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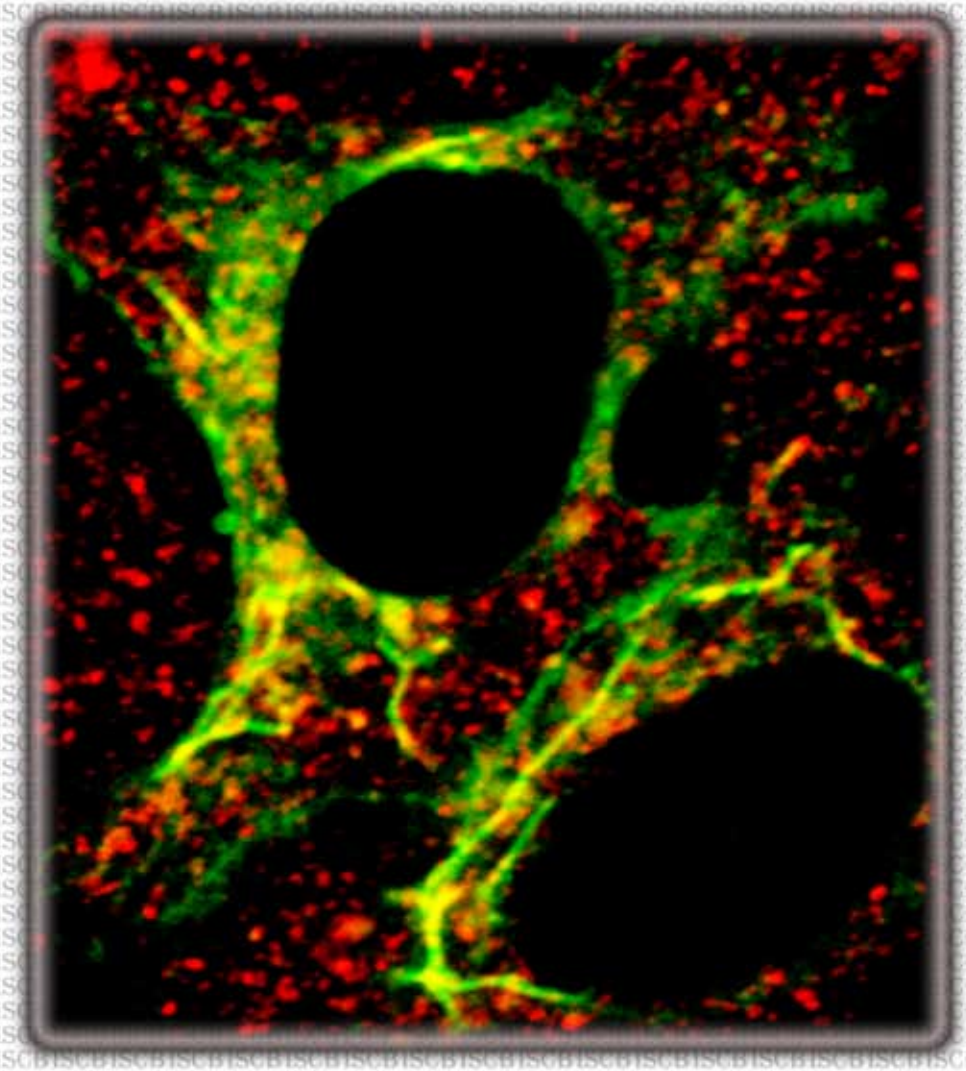
NEWSLETTER



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Dear Members of the ISCB,

Warm greetings to all of you.

We are pleased to bring out the 2014 issue of the Cell Biology News Letter, through which we hope to share information on some of the recent activities of the Society. The News Letter also includes articles on the 2013 Nobel Prize winning work on membrane vesicle traffic, and the bench to bedside story of Trastuzumab-DM1, contributed by Dr. Swarup and Dr Lupus respectively.

We would like to place on record our appreciation, and thank the organizers of the 37th AICBC hosted by inStem, Bengaluru. For those of you who could not attend the meeting at Bengaluru, we have provided a report on the conference. Abstract of the J Das Memorial Award Lecture delivered by Prof. Kanury V.S. Rao and abstracts of award winning student presentations are also included. Considering the very large number of posters presented by students, this year we awarded seven additional prizes to the students. We thank Cell Signaling Technology for sponsoring these additional prizes. It was heartening to see the enthusiastic participation from the students, who submitted over 170 abstracts.

We also request all members to take note that the 38th AICBC is scheduled to be held at CDRI, Lucknow. Details of this meeting will be posted on the ISCB Web Page. Request wide circulation of this information and encourage participation.

Nominations for the S.P. Ray Choudhary 75th Birthday Endowment Lecture for 2014 are invited from members. The announcement and nomination form are given in this News Letter. This lecture will be delivered in the 38th AICBC to be held at Lucknow. Request you to actively participate in sending nominations, when they are called for.

Election for the office bearers for the year 2015-2017 will be held in the 2nd half of 2014. You will all receive a communication for inviting nominations from the Returning Officer in due course.

We encourage contributions to the second issue of the News Letter for the year 2014. Proposals for holding the AICBC in December, 2015 are also invited. Request all of you to go through the ISCB Web Page: www.**iscb**.org.in

Vegesna Radha, Secretary
A.J.Rachel, Joint Secretary

Cover figure:

The Rab8 GTPase regulates various membrane vesicle trafficking pathways in the cell. Activated Rab8 (shown in green) forms tubules which facilitate the transport (recycling) of vesicles carrying transferrin receptor (shown in red) to plasma membrane (Vipul Vaibhava *et al*. J. Cell Sci. 2012). Merging of red and green gives yellow color.

A NOTE ON AICBC 2013, BENGALURU

Day 1: 22nd December, 2013

The XXXVII All India Cell Biology Conference and Symposium on 'Cell Dynamics and Cell fate' was inaugurated at J.N. Tata auditorium, IISc, Bengaluru on 22nd December, 2013. Convener, Jyotsna Dhawan welcomed all the delegates and the invited speakers. The proceedings of the conference commenced with the Presidential address by Prof. B.N. Singh, Banaras Hindu University. Prof. Singh gave a historical account of ISCB and spoke about the changes the annual meetings of the society underwent with the passage of time. He also highlighted the activities of the Indian Society of Cell Biologists. Further, he spoke about few of the unusual genetic features of *Drosophila ananassae*, the model system he has been working on for the last few decades. Natural populations of *D. ananassae* exhibit high mutability and segregation distortion. Prof. Singh and his colleagues observed extensive chromosomal polymorphism and absence of genetic co-adaptation in this species.

