Report of Science Academies' Lecture Workshop on

Progress and Prospects of Biotechnology

June 26 & 27, 2014

INAUGURATION

Thursday, 26th June, 2014 at 10.00 am

Venue: New Seminar Hall

The inauguration of Science Academies' Lecture Workshop on 'Progress and Prospects of Biotechnology' was inaugurated on Thursday, 26th June, 2014 at 10.00 am. The inauguration ceremony commenced with prayer, followed by the welcome address by Dr. Viji Mary Varghese, Head, Department of Biotechnology, St Joseph's college irinjalakuda. The intention of the workshop was then explained by Prof. Edathil Vijayan, FNASc, Convener of the workshop. He also gave a brief description about Science Education Programs of the Indian Academy of Sciences, Bangalore; Indian National Science Academy (INSA), New Delhi and the National Academy of Sciences, India, Allahabad. He also enunciated that 'without science there is no existing of other fields'. The Vice Principal of St. Joseph's College, Irinjalakuda, Dr. Sr. Rosa K. D. CHF delivered the presidential address. The formal Inauguration of the Science Academies' Lecture Workshop was done by Dr. C. S. Paulose, Professor Emeritus, CUSAT. He motivated the young generation to come forward in the research field in order that they may contribute to the betterment of society. After the felicitation by Prof. Polani B. Seshagiri, Indian Institute of Science, Bangalore, Mr. Naijil George, Co-ordinator of the workshop proposed the vote of thanks.

Lectures

First session of the Lecture Workshop began at 11.15 am, chaired by Dr. C. S. Paulose.

Lecture I: Prof. Polani B. Seshagiri on **'PERSPECTIVES AND THE BIOLOGY OF STEM CELLS'**

Mammals like human beings undergo viviparous type of development, in which fertilized egg grows within the maternal body until the young one is capable of independent existence. As sperms approach the ovum, the components around egg induce hyperactivation of sperm motility. Hyperactivation aid in sperm penetration of the zona pellucida of ovum and acrosome reaction. The fertilization between sperm and ovum results in the formation of zygote. Fertilization induced genome activation initiate the zygotic cleavage divisions and formation of morula. Blastocyst derived from the cleavage of morula contain an inner cell mass that is distinct from the surrounding blastula. Stem cells

derived from the developing embryo have the ability of continuous self-renewal and asymmetric cell division. The use of these embryonic stem cells for the treatment of various diseases is hampered by the ethical issues allied to its production. Japanese scientist, Shinya Yamanaka developed induced pluripotent stem cell technology for the conversion of fully developed adult cells into stem cells. The major advantage of induced pluripotent stem cells is its immuno-compatibility. It can be used for the treatment of various other wise incurable diseases.

Lecture II: Prof. K. P. Joy FNA on '**FISH REPRODUCTIVE PHYSIOLOGY**'

Fishes have evolved different strategies to allocate energy budgets to various demands within the limits of their food supply. To ensure adequate energy supply to essential physiological events, energy allocations vary at different times of the year. Fishes ensure maximum reproductive success by adopting different strategies. Differential hormonal levels control various events in fish reproduction. In teleosts, the maturation of primary follicle to postvitellogenic follicle is mediated by follicle-stimulating hormone and luteinizing hormone surge controls the oocyte maturation. Environmental factors like light, rain fall, temperature and sex pheromones play a key role in the regulation of FSH and LH levels by influencing pituitary stimulation. *Heteropneustes fossilis* is an animal model for the study of hormonal influence on fish reproduction. During follicle maturation, LH surge induce ovarian steroidogenesis. Paracrine/autocrine factors including 17,20b-DP, 20b-S, Corticosteroids, Insulin, IGF-I, IGF-II, GnRH, Activin and Hydroxyestrogens act as maturation-inducing substances. The knowledge that dopamine inhibits LHRH-induced preovulatory LH- surge has led to the development of a new generation technique for the induction of spawning in fishes. This method, known as LINPE method uses LHRH (GnRH analogue) and a dopamine receptor- 2 blocker. The current formulations such as OVAPRIM and OVATIDE are based on this concept. OVAPRIM is a commercial spawning kit that contains a synthetic analogue of salmon gonadotropin releasing hormone (20 μg) and dopamine-2 receptor antagonist domperidone (10 mg) extensively used for induced breeding of commercially important fishes. It induces a LH surge causing ovulation and spawning in female fish and spermiation in male fish. Recent experiments show that OVAPRIM induced ovulation cause changes in the expression of all the gonadotropin subunits. GPα transcript level increased compared to the control groups after the first and second injections of OVAPRIM. LHβ expression increased greatly and attained peak levels at 6 and 12 hrs after OVAPRIM injection, however its expression declined subsequently but remained higher, compared to the control group. After the first injection, FSHβ expression moderately increased at 6 and 12 hrs and declined gradually. The second dose given at 24 hrs resulted in an increased expression after the 30^{th} hr and was retained upto the 42^{nd} hr. As expected, plasma and ovarian 17-β estradiol levels decreased during the peri-ovulatory period. There occurs a steroidogenic shift i.e. E2 which inhibits meiotic maturation is decreased and MIS that stimulates GVBD is stimulated.

