BBRC Symposium

Trends in Biochemistry and Biophysics

May 18th, 2015
9:00 AM – 5:00PM
Dasheri Auditorium*
National Centre for Biological Sciences (NCBS) Bangalore, India

ELSEVIER *Elsevier.com/locate/ybbrc*

ncbs.res.in/BBRC_Symposium ncbs

Organizing committee

Wolfgang Baumeister	Max Planck Institute, Germany	*Editor BBRC*
Ernesto Carafoli	Padua University, Italy	*Editor-in-Chief BBRC*
Satyajit Mayor	National Centre for Biological Science, Bangalore	*Editor BBRC*
Dolors Alsina	Elsevier, The Netherlands	*Executive Publisher BBRC*

Venue address

*National Centre for Biological Sciences
GKVK, Bellary Road, Bangalore, Karnataka 560065, India
The Dasheri Auditorium is located in the Southern Lab Complex on the Ground Floor.

Registration

ncbs.res.in/BBRC_Symposium

Free attendance ◆ Registration compulsory ◆ Lunch provided

Morning session (9:00 AM – 12:30 PM)

Chairman: Ernesto Carafoli

9.00 AM Welcome word
Satyajit Mayor, *Director NCBS &* **Ernesto Carafoli**, *Editor-in-Chief BBRC*

9:15 AM **Igor Stagljar**, *University of Toronto, Canada*
Membrane Protein Interactomes in Health & Disease

9:55 AM **Wolfgang Baumeister**, *Max Planck Institute for Biochemistry, Martinsried, Germany*
Structural studies of the 26S proteasome outside and inside cells

10:30 AM – 10:50 AM Break

10:50 AM **Andrew R. Marks**, *Columbia University Medical Center, NY, USA*
Towards a structural basis of complex disorders of heart, muscle and brain

11:30 AM **Katsuhiko Mikoshiba**, *RIKEN, Wako, Japan*
Structural and Biochemical Properties of IP3 Receptor Signaling Complex in Health and Disease

12:10 PM **Annapurna Vyakarnam**, *Indian Institute of Science (IISc), Bangalore*
The long road towards understanding how HIV reactivates Mtb infection

12:45 PM – 2 PM Lunch

Afternoon session (13:30 PM – 5:00 PM)

Chairman: Wolfgang Baumeister

2.00 PM **Bengt Fadeel**, *Karolinska Institutet, Stockholm, Sweden*
Interactions between engineered nanomaterials and biological systems: gene expression profiling as a novel tool to decipher the biological impact of nanomaterials

2:40 PM **Umesh Varshney**, *Indian Institute of Science (IISc), Bangalore*
Mechanism of tRNA selection in the ribosomal P-site and potential to generate proteome diversity in Escherichia coli

3:15 PM – 3:35 PM Break

3:35 PM C**hin Ha Chung**, *Seoul National University (SNU), South Korea*
ISG15 in the Control of DNA Damage Response

4:15 PM **Hisao Masai**, *Tokyo Metropolitan Institute of Medical Science, Japan*
Biological roles of G-quadruplex structures

4:50 PM – 5:00 PM Closing

Igor Stagljar

Membrane Protein Interactomes in Health & Disease

Abstract

In the past decade, numerous comprehensive proteomics studies have allowed researchers to generate detailed protein-protein interaction ("interactome") maps, providing an unparalleled global view of the interplay between the different systems within the cell, and greatly increasing our understanding of the physiological mechanisms involved in both normal and disease states.

My lab is focused specifically on understanding how the interactions of membrane proteins contribute to cellular disease states at a systems level. Despite extensive proteomics research in the past decade, there is a lack of in-depth understanding of protein networks associated with integral membrane proteins because of their unique biochemical features, enormous complexity and multiplicity. This is a major obstacle to understanding the biology of deregulation of integral membrane proteins which leads to numerous human diseases, and consequently hinders our development of improved and more targeted therapies to help treat these diseases.

