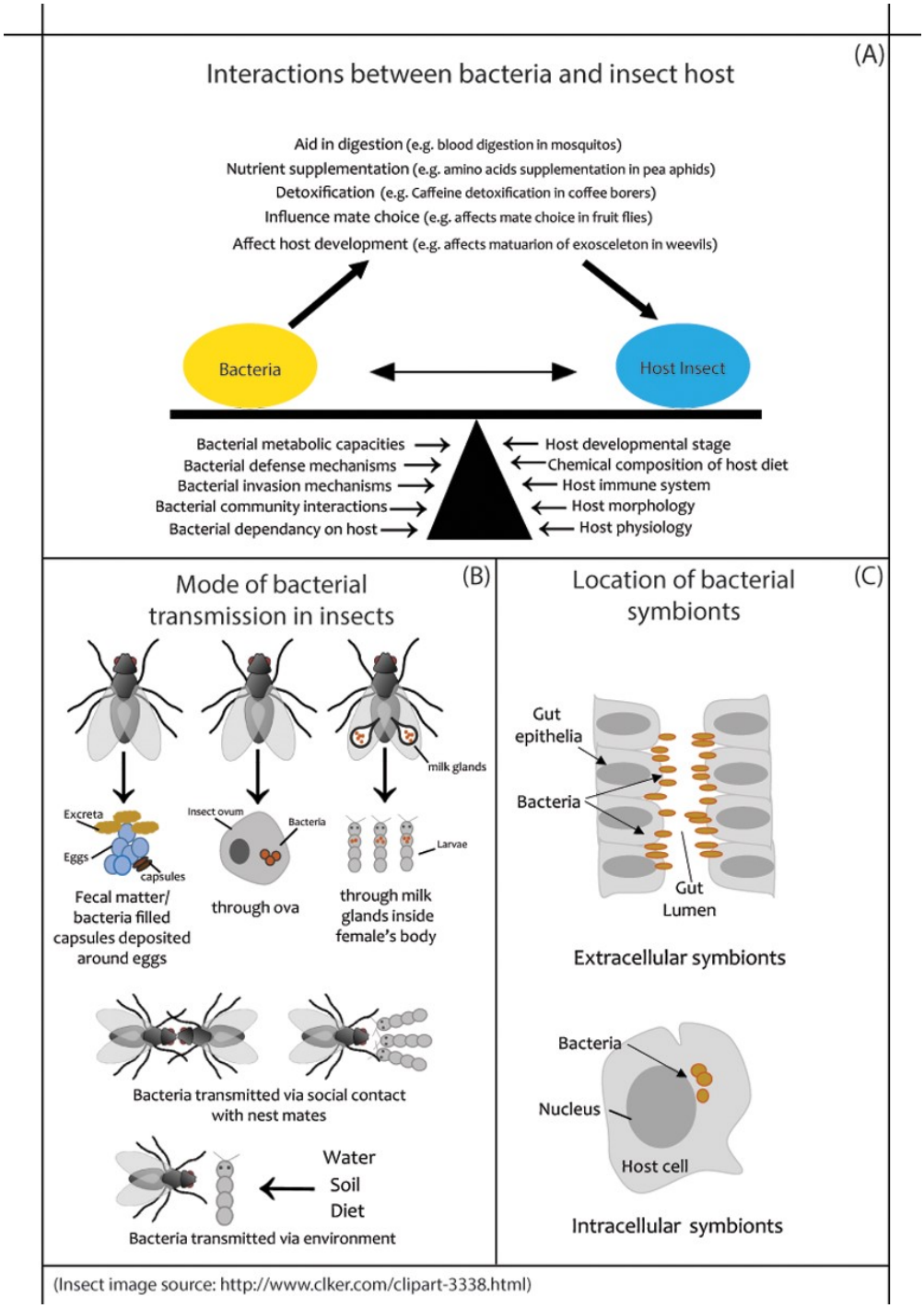

Figure 1: (A) Factors influencing insect-bacterial association, and some benefits provided by bacteria to their hosts. (B) Mechanisms of bacterial transmission across generations. (C) Location of bacterial cells within insect tissues.

physiology and immune system select for certain bacteria, colonization by specific bacteria can also fundamentally alter the host tissue. Additionally, the insect immune system selects for specific bacterial partners. However, colonization by commensal bacteria can often prevent colonization by potential pathogens. For instance, aseptically reared lepidopteran larvae show a 20 fold increase in colonization by the pathogen *Bacillus thuringiensis*, compared to larvae with intact gut bacteria. Similarly, in the locust *Schistocerca gregaria,* gut bacteria secrete antimicrobial peptides that are selectively bactericidal but do not affect the indigenous bacterial species[4]. Thus, the presence of specific bacteria can shape the bacterial community associated with the insect host.

Such interactions have resulted in very specific insect-bacteria associations. In fact, across the insect phylogeny, the divergence of many hosts and their associated bacteria is correlated[40]. In some cases, bacteria can cause their hosts to choose mates feeding on similar diets, or change host preference for specific diets[41,42]. Over generations, such interactions can affect host diversification. Thus, we now understand that insect-bacterial mutualisms are complex interactions that are influenced by several factors such as insect physiology, immune system, morphology, diet, bacterial metabolism and bacterial community structure (Figure 1A). However, many questions still remain unanswered: how are these associations initiated, how quickly can they evolve, and what are the mechanisms governing host-bacterial associations? How frequently do bacterial partners influence host adaptation and diversification? Further investigation of such and many more questions will broaden our understanding of fundamental aspects of the ecology and evolution of both partners.

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Maternal Microbiome: A Macro Dimension of Pregnancy

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Microorganisms are increasingly being recognized to play a crucial role in maintaining human health. Recent advances have broadened our understanding of the impact of resident microorganisms – "the microbiome", on the health of the human host. The microbiome can be defined as the collective genome of all the microorganisms i.e. microbiota that populate a distinct biological or environmental niche. The human microbiome comprises of trillions of microbes (bacteria, fungi, archaea and viruses) dwelling at various sites in our body such as the mouth, nasal cavity, reproductive tract, gut, skin and placenta, outnumbering our own cells by nearly ten times and having many fold more genetic material than our own genome [1]. Despite the availability of the complete genetic blueprint in the form of the human genome since 2003, a deeper understanding of the synergistic associations with endogenous microbiota and their functional implications on human health came to light only upon the completion of the first phase of the human microbiome project in 2012 [2, 3]. This was made possible due to the advancements in sequencing technologies such as next generation sequencing as well as tools for analysis and interpretation of the obtained data.

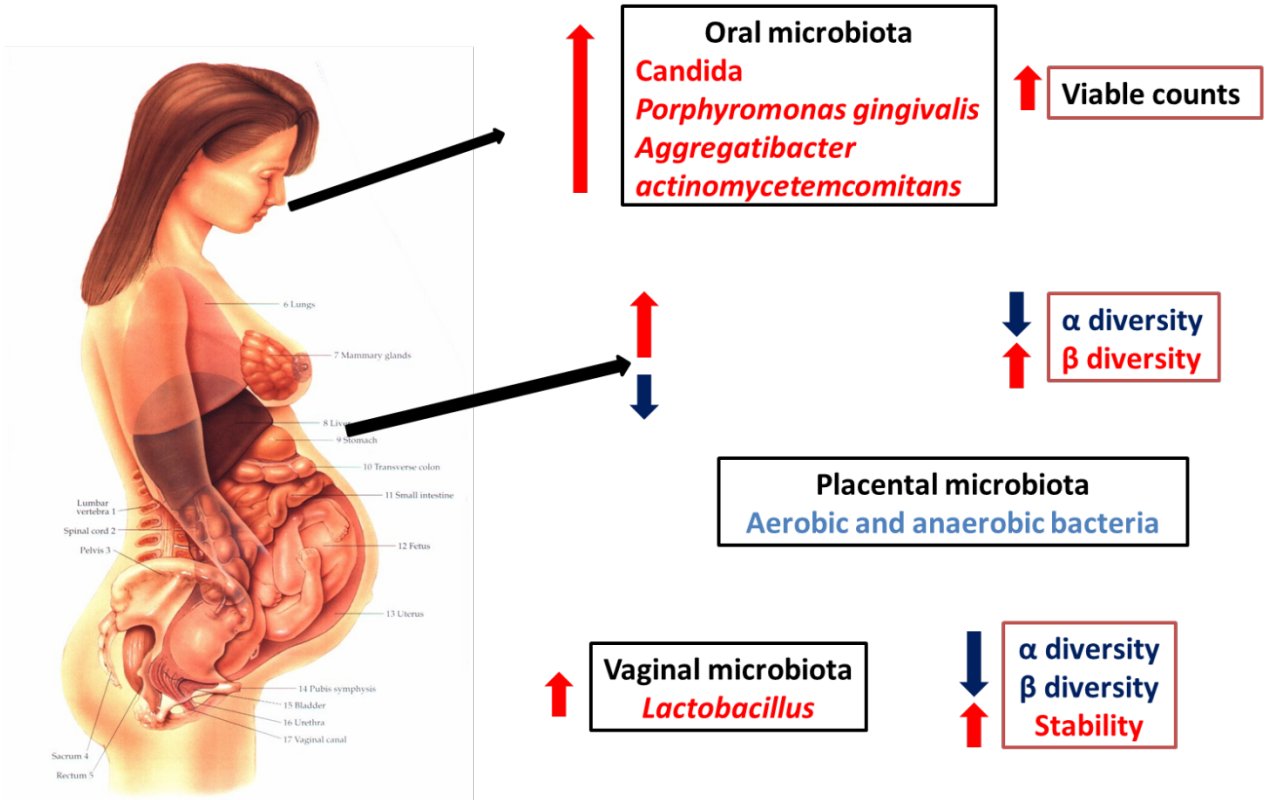
The findings of the human microbiome project revealed that microbiome composition differs between different individuals and also among different sites in the same individual. However, the metabolic signature is preserved despite the observed diversity in microbial species. Some of the factors responsible for the microbial diversity are varying environmental conditions like nutrient availability, humidity, temperature, pH and oxygen demand. Microbiome composition has an influence on diverse physiological processes like metabolism, immunity, signaling processes, hormonal regulation, drug metabolism etc. An alteration in the composition of resident commensal communities relative to the community found in healthy

individuals is called dysbiosis. Dysbiosis can occur on account of a number of factors and is associated with a wide range of disease conditions including obesity, diabetes, inflammatory bowel disease (IBD), metabolic syndrome, ulcerative colitis, Crohn's disease, colorectal cancer and also neurodegenerative disorders [4]. The second phase of the human microbiome project is focused on creating integrated datasets from both the microbiome and the host in a number of microbiome associated conditions such as pregnancy and preterm birth, IBD and diabetes [3]. Besides disease conditions, microbiome composition is also altered dynamically from infancy to childhood to adulthood and important physiological milestones such as pregnancy. The accompanying microbiota transition proves to be beneficial to the host since it enables the host to better adapt to the altered metabolic demands of the particular developmental stage [1, 4].