The first session was chaired by Dr. Apurva Sarin. The inaugural lecture was delivered by Dr. Roop Mallik (TIFR, Mumbai) on phagosomes. He presented a model elucidating dynein pairing in the 'early to late phagosome switch'. The second talk was by Dr. Peter Friedl (Radboud University, Netherlands & University of Texas, USA) who emphasized the importance of tissue niches, and the context of micro- tracks in cancer cell invasion *in vivo*. The second session was chaired by Dr. V. Radha wherein Dr. Supriya Prasanth (UIUC, USA) presented data on the link between Origin Recognition Complex (ORC), replication initiation and chromatin organization. This was followed by the invited talk by Dr. Boudewijn Burgering (University Medical Center Utrecht, Netherland) on DNA damage control by the gerontogene FOXO. His findings revealed a novel function of FOXOs in regulating genomic stability and tumour suppression. The last talk of the second session was by Dr. Jomon Joseph (NCCS, Pune) who presented non-traditional roles of nucleoporins and how NUP358 regulates cell polarity. The third session started with Prof. S.C. Lakhotia as chairperson. In this session, Dr. Satyajit Mayor (NCBS & inStem, Banaglore) spoke about the implications of active control of cell membrane organization via dynamic nanoclusters that are regulated by actin. This was followed by a talk by Dr. Madan Rao (NCBS, RRI) who presented the theoretical implications of active cell membrane on the processing of information. This was followed by inStem Frontier lecture by Dr. Tobias Meyer (Standford University, USA) who captured the decision making processes to enter or exit cell cycle using parallel live cell imaging. The session ended with a special Biocluster talk by Taslimarif Saiyed (C-CAMP, Bengalore) on C-

CAMP's efforts in fostering high-end research and entrepreneurship. One of the highlights of the first day of the conference was large scale participation of young researchers who presented their work in the first round of poster session. The research students interacted enthusiastically with scientists and peers. The fourth session of the first day was chaired by Prof. B.N. Singh, President of ISCB. In this session, the seventh J. Das memorial award lecture was delivered by Dr. Kanury Rao, Head, Immunology group, ICGEB, New Delhi. His recent work is focused on employing systems level approaches to understand communication networks in lymphocytes. He spoke of a network that identifies targets for cancer chemotherapy.

Day 2: 23rd December, 2013

The second day started with the 5th session of the conference chaired by Prof. Jagat Roy wherein Dr. Yamuna Krishnan (NCBS, Bengaluru) displayed her DNA toy as a powerful lens to visualize biology. The next talk was delivered by Dr. Shravanti Rampalli-Deshpande (inStem, Bengaluru) who spoke on the role of EZH2 in pluripotent reprogramming. Dr. Devyani Halder (CDFD, Hyderabad), in her presentation shared her recent data on the regulatory role of histone acetylation/deacetylation in DNA replication. This was followed by lecture delivered by Dr. Jackson James (University of Hyderabad) who presented data on the regulation of Hes-1 gene by Notch during early neocortical development. The last talk of the session was given by Dr. Jagan Pongubala (University of Hyderabad) who spoke on gene regulatory controls during B-cell commitment. The 6th session was chaired by Dr. Sudhir Krishna with Dr. K. Prashant's (UIVC, USA) talk on the role of long non-coding RNAs in cell cycle progression and cancer. The findings from his laboratory revealed that Metastasis Associated Lung Adenocarcinoma Transcript 1 (MALAT1) depletion results in cell proliferation defects during G1/S transition. Dr. Vivek Mittal (Joan & Weill Medical Center of Cornell University, NY, USA) presented data on how blockade of EZH2 histone methyl transferase impairs breast cancer metastasis by depleting cancer stem cell pools. This was followed by an interesting deliberation by Dr. Dasaradhi Palakodeti (inStem, Bengaluru) who works on *Planaria* as a simple multicellular model organism to study stem-cell biology. Recent findings from his laboratory revealed interesting facets of post transcriptional regulation of stem cell function and regeneration in the Planarian *Schmidtea mediterranea*. Dr. Beena Pillai (IGIB, New Delhi) shared her findings on regulatory RNA determinants of neural cell fate using *in vitro* models of neurodegeneration and neurogenesis. The 7th session began post-lunch and was chaired by Prof. Mercy Raman. Dr. Shankar Srinivas (Oxford, UK) spoke on how the division angle during the propagation of pluripotent inner cell mass can influence cell fate during development and differentiation. Dr. Shamik Sen (IIT, Mumbai) presented his

work on the bidirectional relationship between contractility and MMP activity. His findings indicate that extracellular matrix density regulates cancer invasiveness via modulation of cell contractility. Dr. Abdur Rahaman (NISER, Bhubaneswar) spoke on the mechanism of nuclear remodeling in *Tetrahymena*. The 8th session was chaired by Dr. Taslimarif Saiyed and it was highlighted by four sponsored talks by Namita Misra (L'Oreal), Amitabha Majumdar (Unilever), Amit Bhattacharya (Leica) and Clement Khaw (Nikon). The poster session on the second day of the conference was enriched by large number of young researchers from various laboratories of India and there was active interaction between the presenters and the peers. The second day of the conference ended with the General Body meeting of the Indian Society for Cell Biologists.

Day 3: 24th December, 2013

The last day of the conference commenced with 9th Session of the symposium chaired by Dr. Gaiti Hasan. The first talk of the session was delivered by Dr. Sathees Raghavan (IISc, Bengaluru), who elaborated the implications of DNA double-strand breaks in oncogenesis and cancer therapy. He shared data regarding a recent discovery of inhibitors of non-homologous end joining (NHEJ) from his laboratory offering a strategy towards a potential treatment of cancer. Next talk was delivered by Dr. Jayandharan G.R. (CMC, Vellore) who updated the audience regarding various aspects of Adeno-associated virus vector mediated gene therapy and a few interesting findings from his research group. Dr. Krishnaveni Mishra (University of Hyderabad) spoke on sumoylation and the way it can modulate distribution of proteins between sub-nuclear compartments. The last talk of the session was delivered by Dr. Srikala Raghavan who presented the intricacies of Integrin network and its role in epithelial homeostasis and inflammation. Young researchers, students as well as mentors waited eagerly for the10th session of AICBC-13. In this session, chaired by Dr. Akash Gulyani, young research students made oral presentations. Two of the best presentations were awarded prizes. The post-lunch 11th session was chaired by Dr. Maneesha Inamdar and Dr. Siddhartha Jana (IACS, Kolkata) presented data showing the effect of C2 insert of non-muscle myosin II-C on neuritogenesis. This was followed by a talk by Dr. Jonaki Sen (IIT, Kanpur) who presented interesting facets of retinoic acid in neuronal development in the chick. Dr. Aurnab Ghosh (IISER, Pune) spoke on nucleating actin in the neuronal growth cone. He presented interesting findings coming out of his laboratory on how Formin 2 can act as a putative regulator of actin dynamics in neurons. Following this, remaining part of oral presentations by young researchers continued. Finally, delegates reassembled at the 12th session marking the conclusion of the three-day long symposium. The valedictory function

was vibrant with cheering by the audience of the award- winning young participants.

On the whole, over the three days of deliberations at the conference, there were discussions on clusters of localized activity at the cell membrane and cytoskeleton, reorganizations within the cell such as endosomes to lysosomes, nuclear membrane to endoplasmic reticulum, ingenious elucidation of movement of cells and organelles using state of the art techniques, DNA based tools for visual confirmation of cellular physiology etc. The conference ended on an exciting and challenging note with the participants geared up for more excitements in science.

Bimalendu B. Nath,
University of Pune.