To address this problem, my lab has developed two unique technologies specifically suited for the study of full-length integral membrane proteins in their natural cellular context; the classic Membrane Yeast Two-Hybrid (MYTH) 1-5 and the newly created Mammalian Membrane Two-Hybrid (MaMTH)6. Our ultimate goal is to uncover a wealth of information about protein interactions for the majority of "druggable" human membrane proteins, which should in turn greatly facilitate the discovery of new truths about diseases like cancer, schizophrenia, cystic fibrosis, hypertension and Parkinson's disease.

During my talk, I will discuss our recent findings indicating that the application of MaMTH to the human Epidermal Growth Factor Receptor (EGFR) resulted in the identification of Crk II protein as a novel interactor of oncogenic EGFR (L858R), and showed that CRKII promotes persistent activation of aberrant signaling in non-small cell lung cancer (NSCLC) cells. I will also illustrate how MaMTH is a powerful tool for investigating dynamic interactomes of human integral membrane proteins and why it promises significant contributions to therapeutic research.

Wolfgang Baumeister

Structural studies of the 26S proteasome outside and inside cells

Abstract

The 26S proteasome operates at the executive end of the ubiquitin-proteasome pathway for the controlled degradation of intracellular proteins. The 2.5 MDa complex is built of 34 different subunits and comprises two subcomplexes: the 20S core where proteolysis takes place and one or two regulating particles which prepare substrates for degradation. Whereas the structure of its 20S core particle has been determined by X-ray crystallography two decades ago, the structure of the 19S regulatory particle, which recruits substrates, unfolds them, and translocates them to the core particle for degradation, has remained elusive. Recently, the molecular architecture of the 26S holocomplex was determined by an integrative approach based on data from single particle cryoelectron microscopy, X-ray crystallography, residue-specific chemical cross-linking, and several proteomics techniques. Based on a 6Å structure of the Saccharomyces cerevisiae proteasome, and molecular dynamics-based flexible fitting we were able to generate a near-atomic model of the 26 holocomplex. Furthermore, by applying deep image classification to a very large data set, we defined its conformational landscape.

Electron cryotomography allows to perform structural studies of macromolecular and supramolecular structures in situ, i.e. in their functional cellular environments. We applied electron cryotomography with a new type of a phase plate, the Volta phase plate, to hippocampal neurons grown on EM grids and were able to locate 26S proteasomes with high fidelity and nanometer precision in these cells. Subtomogram, classification and averaging revealed the existence of different states of assembly and conformational states. From this we can infer the activity status of individual 26S complexes.

Andy Marks

Towards a structural basis of complex disorders of heart, muscle and brain#

Abstract

RyR channels are required for release of calcium from intracellular stores, a process essential for many cellular functions including excitation-contraction (EC) coupling in skeletal and cardiac muscle, and hormone and neurotransmitter release. They are amongst the largest ion channels, comprised of the four identical ~565 kDa channel-forming protomers, as well as regulatory subunits, enzymes and their respective targeting/anchoring proteins, in a macromolecular complex that exceeds three million daltons. We have obtained high-resolution cryo-electron microscopy (Cryo-EM) reconstructions from highly purified rabbit skeletal muscle RyR1 in the open and closed states. Our data reveal that RyRs are members of the six transmembrane family of ion channels and show a mechanism for channel gating that couples a change in the conformation of the calcium binding site directly to opening of the channel pore, suggesting that calcium binding facilitates mechanical coupling of conformational changes in the cytosolic region to opening of the channel gate. The channel-specific ligand ryanodine shows the channel to be locked in an open state consistent with the know effects of ryanodine on the single channel properties of RyR1. We have mapped disease causing mutations on the structure proving insight regarding disease mechanisms for arrhythmias and muscular dystrophies.