Maternal microbiome during pregnancy

Pregnancy is one of the important milestones in a woman's life considering that there are pivotal changes occurring inside her body with respect to metabolism, immunity and hormonal levels. Immune modulation takes place to support different functions at a time. For example some level of immune suppression is needed to support the growing fetus and this in turn has to be counterbalanced by stringent immune responses to keep any infections at a bay from the mother and the fetus. Pregnancy can be considered as a multi-stage phenomenon where it is said to be inflammatory during implantation and parturition whereas it is has to be anti-inflammatory during pregnancy when the fetus is growing [4]. There is a paradigm shift in metabolic conditions wherein the mother is in an anabolic state in the first trimester where there is lipogenesis, glycogenesis and adipocyte hypertrophy mediated by altered hormonal levels. This is followed by a transition to a catabolic stage in the third trimester where there is surge in the production of estradiol, progesterone and placental lactogen which mediate insulin and leptin resistance, thus enabling higher levels of glucose [5]. This insulin resistance has been associated with elevated levels of immune modulators such as the cytokines TNF-α and IL-6 which are responsible for obesity-associated metabolic inflammation. These persisting conditions are detrimental to long term heath, but on the contrary, they are found to benefit a

normal pregnancy as they support the energy requirements of both mother and the fetus.



Adapted from M. Nuriel-Ohayon, H. Neuman, O. Koren, Microbial Changes during Pregnancy, Birth, and Infancy, Frontiers in microbiology, 7 (2016) 1031.

Fig 1: Microbiome changes associated with pregnancy

The metabolic, hormonal and immune alterations during pregnancy are paralleled by numerous changes occurring in the entire maternal microbiome (Fig 1). Gut microbiome is shown to influence weight gain in pregnancy. Gut remodeling takes place from the first to the last trimester of pregnancy. It is seen that the microbes in the first trimester are similar to those found in non-pregnant women. As the pregnancy progresses to the third trimester, there *is an increase in β diversity i.e. the diversity between individuals and a reduction in α diversity i.e. the diversity within the same individual. There is also a surge in Actinobacteria and Proteobacteria that has been studied for inflammation associated dysbiosis. There is also a reduction in Faecalibacterium*, which is a butyrate producer showing anti-inflammatory effects [6].

The process of birth plays an important role in the microbial colonization of the infant gut, which is shaped by several factors like the mother's microbiome, mode of delivery as well as genetic components. Studies have shown that vaginally delivered babies receive their mother's vaginal microbiota whereas the C-section delivered babies become the recipients of the maternal skin microbiota. This also has an implication on the immunity of the baby [7]. C*omplex microbial communities colonize the neonate within the first week and are mainly dominated by Actinobacteria (Bifidobacterium), Proteobacteria and Bacteroides.*

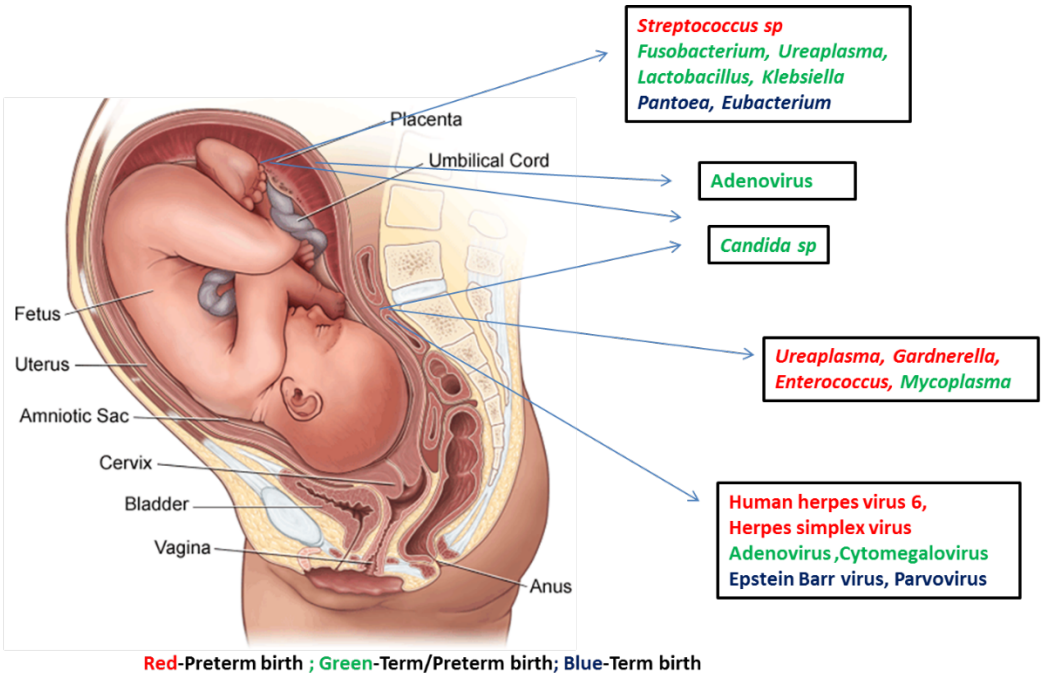
Pregnancy complications and microbiome

There are several complications that can arise in the gestational period. These comprise of preterm birth, cardio metabolic complications like gestational diabetes and gestational hypertension, preeclampsia, eclampsia, intrauterine fetal death, intra-uterine growth restriction (IUGR), placental abruption that affects approximately one in every six pregnancies, hampering maternal and fetal health and survival [1, 4, 8].

Preterm birth and intrauterine infections

Preterm birth (i.e. birth prior to 37 weeks gestation) is the leading cause of infant mortality and adds to severe morbidity and disability among the survivors. Vaginal tract is a host to more than 50 microbial species. T*he vaginal tract is mainly dominated by the lactobacillus species which inhibit pathogen growth through the action of antibacterial compounds and metabolites like lactic acid that helps maintain an acidic environment [9]. Estrogen induced increase in glycogen results in acidic environment which fosters the growth of lactobacilli in pregnancy. About 25% preterm births are associated with the amniotic cavity being the target for microbial invasion [10]. U. parvum*, *U. urealyticum*, *Mycoplasma hominis*, *Gardnerella vaginalis*, *Peptostreptococcus* sp., *Enterococcus* sp., *Streptococcus* sp. (particularly *S. agalactiae*), *F. nucleatum*, *Leptotrichia* sp., *S. sanguinegens*, *Haemophilus influenzae*, and *Escherichia coli* are the most common organisms associated with amniotic fluid infection and preterm birth. *Preterm births are also associated with*

abundance of pathogenic organisms like Gardnerella and *Ureaplasma,* lower proportion of *Lactobacillus* species, and higher alpha diversity which stimulates the proinflammatory cytokines and prostaglandins to cause uterine contractions and weaken the fetal membranes. Most of the studies have detected *Ureaplasma* as the prime species in amniotic fluid from preterm pregnancies. *Intrauterine infections like chorioamnionitis associated with preterm birth have been found to originate in the lower genital tract and ascend into the sterile intrauterine environment.*



Adapted from M.S. Payne, S. Bayatibojakhi, Exploring preterm birth as a polymicrobial disease: an overview of the uterine microbiome, Frontiers in immunology, 5 (2014) 595.

Fig 2: Commonly detected microorganisms in the placenta and amniotic fluid associated with preterm or term pregnancies.

It has now been found that most of the placental taxa resemble the oral microbiota than the vaginal microbiota. It has also been demonstrated that hematogenous dissemination is the probable route for oral microbes to cause intrauterine infection. Fusobacterium nucleatum, a gram negative oral anaerobe facilitates hematogenous transmission during the process of placentation, thus binding vascular endothelium and altering the permeability to enable other microbes like E.coli to invade the placenta. The predominant placental microflora belongs to *Escherichia* sp; *Prevotella tannerae*, *Bacteroides sp*., *Streptomyces avermitilis*, *Propionibacterium*

acnes, *Rhodococcus erythropolis*, *Neisseria polysaccharea*, *Neisseria lactamica*, and *Fusobacterium* sp. also appear in lower numbers [11]. *Paenibacillus* was found in a higher abundance in the placentas of term deliveries while *Burkholderia, Streptosporangium* and *Anaeromyxobacter* were found in a higher abundance in preterm deliveries, although no change in histology was seen in a study [4]. Fig. 2 depicts some of the commonly detected microorganisms in the placenta and amniotic fluid whose composition correlate with preterm or term pregnancies.

E.coli is a common endogenous species present in the maternal gut which can be another potential source. This is due to the ability of *E. coli* to cross the mucosal barrier of the intestine. A prime example of this is *Listeria monocytogenes*, which crosses the intestinal barrier and is able to spread hematogenously to various body sites, particularly the fetoplacental unit. Vertical ascension from the vagina; retrograde through the abdominal cavity, introduction through invasive procedures such as amniocentesis and hematogenously from the placenta are the proposed routes of microbial invasion in the uterine cavity [12].It has been widely seen that periodontal disease is associated with 2 to 7 fold increase in the risk for preterm birth and preeclampsia. One of the speculated studies is that lipopolysaccharides (LPS) from gram-negative periodontopathic bacteria like *Porphyromonas gingivalis* may stimulate inflammatory mediators and prostaglandin production culminating in preterm birth. Periodontal disease and oral microbiota as a causative factor for preterm birth is a well established fact. *Gut microbiota was also seen to be the cause of preterm birth after it was seen in amniotic fluid of the women showing preterm mature rupture of membranes [13].*

Pregnancy associated cardio metabolic disorders

Gestational diabetes mellitus and gestational hypertension are the two most common cardio metabolic complications prevalent in pregnancy. These may increase the risk for C section delivery. Dysbiotic microbiome will lead to the diffusion of gut bacterial endotoxin into the systemic circulation, thus giving rise to low grade inflammatory response which is a common feature of cardio metabolic diseases.