Membrane Vesicle Traffic gets Nobel Prize

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Membrane bound vesicles mediate transport of various materials such as proteins, lipids, nutrients from one sub-cellular compartment to another. Membrane vesicles also mediate secretion of various proteins and other molecules and are also involved in uptake of nutrients by a process known as endocytosis. This year three eminent scientists James E. Rothman (Yale University, New Haven, Connecticut, USA), Randy W. Schekman (University of California, Berkeley, USA) and Thomas C. Sudhof (Stanford University, California, USA) shared Nobel Prize in Physiology or Medicine (about U.S. $ 1.2 million) for their “discoveries of machinery regulating vesicle traffic, a major transport system in our cells”. According to Mellman and Emr (2013) (Ref. 1), this award also provides a recognition to the large community of scientists who established the field of “molecular cell biology”.

The ground work for the Nobel Prize winning work on vesicle trafficking by these three scientists was done earlier by George E. Palade, Albert Claude and Christian de Duve who established the field of Cell Biology. They shared the Nobel Prize in 1974. Biochemical analysis and electron microscopy had identified nearly all the organelles in eukaryotic cells. It was established that protein secretion involves synthesis in the ER (endoplasmic reticulum), addition of sugar (glycosylation) in the Golgi complex, packaging in secretory granules and release at the plasma membrane. However, identification of the vesicles that carry the material from one compartment to another and how this process occurs was not known which was explored by Schekman, Rothman and Sudhof.

These three scientists had taken different approaches to identify and understand the molecular components and mechanisms involved in generating vesicles and transport processes such as fusion of the vesicle to the acceptor compartment. Randy Schekman used a genetic approach to identify the genes involved in secretion. His group isolated temperature sensitive mutants of yeast, *Saccharomyces cerevisiae*, that were blocked in secretion at non-permissive temperature (37ºC), but behaved normally at permissive temperature (22-25ºC). He carried out these studies at a time when most people did not believe that yeast will have the molecular machinery for vesicle transport or secretion similar to the one used by higher eukaryotic cells. These temperature sensitive mutants accumulated vesicles at restrictive temperature and appeared dense microscopically. Accumulation of vesicles was demonstrated by electron microscopy. He screened ‘sec’ (secretory) mutants by using a very simple logic (and assay) that the mutant cells blocked in secretion would become more

dense because protein synthesis and vesicle formation would continue when secretion is blocked. Using this simple logic he isolated 23 complementation groups required for secretory pathway in yeast (2-4). This approach led to identification of genes/proteins involved in various steps of membrane vesicle formation and regulation of transport. This screen identified the small monomeric GTPases of the Rab family (related to Ras) that are involved in providing specificity to vesicle trafficking and also involved in regulating almost all the steps involved in vesicle transport such as budding of vesicles, recruitment of cargo, movement to the target membrane and fusion with the target compartment. Their screen resulted in identification of components of the cytoplasmic coat proteins (COPII) that are involved in transport from the ER to the Golgi. His group also identified cytosolic "tether" proteins that play a crucial role in identifying the target membrane by the transport vesicles.

Several 'sec' mutants identified by Schekman's group coded for proteins that were similar to those identified by Rothman's group using a biochemical approach to understand vesicle formation (budding) and fusion in mammalian cells.

James Rothman used biochemical approaches to reconstitute the transport of proteins to and through the Golgi complex. His group identified and purified various components involved in vesicle formation such as COPI coat protein complex, NSF (N-ethyl maleimide-sensitive factor) and SNAP (soluble NSF attachment protein) (5-9). COPI coated vesicles are involved in transport of proteins from the Golgi to the ER and intra-Golgi transport. He identified the mechanisms of membrane vesicle fusion that involves NSF, SNAP and SNAREs. SNAREs are a family of related proteins that are involved in determining the specificity of membrane traffic and fusion. These are organelle specific and determine the functional and biochemical identity of various membrane compartments in the cell. Several of the proteins identified by Rothman's group showed similarity with those identified by Schekman as 'sec' mutants in yeast. This suggested that essential components and features of vesicle transport machinery were conserved between yeast and mammals.

Thomas Sudhof directed his research towards understanding the mechanisms involved in vesicle fusion in neurons (10-12). Neurotransmitters are released by neurons to communicate with other neurons. They are packaged in vesicles and released from the vesicles when a signal is received in the form of action potential. Action potential triggers the fusion of synaptic vesicles with the presynaptic plasma membrane. Sudhof discovered that Ca^{2+} plays an important role in this process which binds to synaptotagmins.

Synaptotagmins interact with SNARES and, in response to Ca^{2+} binding, they mediate vesicle fusion. Sudhof also discovered another component of vesicle fusion machinery, Munc 18 (in mice) homologous to Sec1 protein in yeast. His work showed that Munc 18 interacts with the SNARE complex and it functions in vesicle fusion. Although Sudhof worked on a very specialized system, his findings about vesicle fusion provide conceptual and

mechanistic insights that are of fundamental importance in other systems.

Acknowledgements:

The schematic (Figure 1) showing steps involved in vesicle transport was provided by Dr Madhavi Latha Somaraju Chalasani.

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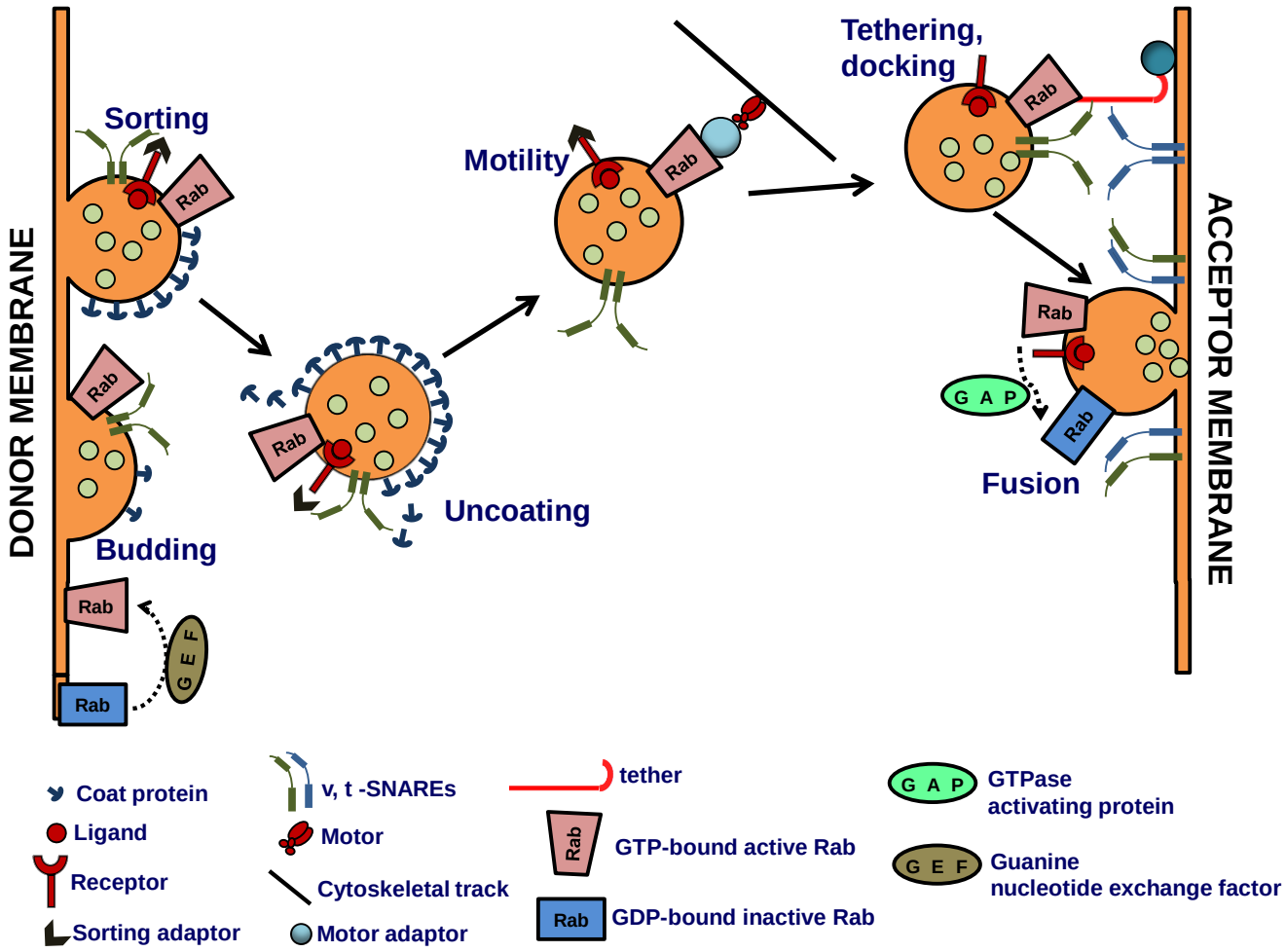