Katsuhiko Mikoshiba

Structural and Biochemical Properties of IP3 Receptor Signaling Complex in Health & Disease

Abstract

IP3 receptors are allosteric proteins comprising four subunits that form an ion channel. It is located on the endoplasmic reticulum and plays an important role in fertilization (Science 1992), growth cone extension (Science 1998), secretion (Science 2005) and behavior (Nature 1996). IP3 receptor is an allosteric protein mainly regulated by Ca2+ and IP3 and dynamic structural change was observed by FRET analysis (PNAS 2011). The IP3 receptors have three isoforms with different IP3 binding affinity. Biochemical and structural analysis showed the IP3 binding affinity is determined by the association of suppressor domain and binding domain of each type of receptor (Nature 2002, Mol. Cell 2005). A critical amino acid in suppressor domain is required for the channel gating (JBC 2010). Furthermore, many of the proteins were associated with IP3 receptors forming a signaling complex that regulate the function of the receptor. These include GIT 1 (JBC 2009), 80H (JBC 2009), CARP (carbonic anhydrase-related protein) (BJ 2003), chaperones such as ERp44, a redox sensor (Cell 2005) and GRP78 (Neuron 2010), bind to the internal loop region. Na, K-ATPase interacts with ligand binding domain of all IP3 receptors (JBC 2006). We named a protein as IRBIT (IP3R Binding protein released with Inositol 1,4,5- Trisphosphate) which binds to the IP3-binding pocket in a resting state and is released with a large amount of IP3 (JBC 2003, Mol. Cell 2006). We identified Na+/HCO3– cotransporter 1 (PNAS 2006) and CFTR (J.Clin. Invest. 2011, 2009) as an IRBIT-binding protein suggesting that IRBIT plays an important role in pH regulation. We found that IRBIT binds to and suppresses the CaMKIIα kinase activity by inhibiting the calmodulin binding to CaMKIIα (PNAS 2015). Calmodulin and IRBIT bind to the same site of CaMKIIα kinase. Defective allostery in IP3R is considered crucial to cellular dysfunction. We found that transglutaminase type 2 targets the allosteric coupling domain of IP3R type 1 (IP3R 1) and negatively regulates IP3R 1-mediated Ca2+ signaling by locking the subunit configurations. The covalent posttranslational modification of the Gln2746 residue that transglutaminase type 2 tethers to the adjacent subunit blocks the IP3 receptor activity (PNAS 2014). IP3 receptor associates with various cytoskeletal proteins such as homer, 4.1N. We recently found that Disrupted-in-Schizophrenia-1 (DISC 1) regulates transport of IP3R1 mRNA for synaptic plasticity (Nature Neurosci. 2015). I will discuss how IP3 receptor exerts multiple functions and how dysregulation causes various diseases judging from the various biochemical and structural analysis.

Annapurna Vyakarnam

The long road towards understanding how HIV reactivates Mtb infection

Abstract

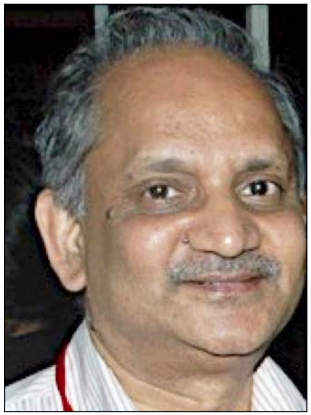
HIV infects and depletes CD_4 T cells that normally coordinate the adaptive T- and B-cell response to defend against intracellular pathogens. The immune defect is immediate and profound: At the time of acute infection with an AIDS virus, typically more than half of the gut-associated CD_4 T cells are depleted, leaving a damaged immune system to contend with a life-long infection. A major consequence of HIV infection is reactivation of latent TB. Whilst most opportunistic pathogens emerge when the immune system is significantly compromised late on in HIV infection, susceptibility to TB can occur quite early on following HIV infection. A unified view of HIV pathogenesis is emerging, but there are many unknowns. Importantly, the factors that predispose the HIV infected host to M.tb are poorly understood. Key to addressing this issue is a better understanding of the quality, rather than just quantity, of CD_4 T-cell responses to both M.tb and HIV, how these responses drive HIV compartmentalization and the impact this has on immune responses that are critical to controlling M.tb infection. This talk will cover these topics.