Thus insulin resistance and chronic sub clinical inflammation proves to be a hallmark pathway for both these disorders [5].

The gut microbiome plays a significant role in metabolizing indigestible polysaccharides and regulating fat storage .The presence and abundance of each taxon determines the differential ability of each microbial taxon to metabolize energy nutrients and harvest the energy. Firmicutes and Bacteroidetes are the two dominant phyla in gut microbiome and alterations in these groups have been seen in obesogenic state as compared to normal individuals. Dietary factors and genetics play a role influencing gut microbial composition. An increased Firmicutes to Bacteroidetes ratio is seen in high fat diet along with increased gut inflammation and intestinal permeability [14].

Pre-pregnancy obesity and weight gain

Pre-pregnancy obesity and excessive maternal weight gain increases the risk of fetal macrosomia, hyperinsulinemia in pregnancy, C-section delivery and metabolic syndrome in childhood. An increase in BMI can also lead to defect in the folate intake which can lead to aberrant fetal neural tube formation. There is mounting evidence on the microbiota-obesity link due to many reasons. The gut microbiota enables hydrolysis of indigestible polysaccharides to easily absorbable monosaccharides leading to the activation of lipoprotein lipase. Thus glucose will be rapidly absorbed and fatty acids will be abundantly stored with de novo synthesis of liver-derived triglycerides; boosting weight gain [1]. Certain specific microbiota compositions were showed to modulate fasting-induced adipocyte factor. Apart from energy harvest, pro-inflammatory and anti-inflammatory properties executed by specific strains of the gut microbiota could also be associated with obesity [15].

Chronic stress also affects gut microbiota by increasing the translocation of bacterial cell components into the blood streams from the gut. Preterm birth is also associated with depression which is also influenced by the gut microbiota.

Table 1: Alterations in microbiota at different sites during certain complications of pregnancy.

Complication	Altered microbiota	Site
Preterm birth[1]	↑ *Streptococcus, Ureaplasma, Escherischia, Gardnerella, Fusobacterium* *Porphyromonas gingivalis*	Vagina Placenta (transferred from oral cavity during periodontal disease)
Obesity and excessive weight gain [1,15]	↑ Firmicutes : Bacteroidetes *Staphylococcus* *Enterobacterium* *E. coli* *Clostridium* ↓ *Bifidobacterium*	Gut

Modulation of microbiota as a therapy

It is quite evident that dysbiosis of microbiome is associated with adverse complications of pregnancy. In view of this, recent studies have focused on exploring the modulation of microbiota as a therapy to control dysbiosis. Consumption of probiotic food during pregnancy has been shown to suppress the rate of preterm birth and preeclampsia [1]. It is important to study and develop the microbial markers associated with pregnancy complications. For this purpose it is important to differentiate between the alterations in microbiota occurring normally during pregnancy with those which may be linked to pregnancy complications. An economic method of diagnosing women with preterm birth could be monitoring the vaginal pH. Targeted therapies can be then implemented by using a vaginal gel containing lactic acid and glycogen or an intravaginal probiotic strain to achieve a healthy level of lactobacilli [4]. Further studies are likely to result in a greater insight with respect to the microbiota alterations that could be targeted for therapy of pregnancy associated complications.

Summary

Human microbiome that occupies different sites in our body has been shown to play several important functions in our body like metabolism, immune protection, signaling etc. Microbiome composition keeps on altering throughout an individual's life and especially in certain dynamic processes like pregnancy. During pregnancy

there are several metabolic and immune changes that are taking place in a woman's body in order to ensure the survival and fitness of the growing fetus. Microbiome has been seen to execute a diverse set of functions in this regard. Dysbiosis can culminate into undesirable complications which at times can be fatal for the growing fetus. Thus it is important to further gain insights and shed light into various molecular pathways mediated by the microbiome to explore several therapies that could prevent such malfunctions and in fact nourish the growing fetus. Although minute, the microbiome efficiently mothers the growing fetus and thus plays a prominent role in pregnancy.

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Mind your gut feelings

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Introduction

The role of microbes as brain manipulator has been observed in past in different animals. Zombie like the behaviour of ants on infection of Cordyceps fungus, rats losing aversion to a cat on Toxoplasma infection and aggressive behavior of dogs in case of rabies are some of the examples. In the year 2006 the term gut microbiota as “forgotten organ” came into the picture (1). Even though relation between gut commensals and human brain was not a very well established area of research, giving a second thought to the fact that there are around 100 trillion microbes reside within a human body, 10 times more than total cells in the human body (2), consisting of ~1000 species, ~7000 different strains which has 150 times more gene pool than the human genome (3), one cannot simply ignore their qualitative and quantitative impact on different aspects of human biology including Gut-brain axis (GBA).

The role of gut microbiota in establishing & maintaining different processes within our body has been a current hot topic of research where researchers are trying to explore their impact on immune system development (4,5), maintaining gut homeostasis, maintaining gut motility (6) and amongst all these is the brain and behavior which we will be emphasizing in this article. In the year 2000, flood incidence in Walkerton led to contamination of drinking water source with the pathogenic strain of *E.coli* and *Campylobacter jejuni* (7). About 2,300 people suffered from severe gastrointestinal infection, and many of them developed chronic irritable bowel syndrome (IBS). Long-term studies (8 years) guided by Stephen Collins showed psychological issues like depression in the residents. It was amongst the earliest link which highlighted the possible microbe-brain relations in humans.

In the past 4-5 years, the interest in the study the Gut-brain axis has increased amongst the scientists, which can be attributed to the connection of altered gut microbiome with brain-related diseases.

Strategies to study Gut-microbiome interaction:

Scientists have used different approaches to elucidate the role of gut microbiota on behavior which includes the use of germ-free animals, animals infected with a particular pathogen, an animal fed with probiotic and fecal transplantation studies. Earlier examinations were mainly based on the on molecular level studies for example change in the level of stress hormone Adrenocorticotropic hormone (ACTH) or corticosterone (9). The molecular level findings of the impact gut microbiota on stress responses were further confirmed by behavioural studies of animals (10, 11, 12). These tests mainly focused on anxiety and depressive behavioural pattern in response to various stressed conditions.

Studies done in 2004 by Sudo et al on germ-free animals showed increased ACTH and corticosterone levels in the blood of germ-free mice as compared to control population (9). The effects were reversed by fecal transplantation with Specific pathogen-free mouse in 9 weeks old mice. But no impact was observed in 17 weeks old mice indicating that the effects of microbiota to stress responses can be age specific. Behavioral studies done by Neufeld in 2010 showed decreased anxiety in germ-free animals by elevated plus maze studies and elevated levels of ACTH (12) . These results are tricky to explain as increased levels of ACTH are indicative of increased stress response and hence the anxiety. Infection studies in mice using *Trichuris muris* showed that anxiety like behaviour was found to be increased as determined by Light dark box test (13). These observations were accompanied by the decreased Brain derived neurotrophic factor (BDNF) expression. The protein is mainly involved in neurogenesis & other important brain functions.

Bravo J. A. et al studied effect of administration of *Lactobacillus rhamnosus* (JB-1) by monitoring central GABAB1b mRNA expression. They observed altered

expression of GABA1b mRNA in different parts of the brain & decreased corticosterone level in *L. rhamnosus* fed mice compared to control (14). One of the early fecal transplantation experiments were carried out by Bercik et al in 2011, where they transplanted the gut microbiota from mice strain which is an introvert type to the extrovert or exploratory type mice strain (15). And the results were quite interesting. After transplantation, the behavior of the exploratory or extrovert type of strain changed to introvert type indicating that gut microflora not just alters the behavior pattern under stress conditions but has an overall influence on behavioral type. Further, many such studies were done using similar strategies to explore more on the Brain-gut commensal communication (16). The output all the studies is as follows

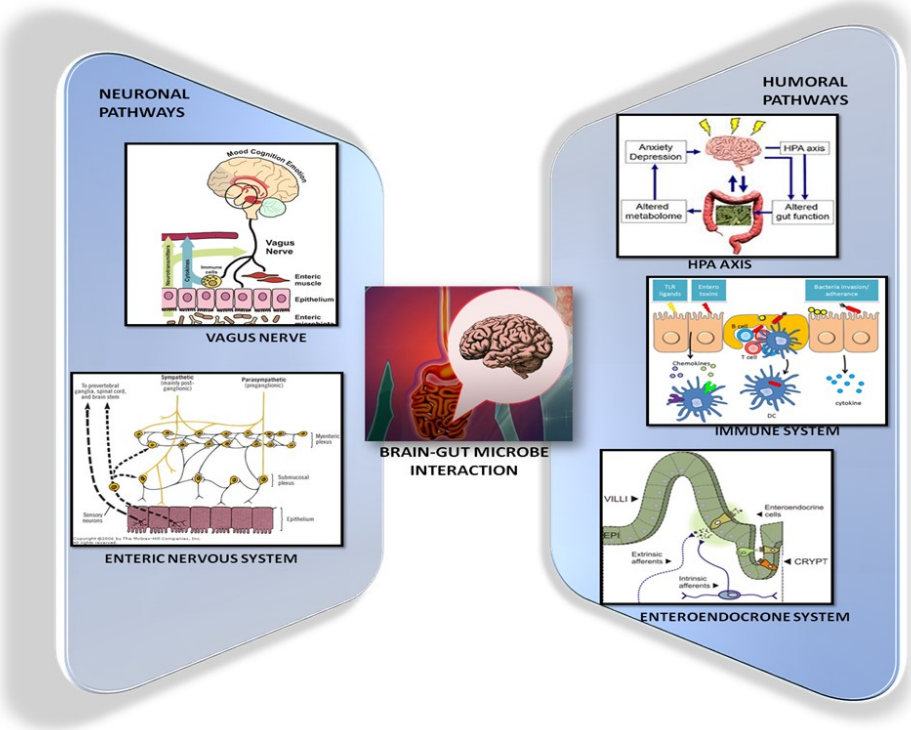
Parameter	**Behavioral**	**Molecular level**
Germ free animals	decreased anxiety like behaviour, impaired memory, increased self grooming,	Decreased BDNF, cFos, NMDA receptor subunit expression, Increased in level of basal corticosterone
Probiotic treated animals	Decreased anxienty and depression like behaviour, increased social interation, improved spacial memory	Increased BDNF and cFos expression
Infected animals	Increased anxiety, impaired recognition & working memory on stress induction	Decreased BDNF & cFos expression

4. Pathways of Gut microbiota- brain interaction:

In order to understand the gut-brain connection, let's have a look at the organization of the nervous system in humans. The nervous system in humans has two main arms, central nervous system and peripheral nervous system each of which has sensory and motor components. The motor component is consisting of a somatic nervous system and autonomous nervous system. The autonomic nervous system has three main branches: sympathetic, parasympathetic and enteric nervous system. We will be focusing on the enteric nervous system & its role in the gut commensal brain interaction.