Figure 1. **An overview of the steps involved in vesicle budding, trafficking and fusion with target membrane:** Coat proteins along with adaptor proteins concentrate cargo molecules and cause deformation of donor membranes to form a bud, which is eventually pinched off to form a vesicle (budding). Thus vesicle formation (budding) and recruitment of cargo (sorting) occur almost at the same time. These processes are regulated by Rab GTPases apart from other factors. Then coat proteins of these transport vesicles are removed (uncoating) and vesicle transport is carried out by motor proteins. The motor proteins are recruited to the vesicles along cytoskeletal tracks by Rab GTPases either by direct interaction with motor proteins or by recruiting adaptors for motor proteins. The vesicle reaches the target compartment and is docked in close proximity to target membrane by tethers and SNARES that are also recruited by Rabs (tethering, docking). The fusion of vesicle (donor) and acceptor (target) membranes is mediated by concerted action of SNARES, tethers and Rabs.

Trastuzumab-DM1: A success story of basic cellular studies translated to the clinic

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In recent decades, microtubule-targeted cancer chemotherapy has been on the forefront of the fight against cancer. Starting with the classical drugs taxol, vinblastine, and their derivatives, microtubule-targeted cancer chemotherapy has witnessed the emergence of novel, more effective therapeutics free of the drawbacks associated with earlier drugs. The major challenges associated with several microtubule-targeted drugs include toxicity to normal cells (non-specific binding of drug molecules), resistance to classical drugs (e.g., taxanes resistance), and drug-induced peripheral neuropathy (1-2). By employing biochemical and cell biology techniques, researchers across the world have made substantial progress in devising means to enhance the tumour-specific binding of drug molecules, in developing drugs effective against tumours resistant to classic anti-cancer drugs such as taxanes, and in contributing to the development of drugs that do not induce severe neuropathy. This mini-review narrates the success story of basic cell biology and chemical biology research that has aided the development of the anti-cancer drug trastuzumab-DM1 (also known as ado-trastuzumab emtansine, or Kadcyla) (3). Manu Lopus Bombay University, India.

Before getting into the details of the development of Kadcyla, it is appropriate to describe microtubules as cancer drug targets. Microtubules are cylindrical, linear polymers of tubulin, a heterodimeric protein that has two slightly different subunits. The GTP-dependent addition of tubulin to microtubules is a reversible process, which contributes to a peculiar phenomenon called *dynamic instability* (4). Microtubules do not exist as solid polymers but frequently switch between phases of growth and shortening. This dynamic instability enables microtubules to precisely execute their cellular functions (5). However, even subtle disruptions in the natural dynamic instability of microtubules can induce cell cycle arrest and promote cell death (6). Several microtubule-targeted drugs work by interfering with the natural dynamics of microtubules (7, 8). Specifically, these drugs suppress the dynamic instability through diverse mechanisms, thereby inducing a loss of tension across the sister kinetochores of dividing cells (7). Mitotic checkpoint proteins then arrest the cells in mitosis (7). This prolonged arrest is often followed by programmed cell death (6, 7).

Returning to trastuzumab-DM1, the author and colleagues investigated the molecular mechanism of action of the drug molecule, drug maytansinoid 1 or DM1,of this antibody-drug conjugate using cellular and biochemical analyses (9, 10). This review first introduces the antibody-drug conjugate Kadcyla and then discusses the molecular mechanism of action of its conjugated drug molecule, DM1.

Trastuzumab-DM1 (T-DM1, Ado-trastuzumab emtansine, or Kadcyla)

In 2013, the United States Food and Drug Administration have approved Trastuzumab-DM1 for the treatment of some but not all patients with HER2+ metastatic breast cancer (a type of breast cancer characterized by an overexpression of human epidermal growth factor receptor 2). T-DM1 may be used only with patients who have already undergone treatment with trastuzumab (an antibody that targets and inactivates HER2+ receptors) and a taxane, separately or in combination (11). *Structure of T-DM1*: In T-DM1, the monoclonal antibody trastuzumab is linked to the maytansinoid DM1 through a cross-linker, succinimidyl trans-4 (maleimidylmethyl) cyclohexane-1-carboxylate (SMCC) (Fig. 1) (12). This thioether linkage is stable in circulation and becomes cleaved once the antibody-drug conjugate enters the target cell (12). The drug, DM1, is an antibody-conjugatable derivative of the natural product maytansine, which itself is a potent anti-proliferative agent originally isolated from plants of the genus Maytenus (12, 13). However, intolerable side effects and a lack of tumour specificity render it ineffective as an anti-cancer drug (14).

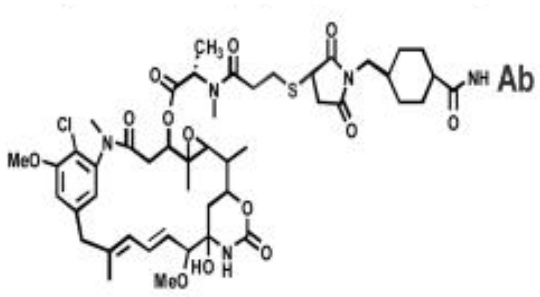

Figure 1: *The antibody-DM1 conjugate*. In trastuzumab-DM1, the drug DM1 is linked to the HER2 – targeted antibody, trastuzumab (Herceptin) through a thioether linkage (10).

Molecular mechanism of action of DM1, the microtubule-targeted drug component of Kadcyla

In order to understand how DM1 works, a comprehensive analysis of DM1, in both its free form and its antibody-conjugated form, was conducted using breast cancer cell lines. DM1 was found to inhibit cancer cell proliferation in sub-nanomolar concentrations and to arrest cells in mitosis (10). Using radiolabeled DM1, the drug has been found to accumulate inside cancer cells in a time-dependent manner (10). When studying the drug's effects on cellular microtubules, DM1 was observed to depolymerize cellular microtubules and purified microtubules *in vitro* in a concentration-dependent manner, while at lower concentrations, it suppressed the dynamic instability of the microtubules (9, 10). Unlike several microtubule-targeted agents, DM1 suppressed nearlyall dynamic instability parameters (such as growth rate, shortening rate, and overall dynamicity) in cells and *in vitro* (9, 10). Investigating the molecular mechanism with which the drug suppresses microtubule dynamics, the drug molecule (DM1) was found to preferentially bind at the tips of growing microtubules and to interfere with the normal, reversible addition of tubulin subunits to existing steady-state microtubules (9, 10).