Bengt Fadeel

Interactions between engineered nanomaterials and biological systems: gene expression profiling as a novel tool to decipher the biological impact of nanomaterials

Abstract

Engineered nanomaterials have unique physico-chemical properties that make them attractive for many different applications. However, close attention to safety issues is needed to enable the safe and sustainable implementation of the nanotechnologies. Indeed, if we accept the notion that materials adopt novel and potentially useful properties at the nano-scale then we must also consider that new toxicological effects, not anticipated from previous studies of larger particles or bulk materials, might occur. Transcriptomics, or gene expression profiling, could aid in the prediction of mechanisms of toxicity. Indeed, the use of global "omics" technologies coupled with computational approaches to determine statistically significant perturbations of genes or pathways represents an attractive method to identity the potential hazards and mechanisms of action of engineered nanomaterials. In this lecture, examples of gene expression profiling studies using cDNA microarray or next-generation RNA sequencing methodologies are presented to illustrate the predictive potential of this approach.



Umesh Varshney

Mechanism of tRNA selection in the ribosomal P-site and potential to generate proteome diversity in Escherichia coli

Abstract

The accuracy of the initiator tRNA (tRNAfMet) selection in the ribosomal P-site is central to fidelity of initiation. A highly conserved occurrence of three consecutive G-C base pairs (3GC base pairs) in the anticodon stem of tRNAfMet in all domains of life (with the notable exceptions in some species of mycoplasma and rhizobia) contributes to the preferential binding of tRNAfMet in the P-site. How this feature is exploited by ribosomes has remained unclear. In a genetic screen, using a plasmid borne copy of a tRNAfMet mutant wherein the 3GC base pairs were changed, we isolated E. coli strains, which allow initiation with the tRNAfMet mutant. We observed that diminished cellular abundance of the chromosomally encoded tRNAfMet permits efficient initiation with the tRNAfMet mutants. Based on this observations, we deleted all four of the native initiator tRNA genes from the E. coli genome, and sustained it on unconventional initiator tRNAs of mycoplasma/rhizobia origin. Further, we observed that under the initiator tRNA depleted conditions, elongator tRNAs also initiate, suggesting a novel mechanism to generate proteome diversity in the cell. Recently, we showed that mutations in either the mRNA or rRNA leading to extended interaction between the Shine-Dalgarno (SD) and anti-SD sequences compensate for the vital need of the 3GC pairs in tRNAfMet for its function in E. coli. In vivo, the 3GC mutant tRNAfMet occurred less abundantly in 70S ribosomes but normally on 30S subunits. However, the extended SD:anti-SD interaction increased its occurrence in 70S ribosomes. We propose that the 3GC pairs play a critical role in tRNAfMet retention in ribosome during the conformational changes that mark the transition of 30S pre-initiation complex into elongation competent 70S complex.

Chin Ha Chung

ISG15 in the Control of DNA Damage Response

Abstract

PCNA (proliferating cell nuclear antigen) plays a crucial role in maintaining genomic stability during DNA replication. When replicating cells are exposed to DNA damage, PCNA undergoes numerous post-translational modifications to activate DNA damage bypass for preventing the collapse of DNA replication forks, which could produces double-strand breaks and chromosomal rearrangements. The mode of DNA damage bypass is dictated by the types of PCNA ubiquitination: mono-ubiquitination for activating error-prone translesion DNA synthesis (TLS) and poly-ubiquitination for initiating error-free template switching. Due to error-prone nature of TLS, it should be tightly regulated to maintain genome stability. However, the precise mechanism for the control of TLS remains unknown. Neither is known how PCNA mono-ubiquitination-mediated TLS is switched to poly-ubiquitination-mediated template switching. ISG15, the product of interferon-stimulated gene 15, is the first reported ubiquitin-like protein. Like ubiquitin conjugation, ISG15 modification of proteins involves three-step cascade enzymes: ISG15-activating E1 enzyme (UBE1L), ISG15-conjugating E2 enzyme (UbcH8), and ISG15 E3 ligases (such as HERC5 and EFP). Here I will present the fascinating role of ISG15 modification of PCNA in the control of TLS and template switching in response to DNA damage.

For faithful transmission of the genetic information, cells must carry out highly processive, error-free replication of the genome and efficient repair of DNA damage or misincorporated nucleotides. The sliding clamp proliferating cell nuclear antigen (PCNA) plays an essential role as a processivity factor as well as a scaffold for recruiting replication machinery. In addition, PCNA plays a crucial role in DNA damage bypass and their repair by serving as a platform for recruiting essential components necessary for DNA damage response. Therefore, PCNA has been regarded as a key mediator for maintenance of genome stability and cell survival.