The communication between the Gut-Brain-Axis is a bidirectional (8) that is the signals are sent from brain to gut and vice versa through components of the nervous system, endocrine system, and immune system. The role of each system will be covered here elsewhere. As mentioned earlier the Gut microbiota and brain communication is bidirectional and involves not just neuronal but other systems as well. To categorize them into groups, they involve mainly the neuronal that is the nervous system and humoral component.In neuronal both CNS and ENS are involved in to and fro signaling between gut microbes and brain. The humoral system includes Hypothalamus-Pituitary-Adrenal (HPA) axis, enteroendocrine system and immune system mainly mucosal immune cells. Role of each is described in this section

The vagus is the tenth cranial nerve. It innervates the pharynx, larynx and visceral organs. It has both afferent and efferent fibers sending signals to & fro. The ratio of afferent to efferent fibers in peripheral nerve bundles is 9:1. Primary afferents innervate the muscular and mucosal layers. The visceral afferent endings in the intestine have the diverse array of mechanosensory and chemical receptors. Results of studies done by Cunningham et al on effects of desipramine (DMI) treatment and vagus nerve stimulation (VNS) showed that vagus nerve stimulation led to the reduction of anxiety and depression associated behavior in mice as observed by forced swim test (17). Enteric nervous system (ENS) is also termed as the second brain of the body as it can work independent of CNS and contains 500 million nerve fibers almost 5 times that of the spinal cord. The ENS in our body contains myenteric and submucosal plexuses which span the mucosal lining of the gut. In vitro experiments by patch clamp method using the mucosal layer from mice showed administration of *Lactobacillus reuteri* enhances excitability of colonic AH neurons, chief sensory neurons in the colon (18). This observation suggests microbiota does have a role in stimulation of neuronal route through the enteric nervous system.



Hypothalamus-pituitary-adrenal axis plays a major role in signaling during stress responses. On perceiving stress signal, hypothalamus secrets corticotrophin releasing hormone (CRH) which signals the pituitary to release ACTH. ACTH triggers adrenal gland to secrete cortisol which deals with the stress responses.

As mentioned in section 3.1, increased corticosterone levels germ-free animals suggest changes in stress response (9). Enteroendocrine system functions through Enteroendochromaffin cells.These are the secretary cells interspersed in the mucosal lining of the gut at regular intervals. They are capable of secreting various signaling molecules like gut hormones & neurotransmitters. These signals can be sensed by the afferent neurons and immune cells residing in the submucosal layer of the gut (19).

Apart from keeping a check on infection the microglial cells, macrophages residing in the brain also plays role in brain development processes. Gut microbiota also found to have an impact on maturation and functioning of astrocytes, where the germ-free, as well as antibiotics, treated mice had abnormal astrocyte morphology, altered gene expression and impaired functional response to a stimulus (20). Along with

regulating function of immune cells of the brain, gut microbiota also has a regulatory effect on the immune cells present in the submucosal layer of the gut. Beneficial bacteria like *Bifidobacterium fragilis* shows anti-inflammatory action by triggering TH17 & Treg cells to produce anti-inflammatory cytokines IL-17A & IL10 respectively. Many of the probiotics like *Bifidobacterium, Lactobacillus* also keeps check on the secretion of pro-inflammatory cytokines by immune cells, whereas microbial dysbiosis and infection with enterobactericeae, *Streptococcus* leads to stimulation of immune cells to secrete pro-inflammatory cytokines and overall activation of immune responses (20). Dysbiosis is a microbial imbalance that is impaired microbiome composition within the body leading to negative impact on overall health.

Possible means of communications at molecular level

Although all above mentioned pathways involve three major systems namely Nervous system, Endocrine system & Immune system in gut microbiota-brain communication, these pathways do not work independently but show interconnections. To describe it in concise manner gut microbiota and brain communication is mediated through an interplay between nervous, endocrine & immune systems. The exact mechanisms underlying this communication are not known at the molecular level but possibilities can be deduced by various research findings. Some of the possible means of these interactions are described in this section.

Many of the gut bacteria found to produce various neurotransmitters for example *Bacillus* produces GABA, *Enterococcus* & *Streptococcus* produces Serotonin, *Lactobacilli* Produces GABA & acetylcholine(21, 22, 23). Neurotransmitters produced by bacteria can directly aid in microbe brain communication. The secretion of short-chain fatty acids (SCFA) also observed by microbiota in the gut. SCFA are the product of metabolism of dietary fibers by microbes which normally cannot be digested by a human digestive system (24, 25). Systemic Na butyrate injection in rat has shown antidepressant effects, increased central serotonin neurotransmission, and BDNF expression (26). SCFAs influence secretion of gut hormones, like

cholecystokinin (CCK), peptide tyrosine kinase (PYY) (27, 28). These hormones have brain penetration properties & their administration in rodents have significant effects on neurotransmitters and & behavior (29, 30, 31).

An interplay between neuronal, endocrine & immune system has been linked to microbiota population in the gut. Interkingdom signalling between enteric microbes and host can be mediated through bidirectional signalling (19). One of the examples of such communication is adrenergic signalling between host and bacterium. Under stress conditions the endocrine system secrets adrenergic hormones some molecules of them can leak through the mucosal lining of the gut entering gut lumen where they bind to Qsec receptors present on the surface of enteric bacteria *Escherichia*. This binding triggers expression & secretion of AL-3 of by bacterium which in turn can bind to the adrenergic receptor 2 on the luminal side of the enteroendochromaffin cells which can send signals to nervous and immune cells. Thes enteroendochromaffin cells play major role in bidirectional signaling as they are capable of receiving signals from luminal side & sending signals by means of signaling molecules on submucosal side & vice versa. That is they act as mediators of this to & fro communication between gut microbes & brain.

Gut Microbiota & Diseases

Seeing the influence of gut microbes on brain functioning, one can predict the impact of dysbiosis on a development of neurological & neuropsychiatric diseases. Some of the most recent links between gut microbiota & neurodisorders are mentioned in this section. Harach and co-workers have showed that mice suffering from Alzheimer's disease are found to have different microbiota composition as compared to the healthy mice (32). Studies on germ-free mice models of Alzheimer's disease showed fewer amyloid plaques as compare to specific pathogen strain free mice. Fewer amyloid plaques in Germ free animals indicate that the microbiota has a role in the development of pathophysiology of the disease. Similarly, the studies were done on synuclein overexpression mice, a model for Parkinson's disease, showed that gut microbes promote a-synuclein-mediated motor deficits and brain pathology while germ-free mice show the reduction in microglia activation (33). The pathology can be

induced by SCFAs even in absence of gut microbes suggesting that modulation of microglia and enhancement of PD pathophysiology is mediated through SCFA's secreted by Gut microbiota. Human gut microbiota from PD patients induces enhanced motor dysfunction in mice. Indicating altered microbiota composition. A recent study by Hsiao and colleagues showed that oral treatment of MIA offspring, the animal model for ASD, with the human commensal *Bacteroides fragilis* resulted in improved gut permeability, altered microbial composition, and amelioration of communicative, stereotypic, anxiety- like defects and sensorimotor behaviors which are the characteristics of ASD (34).

Psychobiotics & Gut commensals

All the above examples show a link between gut commensals & neurological diseases. The information of altered gut microbiota in the diseased condition & information based on studies carried out to see the effect of administration of a specific strain in the reduction of disease pathophysiology will lead to completely new approach for the treatment of neurological or neuropsychiatric disorders. Here we enter the new emerging field of Psychobiotics. Live bacteria (probiotics), which when ingested, confer mental health benefits through interaction with commensal gut bacteria. Burnet and co-workers proposed the definition of psychobiotics be expanded beyond probiotics and prebiotics to include other means of influencing the microbiome (35). Subsequently, several studies use the rodent disease models to see the psychobiotic effect of particular probiotic or prebiotic in reducing disease symptoms. A few studies were also done on humans to check effect of psychobiotics but most of them were done on healthy subjects to see positive behavioral changes caused by consumption of it. But it was in 2013 for the first time scientist showed direct changes in brain activity on administration of probiotic by MRI scan (42). In this experiment they showed sad faces just before MRI scan to all the subjects on psychobiotics & controls. Relative to placebo, probiotic-treated participants showed decreased activity in a functional network associated with emotional, somatosensory, and interoceptive processing, including the somatosensory cortex, the insula.
Some of these examples are mentioned below.