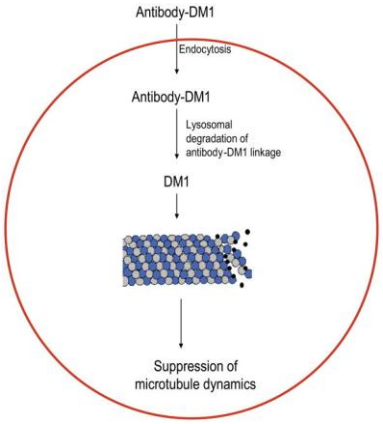

Figure 2. *Mechanism of action of DM1*: Once the antibody-DM1 conjugate binds its target antigen, the conjugate becomes internalized through receptor-mediated endocytosis. Inside the cell, lysosomal degradation liberates DM1 from the antibody. The free drug binds at the tips of dynamic microtubules and suppresses the dynamic instability (14).

Differences between the antibody-DM1 conjugate and free DM1

The free drug and the antibody-DM1 conjugate were observed to suppress microtubule dynamics in a similar manner. However, the antibody-DM1 conjugate had a time lag in suppressing microtubule dynamics compared to the free drug. For example, the SMCC-linked antibody-DM1 conjugate took approximately 24 h of incubation to suppress microtubule dynamics in cells to a similar extent as the free drugs achieved in 5 h of incubation (10). This delay correlated with the time taken to metabolize the antibody-drug conjugate inside the cells. In light of these findings, a general mechanism of action for the antibody-DM1 conjugate was proposed. Accordingly, the antibody-DM1 conjugate binds to its target antigen (HER2, as in the case of trastuzumab-DM1) with the antibody part of the conjugate. Subsequently, the antibody-DM1 conjugate enters the cells through receptor-mediated endocytosis. Once inside, the SMCC linkage is cleaved, releasing the active drug, DM1. DM1 then binds at the tips of microtubules and suppresses dynamic instability (Fig. 2). As described, suppression of microtubule dynamics induces a loss of tension across the sister kinetochores of dividing cells. This loss of tension elevates checkpoint responses, leading to prolonged mitotic arrest often followed by cell death (6, 7).

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Abstract: Prof. J. Das memorial lecture award

Interrogating Cellular Comunication Networks to Identify Targets for Cancer Chemotherapy.

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The molecular components of a cell organize into a complex network of interactions, and biological responses represent emergent features of such networks. The hallmark of dynamic networks, including signaling networks, is non-linear response behaviour. At least one factor influencing the outcome would be the level of functional redundancy in the network. Drug development efforts against cancer are often hampered by the complex properties of signaling networks. Also, the degree of plasticity associated with oncogenic pathway activation, relative to its suppression, also indicates that the ideal targets for pathway inhibition need not necessarily coincide with those that are involved in its activation. Such increments to our understanding of the complex properties of biological systems therefore illuminate that drug development efforts will be significantly aided by a better resolution of the signaling circuitry that controls cell cycle, as well as a description of the least redundant nodes that participate in this process.

The constraints of balancing robustness against sensitivity, however, suggested the existence of *core* signal processing *modules* that calibrate between signal-input and response-output relationships. Here we combined the results of an RNAi screen targeting the cellular signaling machinery, with graph theoretical analysis to extract the core modules that process both mitogenic and oncogenic signals to drive cell cycle progression. These modules encapsulated mechanisms for coordinating seamless transition of cells through the individual cell cycle stages and, importantly, were functionally conserved across different cancer cell types. Pharmacological targeting of their least redundant nodes led to synergistic disruption of the cell cycle in a tissue-type independent manner. Thus targeting combinations of critical nodes from regulatory modules driving distinct phases of the cell cycle provides for a "*multi-module*" targeting based chemotherapeutic strategy that may be more effective against a broad spectrum of cancers.

Prof. A. S. Mukherjee prize for best platform presentation

BIOCHEMICAL ADAPTATIONS DURING LIFE WITHOUT WATER: GLUCOSAMINE PLAYING A PIVOTAL ROLE IN ANHYDROBIOTIC HOMEOSTASIS IN *CHIRONOMUS RAMOSUS*

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Desiccation is one of the closest consequences of an imbalance in environmental humidity and temperature. Growing concerns of desiccation as a stressor and its potential in mediating climate change is gaining attention. To cope such hostile desiccation bouts prevailing in nature, some organisms adopt a strategy of entering into a dry state known as anhydrobiosis. This latent dry state is characterised by the ability of revival and resumption of metabolism upon rehydration. Aquatic organisms, in particular, are frequently challenged to dehydration regimes in their habitats. We thus chose to explore the mechanisms of desiccation tolerance in the larvae of *Chironomus ramosus,* a tropical aquatic midge species. For the first time, we report the identification of glucosamine as a stress metabolite that facilitates recovery of the larvae from desiccation. Further, trehalose, a well- known anhydroprotectant has also been implicated in the desiccation tolerance capacity of the larvae. Experimental validation of these results by FTIR and LC-MS/MS analyses suggested that larvae of *C. ramosusachieve* the anhydrobiotic state with the help of trehalose and glucosamine. These biomolecules not only enable the larvae to sustain the dry state but also help them to revive in response to favourable hydrating conditions. In addition, live bio-imaging studies indicated that larvae also activate their antioxidant defence system in order to overcome the oxidative damage triggered in response to desiccation. Taken together, this work provides insights into the biochemical adaptations in *C. ramosus*, thus deepening our understanding towards the in situ operational mechanism during desiccation. Furthermore, Environmental Scanning Electron Microscopy profiles confirm the transient alteration of chitinous exoskeleton during and after revival from desiccation stress which prompted us to investigate the dynamics of chitin biosynthesis. A hypothetical working model suggests the possible interplay involving key biomolecules, namely, trehalose, glucosamine and chitin that ensure anhydrobiotic survival in *C. ramosus*. This study thus opens up future prospects for the exploration of the role of glucosamine in the survival of other organisms capable of anhydrobiosis.

V. C. Shah prize for best platform presentation

DROSOPHILA SEPTIN, PNUT IS A MODULATOR OF NEURONAL STORE OPERATED CALCIUM ENTRY AND REGULATES FLIGHT IN ADULTS.

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Store operated Calcium entry (SOCE) is a conserved mechanism for uptake of extracellular calcium, following depletion of the intracellular stores. We have previously demonstrated a critical requirement of SOCE during development of the neuronal circuit governing flight in Drosophila. This deficit in the development of the circuit results in flight defects in adult organisms. Septins have recently been identified as novel coordinators of SOCE in HeLa and Jurkat T cells. Using air-puff induced flight behavior in adult animals and direct measurement of SOCE from primary neuronal cultures, we uncover a genetic interaction of Pnut with IP3 receptor, STIM and Orai. These proteins play crucial roles in the signaling pathway involved in agonist induced store depletion and SOCE. The findings of this study show Pnut as a modulator of SOCE in neurons and demonstrate a novel role for this protein in post- mitotic neurons for regulation of flight behavior in adults.