Session chaired by Prof. Edathil Vijayan FNASc

Lecture III: Prof. Anilkumar Gopinath on '**HORMONE RECEPTORS AND ITS SIGNIFICANCE IN HORMONE ACTION'**

A hormone receptor is a molecule that can bind to a specific hormone. They are for the most part proteins with defined structures and definite functional domains. However, in the structure and its 'fate' varies with the type of hormone it binds with. Different hormones have different receptors, with varying locations. In general, water soluble hormones have their receptors in the plasma membrane of target cells and hydrophobic hormones have their receptors within the cell. Cell surface receptors have three functional domains- Extracellular, Trans-membrane and Cytoplasmic or intracellular domains. Binding of hormone to the receptor initiates a series of events that lead to the generation of so-called second messengers within the cell. The second messengers then trigger a series of molecular interactions that alter the physiologic state of the cell. Receptors for steroid and thyroid hormones are located inside target cells, either in the cytoplasm or nucleus, and function as ligand-dependent transcription factors. Being lipids, steroid hormones enter the cell by simple diffusion across the plasma membrane. However, thyroid hormones enter the cell by facilitated diffusion. The receptors 'meet' the hormone either in the cytoplasm or nucleus. The hormone-receptor complex binds to "hormone response elements" within the promoter regions of responsive genes and stimulate or sometimes inhibit transcription from those genes. Receptor activation is the term used to describe conformational changes in the receptor induced by binding the hormone. The receptor then becomes competent to bind to DNA and this affects the state of transcription of the receptor bound genes. Most commonly, receptor binding stimulates transcription. The hormone-receptor complex thus functions as a transcription factor.

Lecture IV: Prof. Polani B. Seshagiri on '**BIOTECHNOLOGY & REGENERATIVE MEDICINE USING STEM CELLS**'

In vitro stem cell research is invaluable to the study of embryogenesis and cell lineage establishment. The use of *in vitro* studies for drug development and testing have reduced the use of animals and hence, cost of research in the pharmaceutical industry. Researchers have identified several hormone receptors responsible for the differentiation of stem cells to fully differentiated cells. Specific and controlled activation of these receptors hold tremendous potential for the treatment of several diseases. Gene based reprogramming helps to convert differentiated adult cells to induced pluripotent stem cells (iPS cells). iPS cells have the ability to differentiate into any type of cell in the body including nerve cells, cardiac cells, hepatocytes and pancreatic beta cells. Unlike embryonic stem cells, iPS research doesn't raise ethical objections and are immuno-compatible as well. Hence, progression of research in the induction of pluripotentcy in adult cells is certain to open doors for the treatment of several otherwise incurable diseases.