Mouse disease model system			
Model	Psychobiotic	Species	Effect
Alzheimer's disease	Prebiotic, chitosan oligosaccharide	Male Sprague–Dawley rats (n = 12)	↑Cognitive function (Morris water maze), ↓ pro-inflammatory cytokines (tumour necrosis factor-/, interleukin-1b) (36)
Amyotrophic lateral sclerosis	Prebiotic, galacto-oligosaccharide	Male transgenic ALZ mice (n = 12)	↓Motor neuron death, ↓ muscular atrophy (37)
Autism spectrum disorder	Probiotic, *Bacteroides fragilis*	Offspring of pregnant C57BL/6N mice (n = 9–75/group)	↑Intestinal permeability, ↑ pro-inflammatory cytokines (interleukin-6), ↓ anxiety (open field test), ↓ repetitive behaviour (marble burying), ↑communication (calling), ↑ sensorimotor gating (startle inhibition) (38)

Studies directly on human	
Psychobiotic	Effect
Fermented milk with *Lactobacillus casei* (n=124)/placebo- 3 week	Self rating as happy (participants whose baseline mood scores fell in the lowest third of the total range) (39)
Probiotic *Lactobacillus helveticus* R0052 and *Bifidobacterium longum*) or a placebo over 30 days (n=55)	Decline in self reported negative mood & distress Decrease in urinary cortisol (40)
Probiotic *Bifidobacterium bifidum* W23*, Bifidobacterium lactis* W52*, Lactobacillus acidophilus* W37*, Lactobacillus brevis* W63*, Lactobacillus casei* W56*, Lactobacillus salivarius* W24*, and Lactococcus lactis* W19 and W58 -4 weeks (n=50)	Reduced reactivity to sad mood (41)

Future prospects:

Psychobiotics is new emerging area and hence opens up a new avenue for treatment of the neuropsychiatric and neurological disorders. A better understanding of the interconnection between the nervous system, endocrine system, and immune system can Humoral which will be helpful to understand and hence treatment of other autoimmune and endocrine-mediated disorders. For exploring further we need a better understanding of exact mechanisms of interaction at the molecular level between gut microbiota and different systems which influence brain functioning. At

the same time, one needs to take care of false positive and false negatives during the studies. There are several questions that one needs to find an answer like an age-specific effect of psychobiotics, dose-response function, time course of an emergence of effects, long term effects, cognitive benefits, detrimental effects, strain specificity & its drug interaction, before going ahead with use of microbiota as psychobiotics.

To summarize, the interaction between gut microbe & brain is quite apparent which can be Germ-free by modulating gut microbiota. Hence better understanding of gut microbe-brain communication does give a new hope for treatment of many neurological & neuropsychiatric diseases. This area opens up new avenues for using beneficial microbes in human health. Understanding the mechanisms underlying microbes - mammalian cells interaction would be most promising area of research worth advocating for younger generation cellular and molecular biologists.

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Prof. A. S. Mukherjee Prize

Regulation of PRL-3 Translation by Plakophilin3.

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Desmosomes are sites of intercellular adhesion that maintain the integrity of epithelial tissues. Loss of desmosomal plaque protein Plakophilin 3 (PKP3) leads to an increase in neoplastic progression and metastasis and is accompanied by an increase in Phosphatase of Regenerating Liver-3 (PRL-3) levels. PRL-3 is overexpressed in metastatic cancers and PRL-3 protein levels are high in cells with decreased PKP3 expression, though there is no change in PRL-3 gene expression(1). These results suggested that the loss of PKP3 leads to an increase in PRL-3 translation.

The process of translation is regulated by RNA binding proteins (RBPs). RBPs identify complex secondary and tertiary structures in the 5' or the 3' Untranslated Region (UTR) of an mRNAs and this association either inhibits or enhances translation(2). PKP3 is also a component of stress granules, which are sites of stalled translational complexes containing ribosomes, RBPS and mRNAs. PKP3 forms a complex with PCBP1 and RBPs such as G3BP1, FXR1 and PABP1 which are known to regulate translation(3). It has been previously reported that the translation of PRL-3 is inhibited by PCBP1, a poly-C binding protein, by its interaction with PRL-3s 5'UTR(4). Our results demonstrate that PKP3 and PCBP1 form a complex in vivo that is dependent on the N-terminal regions of both PKP3 and PCBP1. We hypothesized that PKP3 may regulate the translation of PRL-3, either by directly interacting with the PRL-3 mRNA or by stimulating the ability of PCBP1 to inhibit PRL-3 translation. RNA-IP experiments demonstrate that PKP3 associates with PRL-3 mRNA and along with PCBP1 may regulate PRL-3 translation. Our

results demonstrate that PKP3 loss results in an increase in the levels of a GFP reporter construct that contains the PRL-3 3' UTR. As the 5' UTR of PRL-3 doesn't seem sensitive to PKP3 levels, it is possible that the PKP3-PCBP1 complex cooperate to inhibit the translation of PRL-3.

In addition to PRL-3, PKP3 may interact with a number of mRNAs and possibly alter their translation in response to changing physiological conditions. We are in the process of identifying mRNAs whose translation is altered upon PKP3 and may interact with PKP3. Therefore, PKP3 might play a crucial role in regulating the translation of mRNAs that lead to an inhibition of neoplastic progression and metastasis.

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Prof. S.R.V Rao Prize (Oral)

Paradoxical role of Zic3 in adult neurogenesis unravelled by stem cell model system

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A highly complex transcription factor circuitry regulates the development of eukaryotes in a stage specific manner. There are numerous layers of controls that are imposed on these transcription factors so that the development proceeds unhindered. Information obtained from studies on regulation of lineage specific transcription factors is instrumental in generating *in vitro* cell source of interest from pluripotent stem cells (PSCs) for therapeutic purpose. How a pluripotent stem cell, receiving numerous signals from hundreds of transcription factors, makes the decision of either self renewal or differentiation is a compelling research query. Owing to its paradoxical role in maintenance of pluripotency and neural lineage commitment, Zinc finger transcription factor in Cerebellum3 (*Zic3*) gains our research interest. *Zic3* is a member of Gli super-family and is the earliest Zic proteins to be expressed in pre-implanted blastocyst. ZIC3 maintains self-renewal by governing the expression of pluripotent factor Nanog. Post gastrulation, ZIC3 has been shown to be associated with left-right axis symmetry and in generation of ectoderm and mesoderm tissues. However, in adult life, Zic3 expression is restricted to brain suggesting its probable requirement in neural commitment. The lacunae in the field arise due to the lack of studies about role of ZIC3 in neural development and in its poorly built interactome. In an attempt to fill these lacunae, we developed a model to study neurogenesis using adult dental pulp stem cells and successfully generated Olfactory bulb like neurons exhibiting co-expression of Tyrosine Hydroxylase (TH) and Glutamate decarboxylase (GAD65). Interestingly, ZIC3, whose expression was undetected in undifferentiated DPSC, showed an up-regulation upon neural differentiation. Further, upon genetic manipulation of *ZIC3*, we found that ZIC3

expression positively correlates with that of TH. This intrigued us to decipher the mechanism underlying this *ZIC3* mediated TH regulation. By performing Chromatin immunoprecipitation analysis, we found that ZIC3 directly binds to *TH* promoter and regulates its expression. To authenticate this in a developmentally relevant model system, mouse embryonic stem cells (mESCs) were used and it was observed that ZIC3-TH axis holds true also in olfactory bulb like neurons derived from mESCs. This was further reiterated by Zic3 knockdown experiments in mouse olfactory bulb derived primary neurons which also showed a down-regulation of TH. In all, using three different model systems, our study conclusively illustrated a direct relationship between ZIC3 and TH in olfactory bulb like neurons.

TH is the major rate limiting enzyme in the synthesis of Dopamine and finds high relevance in context of Parkinson's disease which is characterized by curtailed Dopamine producing neurons. As our study has shown a reliable regulation of TH by ZIC3, we intend to understand if an up-regulation of ZIC3 would restore TH expression in unhealthy neurons and reverse Parkinson's phenotype. A small molecule which can enhance ZIC3 expression leading to subsequent increase in TH activity would be screened for in the study. By and large, this study aims at contributing to the existing knowledge in the field of transcriptional regulation of *ZIC3* in neurogenesis, while trying to also find its relevance in context of Parkinson's disease.

Prof. S.R.V Rao Prize (poster)

Chronic vs Acute gamma radiation stress in Chironomus*:* a decisive factor for apoptosis

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In a current scenario, research based on basic interaction of ionizing radiation with biological systems has gained tremendous attention as it has applications in medicine, agriculture, space reasearch and other technologies. *Chironomus ramosus* has proven to be an excellent model organism in the field of radiation biology as these ancient- dipteran insects can efficiently tolerate upto 3500 Gy of gamma radiation exposure. Although exact mechanism for its gamma radiation tolerance is still not completely understood some key features like efficient oxidative machinery, role of HSP 70, ability to control damaged DNA, presence of more aromatic amino acids at heme pocket of its extracellular hemoglobin all together contributes for its better adaptive nature against gamma radiation stress. This study was designed to investigate consequences of effect of different dose rates of gamma irradiation on the midge larvae. Change in the dose rates from 5.5 Gy/min (i.e. chronic) to 55.2 Gy/min (i.e. acute) led to enhanced larval lethality and shift of mortality curve. These initial findings emphasized us to look into the mode of cell death in response to change in dose rates. Fate of *Chironomus* larvae after increase in dose rate checked using Annexin-V fluorescence staining which could distinguish healthy, apoptotic and necrotic populations of hemocyte cells. These experiments revealed dose dependant apoptosis in *Chironomus* larvae, which was further validated by biochemical assays for involvement of intrinsic Caspase 8, Caspase 9 and effective Caspase 3 activities for the execution of apoptotic machinery. Maximum activity for Caspase 9 indicated intrinsic pathway of apoptosis which was

further validated after measuring mitochondrial membrane potential. Fluorescent dye JC 1 showed reduction in signal for irradiated samples which directly correlated with decrease in mitochondrial membrane potential. Differential tail length pattern was observed for alkaline and neutral comet assays. Larvae exposed to chronic dose of gamma exposure showed DNA single strand damage in alkaline comet assay while maximum percentage of cells showed neural comet which indicated DNA double strand breaks. Comet data supported our hypothesis that damage beyond the repair activates apoptosis machineries in these midge larvae. Although few polydispersity peaks were observed in dynamic light scattering, interestingly typical apoptotic DNA ladder formation was not so evident in this organism; rather we have seen fragmentation of RNA as a result of irradiation. However, all the studied parameters suggested dose rate dependant gamma radiation induced apoptosis, we further validated apoptotic cells using atomic force microscopy and transmission electron microscopy. Surface topographical changes in hemocyte cells were evident after acute gamma radiation exposure while typical apoptotic blebbing was also clearly seen using AFM and TEM respectively. The findings obtained in this study clearly indicates that unwanted and damaged cells in *Chironomus* larva are removed through a regulated cascade of molecular events after exposure to acute gamma radiation stress.