B. R. Seshachar Memorial prize for best Poster presentation

WHAT IT TAKES TO MAKE A NICHE?

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Stem cell niche provides structural and functional cues to its resident stem cells. Within this microenvironment the stem cells achieve a balance between proliferation inhibiting and proliferation stimulating signals and are thus able to take care of tissue maintenance and regeneration. Therefore, what maintains and regulates the niche is climacteric to our understanding of stem cell development. Although, a myriad of signaling

networks from the niche has been identified for regulation of stem cell state and function, the information regarding niche maintenance is still in its infancy. Using Drosophila's hematopoietic organ as our model, we conducted an unbiased genome wide RNAi-screen to reveal the key regulators of niche maintenance. Our effort has yielded a variety of molecules like transcription factors, signaling molecules, chromatin remodeling factors, and mitochondrial components whose interplay maintains the hematopoietic niche in Drosophila.

The dynamicity of these factors and the temporal requirement for their functionality is also being explored. We intend to unravel the signals and their crosstalk required for specification and functionality of the hematopoietic niche. Data regarding this will be presented.

Manasi Ram Memorial prize for best Poster presentation

IN VITRO RECONSTITUTION OF DYNEIN DRIVEN MICROTUBULE MOTILITY

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The role of mechanical forces as an important regulatory factor in generation of cellular responses is being increasingly realized. ATP-consuming translational motors are a prime example of active force generators in cells. We are examining the role of collective behaviour on microtubule (MT) motility using an in vitro reconstitution assay in microscopy. Previous experiments with meiosis II arrested Xenopus egg extracts have shown that centrosomal MTs grow preferentially towards chromatin [1] due to a RanGTP gradient. Experiments over a time period ~1h indicate that these centrosomes move towards and merge with surface immobilized chromatin mitotic extracts. In order to quantitatively understand this process, a microtubule-motor simulation with surface-immobilized minus-ended motors (dynein) and a directional gradient were developed that match experiments [2]. This model predicts a motor density dependence of centrosomal motility. To experimentally test the ideas from the model we are developing an in vitro reconstitution assay of surface immobilized dynein motors and microtubule arrays. The motor used is a previously described [3] GFP- tagged non-essential cytoplasmic dynein from S. cerevisaeprepared by recombinant protein expression and purification based on a 6xHis-tag. We test the activity of the motor by an ATPase assay. The microtubules are assembled using a polymerization-depolymerization approach to purify goat-brain tubulin. MTs are labeled using a Rhodamine-tubulin labeled porcine tubulin. The system is assembled in a flow chamber and observed using time-lapse microscopy in DIC and fluorescence

modes.Usingtaxol MTs of a fixed length are obtained. MT gliding assays are performed in a flow-cell made with double backed tape. Currently we are in the process of optimizing the assay and expect to track the movement of microtubules using single particle tracking and kymography.

References:

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V. C. Shah prize for best Poster presentation

GLOBAL ANALYSIS OF B-CATENIN OCCUPANCY IN QUIESCENT MYOGENIC CELLS REVEALS DIVERGENT ROLES FOR WNT MEDIATED TRANSCRIPTION IN REGULATION OF CELL FATES

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The Wnt signaling pathway is known to regulate cell fates by controlling the activity of proliferative genes as well as differentiation genes in a variety of cell types. Here we show that Wnt/β-catenin signaling regulates cell quiescence, proliferation and differentiation in a stage dependent fashion in myogenic cells. Our analysis of β-catenin occupancy shows a very diverse action on various genes by Wnt pathway under various states. Comparison with gene expression show that β- catenin bound targets are not necessarily trans-activated and a large population of β -catenin bound genes is also transcriptionally repressed. Knock down/ pharmacological inhibition of β-catenin mediated transactivation de-regulated the quiescent state. Additionally β-catenin bound genes are differentially regulated by histone acetyl transferases CBP/p300. CBP and p300 have a stage specific role in determining the transcriptional response of a gene towards β-catenin mediated regulation showing a dichotomy in proliferative versus differentiation genes under different conditions allowing a fine tuning of a pathway into specific needs according to the cells environmental cues and physiological needs.

LYSOSOMAL GTPASE ARL8B GOVERNS HOPS TETHERING COMPLEX MEDIATED LATE ENDOSOME-LYSOSOME FUSION IN MAMMALS

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Vesicular fusion mechanisms have been conserved from yeast to humans but what determines the specificity of this fusion, is still a question. Our research focuses on answering the question in the context of late endosome-lysosome fusion. Fusion at lysosomes relies on a complex interplay of small GTPases, HOmotypic Fusion and vacuole Protein Sorting (HOPS) complex and SNARE proteins. The putative mammalian HOPS complex is composed of six different subunits, namely, Vps11, Vps16, Vps18, Vps33, Vps39 and Vps41. Thus far the mechanism of recruitment and assembly of mammalian HOPS complex to late endosomes and lysosomes is not known. Here we describe that a small GTP-binding protein, Arl8b, recruits Vps41 through its N- terminal WD-40 domain. Immunofluorescence experiments have shown that WD-40 domain of Vps41 also bears the site for binding with Vps18. This is followed by subunit-wise assembly of entire HOPS complex on lysosomes. Subunit-subunit interactions of the different Vps proteins of the HOPS complex were determined using yeast-two-hybrid analyses. Our studies reveal that despite very less sequence similarity from yeast to mammals, the architecture of the HOPS complex has stayed remarkably conserved. The long-term goals of the study revolve around deciphering the mechanism by which mammalian HOPS complex regulates lysosomal trafficking, proper functioning of which is critical for cellular survival and defence against pathogens.

ROLE OF KERATIN 8 PHOSPHORYLATION IN NEOPLASTIC PROGRESSION OF SQUAMOUS CELL CARCINOMA

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Keratins are the largest subgroup of cytoplasmic intermediate filament proteins expressed in tissue specific and differentiation state specific manner. Keratins 8/18 (K8/18) are predominantly expressed in simple epithelial tissues and perform both mechanical and regulatory functions. Aberrant expression of K8/18 is associated with neoplastic progression, invasion and poor prognosis in human oral squamous cell carcinomas (OSCCs). Our recent data suggests that K8/18 promote cell motility and tumor progression by down-regulating β4 integrin signaling in OSCC. K8/18 undergoes several post translational modifications including phosphorylation, which is known to regulate various cellular processes; however its significance in neoplastic progression is still immerging. Our mutational studies suggest that loss of K8 phosphorylation leads to increased migration and tumorigenicity in OSCC cells although the mechanism responsible for these phenotypes is still unidentified. Currently we are investigating role of K8 phosphorylation in neoplastic progression using skin epidermoid carcinoma cell line (A431) so as to understand whether these effects are cell line specific and also the mechanism underlying the same. K8 knockdown in A431 led to decrease in cell tumorigenic phenotype. Experiments are in progress to check the effect of K8-S73A/S431A phosphorylation deficient-mutants and K8-S73D/S431D phospho-mimetic mutants on tumorigenic behaviour of these cells.