When replicating cells are exposed to DNA damage, PCNA undergoes numerous post-translational modifications, such as ubiquitination and sumoylation, for the control of DNA damage response (Bergink and Jentsch, 2009; Jackson and Durocher, 2013; Mailand et al., 2013; Ulrich and Walden, 2010). UV, to which human epithelial cells are persistently exposed, induces mono-

ubiquitination of a highly conserved Lys164 residue in PCNA by the RAD6-RAD18 complex (Hoege et al., 2002). This modification of PCNA triggers the exchange of replicative DNA polymerases, such as Pold, with damage-tolerant, Y family of DNA polymerases, including Polh, for TLS (Bienko et al., 2005; Kannouche et al., 2004; Lehmann et al., 2007; Stelter and Ulrich, 2003). TLS polymerases bypass damaged DNA and therefore replication fork progression can occur without the need of removal of the damage and the risk of fork collapse (Sale et al., 2012). However, TLS polymerases, lacking proofreading activity, can introduce incorrect nucleotides, hence are potentially mutagenic (Loeb and Monnat, 2008; Matsuda et al., 2000; Sale et al., 2012). Thus, error-prone TLS polymerases need to be released from PCNA after DNA lesion bypass for preventing excessive mutagenesis.

While PCNA is mono-ubiquitinated under DNA damage conditions, Polh is mono-ubiquitinated in the UBZ domain under normal conditions and this modification prevents its interaction with PCNA (Bienko et al., 2005, 2010). Upon DNA damage by UV, however, Polh is deubiquitinated by an unknown USP, binds to mono-ubiquitinated PCNA, and carries out TLS. PCNA could also normally be mono-ubiquitinated by the CRL4cdt2 ubiquitin ligase complex (Terai et al., 2010), but this process is rapidly reversed by USP1 for preventing unnecessary TLS (Huang et al., 2006). Upon DNA damage, however, USP1 is degraded by auto-cleavage for abrogating the negative regulation of PCNA mono-ubiquitination, implicating the role of USP1 in prevention of TLS under normal, but not under DNA damage conditions. Therefore, a key unanswered issue is how TLS is turned off after DNA lesion bypass to prevent TLS-mediated mutagenesis. Neither is known how replicative DNA synthesis can be resumed after TLS termination.

ISG15, the interferon-stimulated gene 15, is the first reported ubiquitin-like protein (Haas et al., 1987). ISG15 is robustly induced by type-I interferon, lipopolysaccharides, and viral infection (Kim et al., 2002; Yuan and Krug, 2001). Like ubiquitination, protein ISGylation is catalyzed by 3-step enzyme system: UBE1L as an ISG15-activating E1 enzyme, UBCH8 as an ISG15-conjugating E2 enzyme, and EFP and HERC5 as ISG15 E3 ligases (Dastur et al., 2006; Kim et al., 2004; Yuan and Krug, 2001; Zhao et al., 2004; Zou and Zhang, 2006). In addition, protein ISGylation can be reversed by a deISGylating enzyme, UBP43 (Malakhov et al., 2002). Although more than 300 cellular proteins were identified as targets for ISGylation, only a few cases of their functional significance have been uncovered (Jeon et al., 2010). We have recently shown that DNA-damaging agents, such as doxorubicin, induce the expression of ISG15, UBE1L, and UBCH8, leading to ISGylation and down-regulation of ΔNp63a (Jeon et al., 2012), which acts as a mitotic and oncogenic protein by suppressing the transactivities of proapoptotic p53 family members, including p53 and TAp63 (Yang et al., 1998). Therefore, we have suggested that ISG15 serves as a tumor suppressor via its conjugation to Np63a.

Hisao Masai

Biological roles of G-quadruplex structures

Abstract

G-quadruplex structures are stable DNA structures that can be generated on single-stranded DNA containing stretches of guanine residues. In spite of its predicted prevalence on genomes from various species, its general biological roles have remained elusive. In this presentation, I will present evidence for two novel and potentially conserved roles of G-quadruplex structures and would like to discuss its general biological significance.