Prof. B R Sheshachar Prize

On The Importance Of Fidelity Of DNA Content On Vertebrate Development Using Zebrafish As A Model Organism

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Metazoan development begins from a single cell zygote that undergoes iterative rounds of cytokinetic events that generate a multicellular organism which undergoes morphogenetic movements to give rise to a complex three dimensional animal. Early cytokinesis events are rapid thereby demanding intricate coordination of multiple events like DNA replication, nuclear envelope breakdown, assembly of the mitotic spindle etc, in a temporally controlled manner. Therefore, perturbation of the rhythmicity or dynamicity of this process may lead to embryonic death. Zebrafish embryos subjected to transient heat shock during the first mitotic division result in whole organism tetraploidy. Surprisingly, we found that heat shocks administered at specific two minute intervals during the first zygotic mitosis resulted in higher survival of tetraploid embryos until 24 hours post fertilization. We hypothesised that variations in survival of embryos after heat shock could result from perturbation of specific phases of mitosis at the time of the heast shock. Immunofluorescence analysis of heat shocked embryos confirmed our hypothesis and revealed that heat shocks administered at mitotic phases with intact DNA-centrosome interactions, such as prophase and telophase, resulted in high lethality indices (~90%) in the population. However heat shocks performed after nuclear envelope breakdown (prometaphase, metaphase) results in ~50% survival until 24 hours post fertilization. Thus heat shocks performed at phases of mitosis when the nuclear envelope:centrosome interactions do not exist are conducive to embryonic survival. These findings suggest that early vertebrate embryos undergoing iterative rounds of cell divisions possess temporal windows of resilience to perturbations that coincide with the naturally dynamic mitotic phases.

It is known that vertebrate haploids and tetraploids do not survive to adults, which is often attributed to gene dosage errors. However, all eukaryotic embryos immediately after

fertilization are in a transcriptionally quiescent phase, which is relieved only at zygotic genome activation. Thus, the immediate response to changes in ploidy cannot be gene dosage or transcription related. We hypothesized that early zebrafish embryos must respond to alterations in ploidy by altering cell biological features, if at all. We immunolabelled in diploid, haploid and tetraploid embryos components of the mitotic spindle and measured various parameters like length, width and centrosome areas. Surprisingly, we found that absolute values of spindle length and width do not change at all. However centrosome areas vary significantly across different ploidy conditions. As development progresses, these early responses appears to converge into variations in cell sizes in altered ploidy embryos. Haploid embryos tend to have smaller cells while tetraploid embryos have larger cells compared to diploids initially and eventually both haploids and tetraploids tend to have smaller cell sizes in comparison to diploids. While these cell size variations are manifesting, signaling mechanisms that pattern the embryo unfold. We hypothesise that cell size variations in non-diploid embryos may perturb appropriate partitioning of morphogens across fields of cells in an embryo thereby resulting in global patterning and axis specification defects. These in combination with eventual gene dosage errors may result in lethality of non-diploid embryos. Taken together our work may provide insights into what goes wrong when the DNA content of a developing vertebrate embryo is altered.

Prof. Manasi Ram Memorial Prize

Pleiotropic functions of *CG34422/hat-trick* as a maternal gene during oogenesis in *Drosophila*

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Chromodomain (chromatin organization modifier) containing proteins are involved in chromatin remodeling and transcriptional regulation of gene expression in eukaryotes during development (Eissenberg, 2001; Cavalli and Paro, 1998). Although molecular and structural analysis of various *Drosophila* chromodomain proteins such as Heterochromatin Protein 1 (HP1), Polycomb (Pc) have been carried out over last decades, very little is known about the chromodomain protein CG34422. It has recently been named as *hat-trick* for its putative role in heterochromatin association and its influence on TDP-43 toxicity (Sreedharan et al, 2015). The gene *hat-trick* encodes two annotated transcript variants which translate into two polypeptides of size 186 kDa and 259 kDa, respectively. It harbors an AT-rich interacting domain (ARID), chromatin organization modifier (CHROMO) domain, Retinoblastoma Binding Protein 1 N-terminal (RBB1NT/NUC162) domain, Tudor-Knot Domain. Given the putative role of *hat-trick* in chromatin modeling, it is tempting to speculate its pleiotropic functions in modulating an astounding variety of processes. Since oogenesis in *Drosophila* has been an excellent model system to study various aspects of cell and developmental biology, including cell fate determination, cell differentiation, signal transduction, cell migration, etc., we were keen to explore the pleiotropic functions of *hat-trick* during oogenesis.

In an attempt to functionally characterize *CG34422*/*hat-trick*, we commenced with dissecting its role in oogenesis. Fluorescence *in-situ* hybridization revealed that the

hat-trick transcripts are present at all stages of oogenesis and in all cell types. We have generated polyclonal anti-Hat-trick antibody to check its expression at the protein level. Immunostaining experiments confirmed its dynamic expression throughout the oogenesis. The protein is predominantly expressed within the oocyte nucleus, specifically within the karyosome after the oocyte arrest. It co-localizes with Heterochromatin Protein1 marking the heterochromatinized pericentromeric region. Further, co-localization of Hat-trick with C(3)G, a synaptonemal complex component, confirmed its role in heterochromatin clustering and karyosome maintenance. We used FLP-DFS technique to generate loss-of-function *hat-trick* mutant clones. The phenotypes observed in *hat-trick* null egg chambers highlighted its obligatory role in oocyte determination, arrests, positioning, maturation, transport from nurse cells to oocyte, Gurken mediated dorso-ventral polarity maintenance, etc.

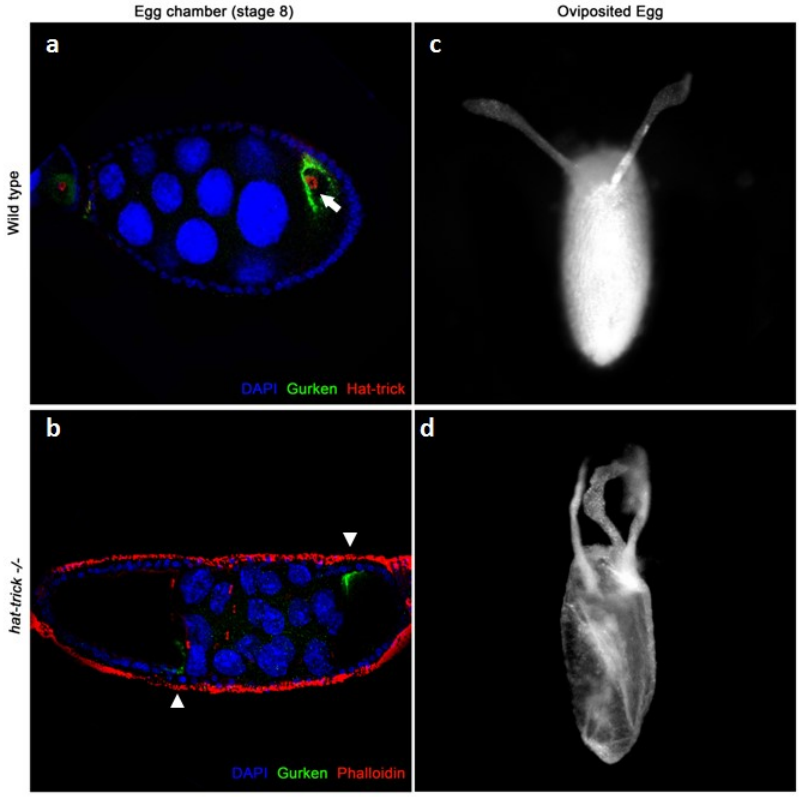

Fig: (a) The specific localization of Hat-trick within oocyte nucleus (arrow) of wild type egg chamber. Gurken protein is localized at dorso-anterior region of wild type oocyte. (b) htk^{71} mutant egg chamber show presence of Gurken at two opposite poles of egg chamber (arrow-heads). This misregulated Gurken might be the probable cause of dorsalized eggs having extra antler-shaped dorsal appendages (d) in comparison to wild type (c).

Being a chromatin modelling protein, Hat-trick seems to have pleiotropic functions in regulating different aspects of developmental and cellular processes. Here we present that *hat-trick* has profound pleiotropic functions in *Drosophila* oogenesis. Germline mosaic analysis revealed that *hat-trick* is required for cytoblast proliferation, oocyte determination, nurse cell endoreplication, migration, germ cell positioning and cyst encapsulation inside the germarium. It has been illustrated here that *hat-trick* is required for axis specification and germline formation by circuitously affecting the localization and translation of Gurken. Chromatin binding and modeling property of Hat-trick protein is accountable for its speculated role in regulating a wide range of processes in a direct or indirect fashion. Given to its obligatory maternal contribution during egg maturation and fertilization, *hat-trick*^{71}can be considered as a maternal effect lethal mutation or mutation that prevents proper oogenesis.

Suggested readings

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XL AICB Conference Prize

Functional analysis of conserved noncoding DNA sequences in zebrafish

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Evolution has been the key player in the generation of complex functions from lower to higher organisms. Lower organism generally contains a large fraction of coding DNA sequences, while in the case of higher organism non-coding DNA constitutes the major part of the genome. In human, protein-coding DNA makes up to the 2% of the genome and rest is non coding DNA. Noncoding DNA part of the genome shows a great level of conservation among different organisms. DNA sequences, which are identical up to 200 bps are known as ultraconserved sequences. Human genome constitutes up to 5% of the ultraconserved sequences. Although the function of ultraconserved sequences is not known but the cell is maintaining these sequences in the genome throughout the evolution. Ultraconserved DNA sequences are known to clustered near developmental genes in high density. We have earlier reported three ultraconserved noncoding DNA sequences, **C**onserved **R**egions (CR1, CR2 & CR3), associated with Hox cluster. Hox genes are a set of transcription factors, which regulate early embryonic development. These genes show a collinearity of genomic organization and function along the anterior-posterior body axis. Hox genes and their organization are conserved across phyla. We performed many assays to find out the function of CRs in cell lines as well as in zebrafish.

Our results from reporter gene assays in different cell lines using transient transfection assays and FACS suggest minimal or no activity of CRs. Stable cell line transformants in different cell lines suggest that CRs work as repressors. To show the effect of CRs at the organism level, we used zebrafish as an animal model.