Further we propose to elucidate the mechanisms involving; phosphatases/kinases mediated regulation of K8 phosphorylation and other signalling pathways responsible for these phenotypes. Thus this study will give us better insights into the role of K8 phosphorylation in tumour progression. This information will be useful in establishing prognostic value of K8 phosphorylation in SCC.

THE CHARACTERIZATION AND STUDY OF RNA BINDING PROTEINS IN THE PLANARIAN SCHMIDTEA MEDITERRANEA

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Although all organisms display the ability to regenerate to varying extents, planarians steal the show- with even the smallest fragment having the capacity to regenerate into an entire animal. Planarian regeneration depends on the presence and precise regulation of pluripotent adult somatic stem cells - Neoblasts, which differentiate to replace cells during regular homeostasis or in case of injury. The molecular mechanisms by which these unique cells virtually differentiate into specific cell types are not clearly understood. While cell-fate regulators often act at the transcriptional level, post- transcriptional regulation finely tunes gene expression and potentially controls the stem cell function and regeneration. Recent transcriptome analyses at our lab showed enrichment of several genes encoding for RNA binding proteins in neoblast populations suggesting the role of post-transcriptional regulation in controlling the stem cell function. RNA binding proteins (RBPs) interact with nascent RNA transcripts in order to carry out important functions such as splicing, nuclear export, stability and translation; so that proteins are efficiently produced to meet the needs of the cell. Thus, an understanding of the general principles of the cell biology of RBPs is crucial, to highlight their central role in gene expression. We are currently looking at candidate RBP's that are differentially expressed in certain cell types of the planarian, Schmidtea mediterranea.

Smed-PABPC2, an RRM-1 protein, is necessary for neoblast proliferation and function. Smed-DDX-24, an RNA helicase, on the other hand, does not play a role in proliferation, but is absolutely essential for terminal differentiation of neoblasts. Our goal is to understand the role of these RNA binding proteins in order to decipher the chain of events that leads to the regeneration of the entire animal.

GENETIC ALTERATIONS IN TLR4 SIGNALING PATHWAY AND IMPAIRMENT OF WOUND HEALING IN TYPE 2 DIABETES (T2DM) PATIENTS

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The Toll-Like receptor 4 (TLR4) plays an important role in immunity, tissue repair, and regeneration. TLR4 is also an important regulator of wound inflammation and plays an important role in restoring damaged tissue integrity during normal wound healing. Any imbalance in TLR4 mediated signaling may abrogate the proper wound healing cascade. Diabetic wounds are hard to heal and altered TLR4 mediated signaling may be contributing the same. In present work we evaluated the association of TLR4 single nucleotide polymorphisms (SNPs) rs4986790, rs4986791, rs10759931, rs1927911, and rs1927914 and their effect on TLR4 expression with increased diabetic foot ulcer (DFU) risk in patients with type 2 diabetes mellitus (T2DM). PCR-RFLP was used for genotyping TLR4 SNPs in 125 T2DM patients with DFU and 130 controls. Expression analysis of TLR4 was done using semi-quantitative RT-PCR, quantitative Real-time PCR and western blot. DNA methylation is an important regulator of gene expression therefore methylation status of promoter of TLR4 gene was analysed in DFU and control wounds using specific methylation-sensitive restriction enzyme methodology. The haplotypes and linkage disequilibrium between the SNPs were determined using Haploview software. Risk genotypes of all SNPs except rs1927914 were significantly associated with DFU. Haplotype ACATC (p value = 9.3 e-5) showed strong association with DFU risk.

Two haplotypes ATATC (p value = 0.0119) and ATGTT (p value = 0.0087) were found to be protective against DFU. TLR4 message and protein were significantly down-regulated in DFU patients as compared to controls. In conclusion genetic alterations in TLR4 mediated signaling may be a contributing factor to the impairment in healing of diabetic wounds.

EHARPC1, A 41KDA SUBUNIT OF ARP2/3 COMPLEX IS INVOLVED IN ERYTHROPHAGOCYTOSIS IN ENTAMOEBA HISTOLYTICA

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Entamoeba histolytica causative agent of amoebiasis has two developmental stages, cysts that initiate infection and trophozoites that cause pathology observed during invasive disease. The latter are highly motile invading epithelial tissue and phagocytosing erythrocytes, immune cells and commensal bacteria. Phagocytosis and high motility of trophozoites are due to high activity of cytoskeleton machinery playing an indispensable role in its survival and virulence. Arp2/3 complex participates in actin dynamics by promoting actin nucleation and is composed of 7 proteins i.e. Arp2, Arp3, p41, p34, p21, p20 and p16. As compared to mammalian systems signaling cascade involved in activation of cytoskeletal dynamics of *E. histolyticais* not yet systematically dissected. So here we show the role of EhARPC1, 41KDa subunit of Arp2/3 complex in erythrophagocytosis of E.histolytica.

Our group has previously shown that calcium binding protein EhCaBP1 and its binding partners EhC2PK (C2 domain protein kinase) and EhAK1 (alpha kinase) is involved in erythrophagocytosis. Further to understand the process of actin nucleation during phagocytosis we cloned EhARPC1 another binding partner of EhCaBP1. Surprisingly, we found EhARPC1 binds to EhCaBP1 indirectly via EhAK1 through SH3 domain of EhAK1. Further conditional suppression of EhARPC1 resulted in delayed phagocytic cup formation. Over expression of a kinase dead mutant of EhAK1 or conditional suppression of EhAK1 resulted in inhibition in enrichment of EhARPC1 at the site of RBC attachment, thus delaying phagocytosis.

We have also found that over expression of EhARPC1 increases F-actin content in cells. Overall these results suggest that EhARPC1 may be involved in regulation of actin nucleation during phagocytosis and likely to be a promising player to understand mechanism involved in actin dynamics.

HSA-MIR-4485, A TUMOR SUPPRESSOR MIRNA, ASSOCIATES WITH HUMAN MITOCHONDRIA BY TARGETING MITOCHONDRIAL GPD2

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miRNA are ~20 nt long small non coding RNA which regulates post transcriptional genes expression and fine tunes the cellular processes like proliferation, differentiation and death. Their aberrant expression is observed in various pathologies including cancer. The miRNAs also have spatial and temporal regulation in cellular milieu. In our previous study, we performed deep sequencing of small RNA associated with mitochondria of HEK293 and HeLa. hsa-miR-4485, was observed to be associated with mitochondria of both HEK293 and HeLa. Interestingly, the sequence of this miRNA aligns with mitochondrial genome in the region encoding 16S rRNA. The association was validated by real time PCR and confocal microscopy using labeled miRNA. The targets of hsa- miR-4485 were determined using Starbase. The mitochondrial GPD2 which is abundant in most cancers was identified as a putative target which was validated by real time PCR. Further, we examined its levels in cancer cell lines as they are excellent experimental models to investigate biological significance in vitro wherein it was observed to be significantly upregulated. The over expression of this mitochondrial associated miRNA decreased clonogenic ability suggesting its anti- tumor nature.