Transient reporter assays in zebrafish also confirm the time-dependent repressor activity of CRs. To check the regulatory effects of CRs in the regulation of development, we need to generate transgenic and knockout of CR sequences using CRISPR/Cas9 systems. Since CRs are conserved in the different vertebrate system, we want to check the functional conservation of CRs in different organisms. Finally, we will try to decipher the mechanism of CRs during early embryonic development for that first we need to test the transcription potential of CRs.

Overall this study for the first time suggests that ultraconserved DNA sequences work as repressors at the cell as well as organism level and opens up a new dimension of research for ultraconserved sequences with respect to their function.

40th AICB Conference 2016, Jiwaji University, Gwalior, India.
Nov. 17-19, 2016

XL AICB Conference Prize (Best Poster Award)

Yeast Crg1 is required for mitochondrial integrity and TOR signalling by modulating redox homeostasis

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Yeast Crg1 acts as methyltransferase required for resistance against cantharidin. It inactivates Cantharidin by methylation. However, cellular targets of Crg1 are not well characterized. In this study, we have identified the function of Crg1 in regulation of lipid homeostasis and in TOR signalling. From chemical genetic screen, we have found *CRG1* deletion strain highly resistant to inhibitors of TOR (Rapamycin), sensitive to non-fermentable carbon sources (NFC) and oxidative stress (hydrogen peroxide). Rapamycin resistance usually develops due to hyper activation of TOR pathway. The TOR signalling is involved in regulation of many cellular processes; ribosome biogenesis, protein translation, autophagy and cell growth. The interplay between mitochondria and TOR has also been shown to modulate cellular homeostasis in response to oxidative stress. One of the major functions of mitochondria is regulate cellular redox homeostasis. Therefore, we hypothesized that Crg1 may play a key role in regulation of mitochondrial integrity and TOR signalling. The function of many proteins such as such as Sch9, Sfp1, Tap42, Gln3, Gat1, eIF4E, Atg13 is mediated through TOR signalling via phosphorylation. We found significant down regulation of TOR signalling in *CRG1* deletion strain. Compared to an isogenic wild type, in *CRG1* deletion strain, we detected decrease in Sch9 phosphorylation, increase in acidic vacuoles, educed cell size, cell cycle arrest in G1-phase, cytosolic localization of Sfp1 and down regulation of ribosome biogenesis genes. Furthermore, we also observed nuclear localization of Gln3 and Gat1 and upregulation of their targeted genes upon *CRG1* deletion. Thus we concluded that *CRG1* deletion mutant exhibit resistant to rapamycin and promote inhibition of TOR signalling through an unknown mechanism. Since TORC1 is a central kinase in TOR pathway that senses the nutrition availability or starvation, we challenged the

CRG1 deletion cells with glucose starvation and NFC and found that the mutant is unable to grow in both the conditions. We further identified severe loss of mtDNA in the mutant indicating defect in mitochondrial dynamics. To rescue the function, we complemented *CRG1* deletion mutant with a wild type Crg1 but didn't observe rescue in response to Rapamycin and oxidative stress. However, resistance to cantharidin was completely restored. We reasoned that CRG1 deletion might result in irreversible damage to mtDNA which could not be recovered by complementation. The loss of mtDNA can occur due to accumulation of ROS in mitochondria. Interestingly we observed accumulation of ROS upon *CRG1* deletion which got suppressed by overexpression of superoxide dismutases; *SOD1/SOD2* and supplementation of antioxidants; GSH/NAC. Furthermore to study role of Crg1 in mtDNA integrity, TOR signaling and resistance to rapamycin, we screened petite strains with rho^0/rho^- phenotypes with rapamycin and found that loss of mtDNA causes resistance to rapamycin. Thus we concluded that rapamycin resistance of *CRG1* deletion cells might be due to loss of mtDNA. Interestingly we also found that *CRG1* haploid deletion doesn't show loss of mtDNA and rapamycin resistance but the diploid deletion does. The role Crg1 in regulation of mtDNA integrity and redox homeostasis in haploids is not clear. Either it happens via ageing or a specific mechanism that operate in diploid but not in haploid. Altogether we have identified a new function of Crg1 in maintenance of mtDNA and TOR signalling.

"Yeast Crg1 is required for mitochondrial integrity and TOR signaling by modulating redox homeostasis"

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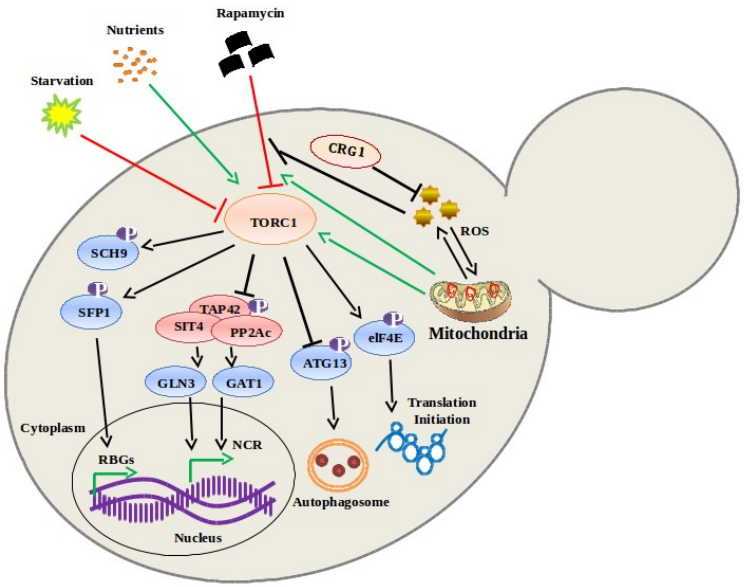

Concluding Model: Crg1 maintains mitochondrial integrity by modulation of redox homeostasis. Mitochondria regulates TORC1 function. Active TORC1 regulates various pathways via downstream proteins such as Sch9, Sfp1, Gln3, Gat1, Atg13, elF4A etc. The *CRG1* deletion alters redox homeostasis and accumulate ROS, causing damage to mtDNA. The damage or loss of mtDNA inhibit TORC1 signalling and develops resistance to rapamycin. In conclusion, Crg1 regulates TORC1 function via modulation of redox homeostasis and mitochondrial DNA integrity.

40th AICB Conference 2016, Jiwaji University, Gwalior, India.
Nov. 17-19, 2016

XL AICB Conference Prize (Best Poster Award)

Rab11 is essential for dorsal closure and epithelial morphogenesis and in developing *Drosophila* embryos.

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The process of epithelial morphogenesis in developing *Drosophila* embryos is accomplished after the execution of two critically important events. First being Gastrulation and the second being Dorsal Closure. Both these events show a striking feature in common which is of collective cell migration, a feature which involves stringent orchestration of the dynamics of cell membrane components and intracellular signaling events. A critical signaling event which occurs during this process is the Basket or JNK and Dpp signaling which when perturbed results in the failure of this process and consequently a dorsal open or basket phenotype is evident. The regulation of Basket or MAPK signaling in migrating epithelia is an enigmatic process as it is modulated by a vast array of upstream regulators, comprising of membrane proteins and also parallel signaling cascades which synergistically complete the process of embryonic morphogenesis. Here we report that Rab11, a small Ras like GTPase associated with Recycling Endosomes and Trans Golgi Network, when mutated show the dorsal open, basket or puckered phenotype illustrating that *Rab11* mutations typically perturb the components of JNK signaling pathway. Different alleles of *Rab11* when conditionally driven by *Pannier-Gal4* (Embryonic epithelium specific Gal4) show phenotypes similar to mutants of JNK signaling pathway. This was confirmed from the cuticle preparations of the mutant embryos under the influence of different alleles of *Rab11*, viz., $Rab11^{RNAi}$, $Rab11^{N124I}$or $Rab11^{DN}$ (dominant negative), and $Rab11^{Q70L}$ (*Rab11* constitutively

active). Owing to the membrane organizing properties of Recycling Endosomes we have already established that Rab11 is critically important for the Recycling of βPS-integrins and Growth factor receptors at the Leading Edge Cells or the Dorsal Most Cells of the lateral epithelia of the fly embryo. There are however a few reports which suggest that Rab11activity is a must for the definition of cell polarity determining proteins which also have a critical role to play in determining the anterior-posterior axis of migrating cells. Here we are showing that *lgl*, which is a potential tumour suppressor gene and also an epithelial polarity determining protein, when perturbed, again show the dorsal open or basket phenotype. Not only this, conditionally driven RNAi lines of both *lgl* and *Rab11* show strikingly similar results in both embryonic and adult stages, where *pnrGal4* driven $Rab11^{RNAi}$ and lgl^{RNAi} flies develop thoracic closure defects. Based on these phenotypic observations we speculate that *Rab11* could be a potential regulator of the JNK signaling pathway in migrating epithelium of *Drosophila* which it executes probably with its interaction with *lgl*, a classically documented and conserved tumour suppressor gene across invertebrate and vertebrate phyla.

ISCB Sponsored Workshop

A 2nd One-day Seminar & 4th Training Workshop in "Recent Techniques in Molecular & Cell Biology" was held in the Department of Zoology, Lucknow University from Nov 23-26, 2016. It was convened and organized by Prof. Monisha Banerjee and her research team of Molecular & Human Genetics Laboratory as well as Dr. Mohd Arshad, Molecular Endocrinology Lab and Dr. Suchit Swaroop in the Department of Zoology, Lucknow University. The seminar cum workshop was organized under the Centre of Excellence programme of Higher Education, Govt of UP, Lucknow and Indian Society of Cell Biology (ISCB).

The Seminar cum Training Workshop was inaugurated and the Protocol Book was released on 23rd Nov, 2016 by Prof. Abbas Ali Mahdi, King George's Medical University, Lucknow; Prof. U.D. Mishra, Dean, Faculty of Science, Lucknow University and Former Head of Department, Zoology, LU. The Prof. Abbas Ali Mahdi delivered the inaugural lecture where he highlighted the immense development that has taken place over the years in the field of disease diagnostics. The pre-lunch session was dedicated to lectures by other eminent speakers' *viz.* Prof. SK Saxena and Dr. Neetu Singh of KGMU, Lucknow, Dr. BN Singh and Dr. SK Rath from CDRI, Lucknow. All of them spoke about their respective research work in dengue, cancer genomics, *Mycobacterium* and basic genetics respectively.

Twenty three (23) participants registered for the Training Workshop from different universities and colleges *viz.* Allahabad University; SRM University, Chennai; Shibli College, Azamgarh; GGDSD College, Chandigarh; Integral University and Lucknow University. There were lectures and extensive laboratory work which included all modern techniques of cell culture, molecular biology and genetics. The excitement amongst participants was immense. Twenty (20) experiments were demonstrated and hands-on training was provided in 3.5 days. Each participant was provided with Protocol Book, was given lunch and refreshments on all 4 days. The training workshop ended successfully on 26th Nov, 2016 with a short Valedictory ceremony where Prof. Chandan Haldar, Head, Department of Zoology, BHU, Varanasi and Prof. Omkar, Head, Zoology, Lucknow University distributed certificates to all participants and research team members.

Dr. Monisha Banerjee

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A strong recommendation to become members of Indian Society of Cell Biology

As a bonafide life members of ISCB and as a members of several academic and professional societies, I can very confidently say that Indian Society of Cell Biology is one of the finest scientific societies having very high reputation not only in India but worldwide. Its growing exponentially in terms of its membership strength as well as quality of science its members doing in India. So, joining this society will be a wise and a very judicious step for knowing best science in the country.

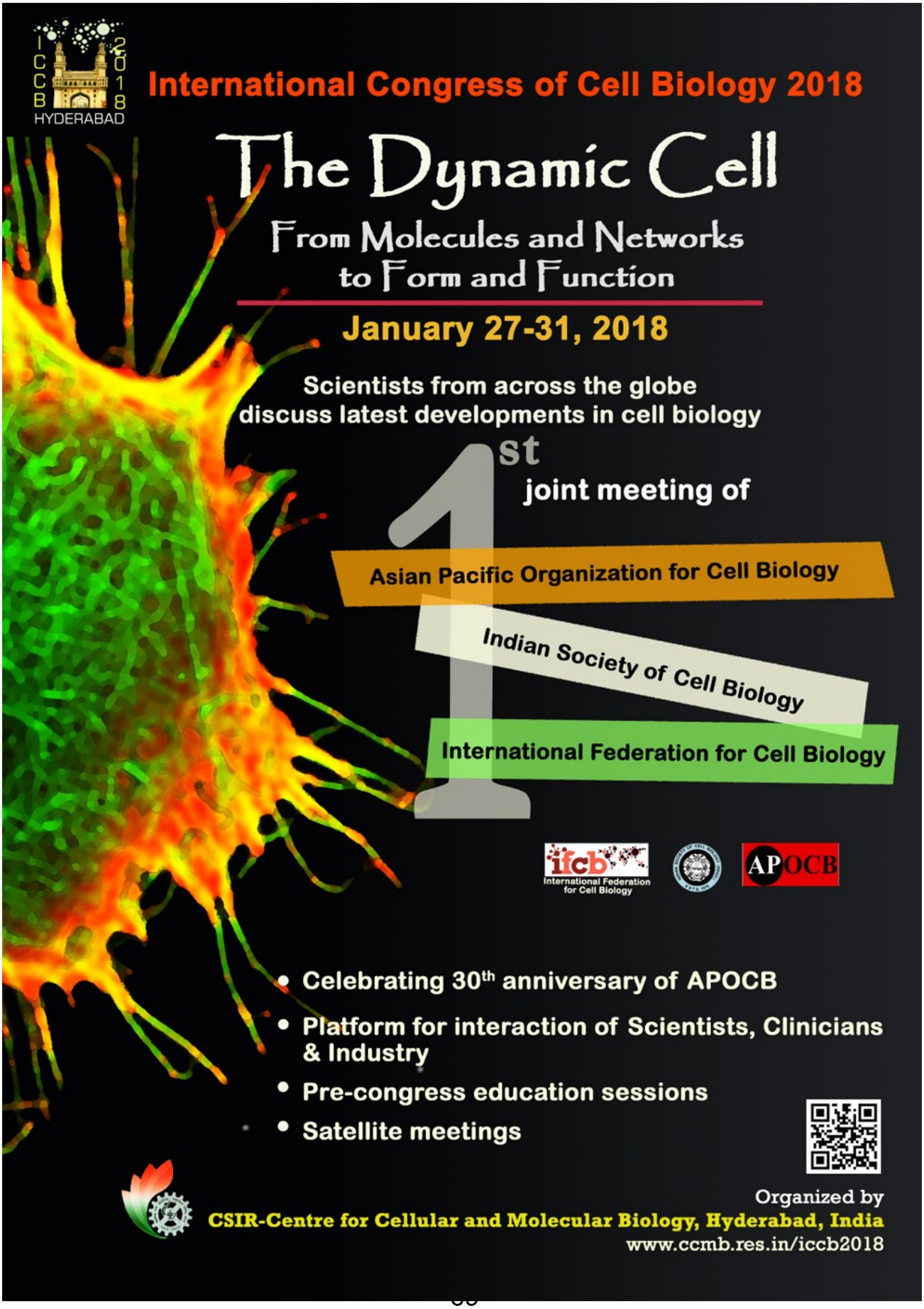
Please note and tell those interested that membership fees has been revised and approved in last Executive Committee meeting and implemented from 1st April 2017.

New Membership slabs will be as below

Life membership	Rs. 3000=00	+ Rs 50=00 (admission fee)
Ordinary membership	Rs. 500=00	+ Rs. 50=00 (admission fee)
Student membership	Rs. 300=00	+ Rs. 50=00 (admission fee)

Membership form can be downloaded from ISCB website, www.iscb.org.in

EC 2015-17


ICCB 2018 HYDERABAD
International Congress of Cell Biology 2018
The Dynamic Cell
From Molecules and Networks to Form and Function
January 27-31, 2018
Scientists from across the globe discuss latest developments in cell biology
1st joint meeting of
Asian Pacific Organization for Cell Biology
Indian Society of Cell Biology
International Federation for Cell Biology
ifcb International Federation for Cell Biology
APOCB
• Celebrating 30th anniversary of APOCB
• Platform for interaction of Scientists, Clinicians & Industry
• Pre-congress education sessions
• Satellite meetings
Organized by
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www.ccmb.res.in/iccb2018

Statement of accounts

[Due to delay in auditing of the accounts, which is underway, statement of account will be produced at later stage].

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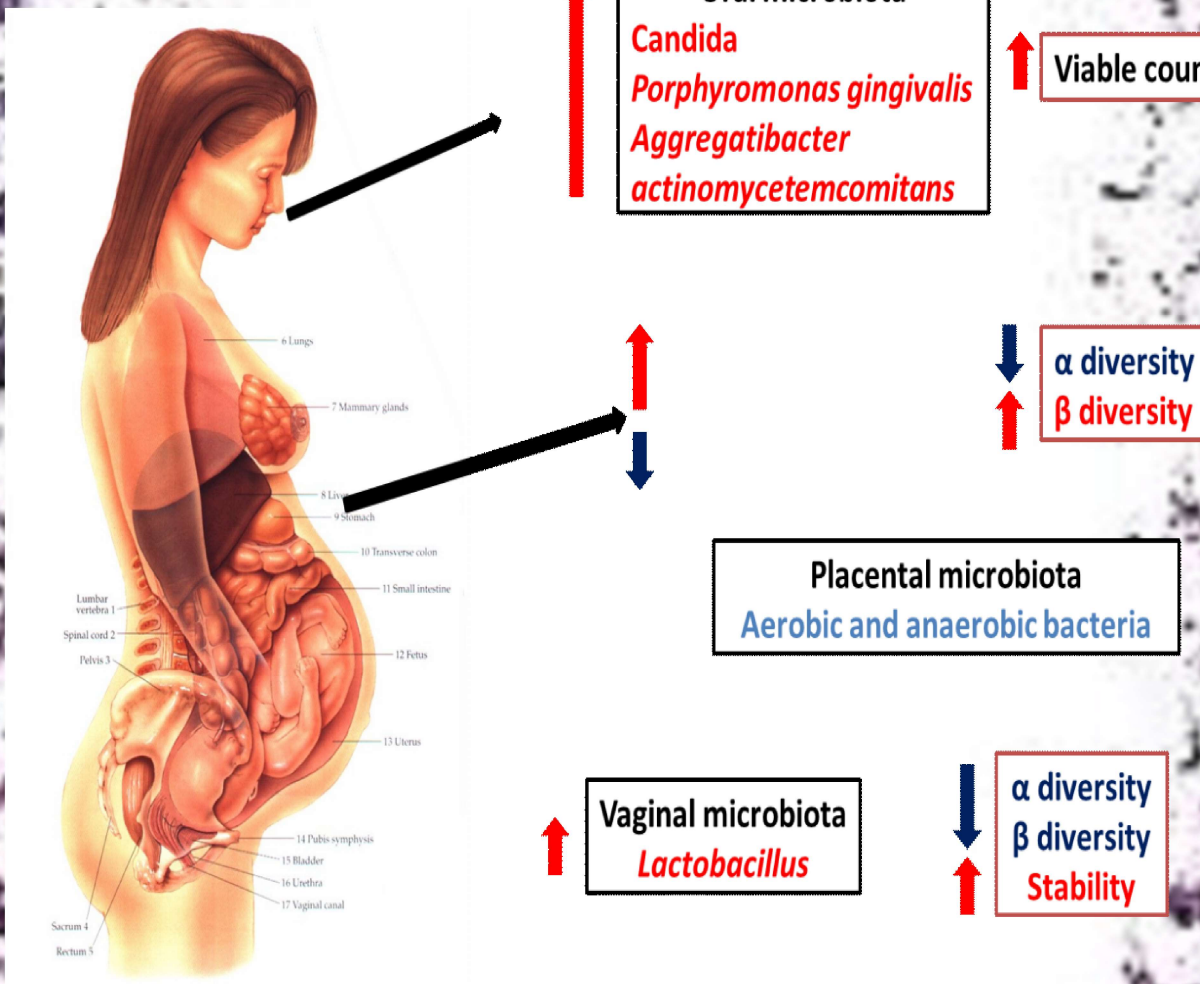
Cell Biology Newsletter



Adapted from M. Nuriel-Ohayon, H. Neuman, O. Koren, Microbial Changes during Pregnancy, Birth, and Infancy, Frontiers in microbiology, 7 (2016) 1031.

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