ROLE OF NONAMER BINDING DOMAIN AND CENTRAL DOMAIN OF RAG1 IN SEQUENCE AND STRUCTURE SPECIFIC ACTIVITY OF RAGS

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RAG complex is a site specific endonuclease responsible for the generation of antigen receptor diversity and nicks at 5' end of the heptamer present in the recombination signal sequence (RSS). It also acts as a structure specific nuclease by cleaving non B-DNA structures. Nonamer binding domain (NBD) of RAG1 plays a central role in the recognition of RSS during its sequence specific activity. To investigate the DNA binding properties of NBD and to elucidate the role of nucleotide sequence governing the NBD-non B-DNA interaction, NBD of murine RAG1 was expressed and purified from E. coli. Electrophoretic mobility shift assays showed that NBD binds with high affinity to nonamer in the context of 12/23 RSS as well as heteroduplex DNA. Besides, NBD efficiently bound to single-stranded oligomers harboring thymines with a minimum stretch of 28. It is known that NBD is indispensable for sequence specific activity of RAGs. However, we show that external supplementation of purified NBD to NBD deleted RAG1 and RAG2 could not restore the sequence specific activity, it shows in the context of the core protein. In addition, we show that NBD along with central domain (CD) of RAG1 binds preferentially to single-stranded DNA, like CD alone. Therefore, our results show that NBD binds to single-stranded DNA substrates harboring thymines and duplex DNA containing poly A/T base pairs, besides its physiological substrate, RSS. Hence NBD might have an impact on RAG mediated cleavage at regions outside antigen receptor loci.

NOMINATIONS ARE INVITED FOR the 15th. PROFESSOR S. P. RAY-CHAUDHURI 75TH BIRTHDAY ENDOWMENT LECTURE

The Indian Society of Cell Biology has instituted the Prof S. P. Ray Chaudhuri 75th Birthday Endowment Lecture as a mark of its respect to Prof Ray Chaudhuri in recognition of his immense contributions to Cytogenetics and Cell Biology. Nominations for the lecture are invited from Life and Ordinary members of at least three years standing.

The person to be nominated need not be a member of the Society when nominated but may be requested to become a member in due course of time. The person to be nominated will ordinarily be an Indian citizen at the time of nomination/selection and should be an eminent scientist who would have made outstanding original contributions to Cell Biology or contributed substantially to growth of the subject in India.

The following is the list of the past speakers of Prof S.P. Ray Chaudhuri 75th Birthday Endowment Lectures

Number	**Speaker**
First	Dr O Siddiqi (TIFR)
Second	Dr A T Natarajan
Third	Dr G Padmanaban (IISc)
Fourth	Dr H Sarat Chandra (IISc)
Fifth	Dr Lalji Singh (CCMB)
Sixth	Dr K P Gopinathan (IISc)
Seventh	Dr A N Bhaduri (IICB)
Eighth	Dr A N Bhisey (CRI)
Ninth	Dr S C Lakhotia (BHU)
Tenth	Dr R Raman (BHU)
Eleventh	Dr K VijayRaghavan (NCBS)
Twelvth	Dr S K Sopory (ICGEB)
Thirteenth	Dr Veena K Parnaik (CCMB)
Fourteenth	Dr Tapas Kundu (JNCASR)

Nominations may be sent to: Dr Vegesna Radha, Secretary ISCB

Chief Scientist,
Centre for Cellular and Molecular Biology
Uppal Road, Hyderabad 500 007
Tel: +91-40-27192619 / +91-40-27160222
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NOMINATION FORM FOR THE PROF. S.P. RAY-CHAUDHURI 75th BIRTHDAY ENDOWMENT LECTURE

Name & Address of the member making the nomination:

Nomination:

I wish to nominate.....
(address.....
.....)
for the PROF. S.P. RAY-CHAUDHURI 75th BIRTHDAY ENDOWMENT LECTURE I have obtained consent of the nominee for the purpose. The biodata etc of the nominee are enclosed herewith.

Date Signature of the nominating member

Obituary

Professor V. C. Shah

Professor Vinodkant Chunnilal Shah passed away on 20th March 2013 in Chicago. Prof. Shah was a renowned educationist and a leading cell biologist of the country. He contributed, as a Founder member, significantly to establishment and growth of the Indian Society of Cell Biology.

Professor Shah was born on 27th November 1929 at Sankheda in Gujarat. He obtained his B.Sc. and M. Sc. Degrees from the M. S. University, Baroda, receiving the Chancellor's Gold medal for his excellent performance at M.Sc. He further obtained M.S. (Zoology) degree from the University of Minnesota in 1957 and Ph.D. in 1961 from Columbia University for his autoradiographic studies on DNA replication in cultured mammalian cells under the supervision of Professor H. J. Taylor, who is well known for studies on semi-conservative replication of chromosomal DNA. Subsequently, he worked at Brookhaven Laboratory, Long Island (1961-62) and at Tufts University, Boston (1962-64., before returning to India in 1964 as Reader at the Department of Zoology of Delhi University. He moved to the Zoology Department of Gujarat University as Professor and Head in 1970, where he worked till 1988. He served as Vice-Chancellor of Bhavnagar University (1988-90) and the M.S. University Baroda (1990-93). Later, he was associated with the Pharmaceutical & Educational Research Development Centre, Ahmedabad as its Director. For the past several years, he mostly stayed in the US with his three daughters.

Following his doctoral and post-doctoral work in the US, Prof. Shah developed active groups for cell biological and cytogenetic studies, initially at Delhi and later at Gujarat University. In 1960s, he was one of the first scientists in the country to use the technique of cellular autoradiography to study amphibian and mammalian chromosomal replication, especially of the heterochromatic regions. Working in close collaboration with Prof. S. R. V. Rao at the Zoology Department of Delhi University, he contributed to interesting studies on sex chromosomes in some Indian rodent species. He also examined DNA synthesis in isolated chloroplasts of Euglena and effects of small metabolites on cell cycle progression. After moving to Gujarat University, he established a Human Cytogenetics laboratory for studying human birth defects using molecular cytogenetic methods. He also developed interest in factors underlying vitiligo. He guided doctoral research of 28 students and published 185 research papers besides authoring three books on Radiation Biology, Genetics and Cell Biology. He also served as member of Editorial Boards of several journals.

Besides his active involvement in research, Professor Shah also contributed significantly to development of Biology education, especially in the state of Gujarat. Professor Shah was involved in organizing the Cell Biology Conferences at the Zoology Department of Delhi University in 1960s and

early 1970s. It was at these meetings that the idea of having an Indian Society of Cell Biology took shape. Professor Shah, together with several others, played an important role in defining the basic framework of the ISCB, which finally came into existence in 1976. He remained associated with ISCB for a long time and served its Executive Committee as Member (1977-78, 1983-84, 1989-90, 1993-94), Secretary (1981-82), Vice-President (1979-80), and President (1987-88).

I had the privilege and pleasure of knowing him personally since 1969 and of having worked at the Department of Zoology, Gujarat University from 1972-76. As Head of the Department, he was instrumental in getting me at the Gujarat University. He extended all possible facilities and help so that I could develop my teaching and research activities. His encouraging and caring approach was indeed remarkable. I and my wife greatly enjoyed good friendship with his entire family and still remember the delicious Gujarati dishes that Mrs. Shah would so caringly prepare for us.

Professor Shah is survived by his wife and three daughters, who currently live in the USA. Professor Shah would be remembered by his colleagues, students and friends for his significant contributions to the growth of disciplines of Cytogenetics and Cell Biology in the country. The ISCB also remembers him for his role in shaping the Indian Society of Cell Biology and nurturing it during its infancy.

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INDIAN SOCIETY OF CELL BIOLOGY