Day 2 - Lectures

Thursday, 27th June, 2014

Session chaired by Dr. Viji Mary Varghese

Lecture VI: Prof. Edathil Vijayan FNASc on '**PASSAGE THROUGH THE HISTORY OF NEUROENDOCRINOLOGY**'

The human brain has mystified people throughout history. Though it weighs a mere 1.5 kg and is small enough to be held in our hands, it is our body's most vital organ. Its complex network of 100 billion or more nerve cells orchestrates every aspect of our thoughts, perception and behavior. Functions of the brain include production of transmitter molecules, peptides and other signal molecules, reception of inputs, their processing or integration, signal transduction, and neurotransmission. Discovery of synthetic/secretory role of neurons in the central nervous system during 1930's gave rise to the concept of neurosecretion in 1940's. 1950's and 60's witnessed the emergence of releasing/inhibitory factors-peptide hormones, culminating in the award of Nobel Prizes in the 70's to the new discipline of neuroendocrinoloy. 1970's and 80's saw the explosion of a number of brain peptides, hormones and other molecules suggesting brain as an endocrine organ. The role of brain as an endocrine organ was established in the 1990's with the discovery of exciting molecules like cytokines, nitric oxide and a variety of other signal molecules. Nitric oxide (NO), a ubiquitous molecule plays a crucial role in a host of biological systems. NO is produced by and intimately involved in the functions of female reproductive tract. The hippocampus in the brain appears to be the target for physiological levels of estradiol in decreasing anxiety and depression. Estrogen alpha and beta receptors are localized in the hippocampus and ER-beta is associated with anti-anxiety and anti-depressive responses. Administration of estrogen receptor (ER) blockers increase anxiety and depression and selective ER modulators with greater affinity for ER-beta than ER-alpha indicate that ER-beta is required for estradiol's modulation of anti-anxiety and anti-depressive actions. Vasopressin is another hormone with a large number of biological functions such as control of body temperature, blood pressure, brain development, circadian rhythmicity, modulation of memory, aggression and sexual behavior. Alzheimer's disease is the most common form of dementia and one of the most complicated neurodegenerative diseases. This is associated with altered function and concentration of many neurotransmitters, especially acetylcholine and substance P (SP). Blockage of sustained release of substance P from hippocampus and mamillary bodies helps to reduce neuropathology of Alzheimer's disease. Increased production of substance P by the mamillary bodies lead to initiation of Alzheimer's disease by altering the function of NMDA receptors. Thus, blocking sustained release of SP from mamillary bodies and hippocampus can prevent Alzheimer's disease.

Lecture V: Prof. P. R. Sudhakaran on '**BIOSIGNALING**'

Two types of information are sensed by cells: direct physical aspects of the environment and symbolic information encoded by other cells. Cell signaling pathways regulate: cell proliferation, migrationdifferentiation, exocytosis, cell survival, death and development. Response often depends on multiple signals and dysregulation of signaling plays a major role in disease. Cell signaling can be activated by either physical or chemical stimuli. Chemical signals include steroid and peptide hormones. Cell surface receptors and intracellular receptors interact with specific cell signaling molecules with high affinity. Cell surface receptor class is spatially restricted, and can therefore convey information on the signal source. There are three known classes of cell-surface receptor proteins; ion channel-linked, G protein-linked and enzyme-linked. Turning-off mechanisms of signaling include receptor sequestration, receptor down regulation, receptor inactivation by its modification, inactivation of signaling proteins, production of inhibitory proteins and cross-inhibition of different signaling pathways. Cell signaling is central to modern medicine. For instance, Dasatinib is a Tyrosine Kinase inhibitor used in the therapy of cancer and sunitinib is another one that targets a variety of TK activities associated with many proteins (VEGF, c-kit, flt3, PGDF). Gefitinib and Erlotinib are the inhibitors of the epidermal growth factor receptor tyrosine kinase, associated with lung cancer.

Lecture VII: Dr. Sarita G. Bhat on '**THE PROKARYOTIC IMMUNE SYSTEM**'

Horizontal gene transfer (HGT) is a major contributor to genome evolution. HGT occurs by the action of mobile genetic elements like viruses, plasmids and transposons. As they are incapable of independent replication of their information, suitable host is a requirement for their existence. In the absence of integration or when recombination is neutral, there is less burden on host due to mobile genetic elements. Anti–invasion defense mechanisms and Anti–defense-invasion mechanisms is a biological arms race. Eukaryotes possess both generic and specific defense systems. In prokaryotes, Restriction/modification system recognizes and degrades DNA with nonnative methylation patterns. Other prokaryotic defense systems at the level of viral lytic cycle include impaired viral adsorption and the altruistic process of abortive infection. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) are perfectly conserved short sequences, typically of 20 to 40 base length, separated by unique sequences known as spacers. The spacer length in a given array is sometimes approximately conserved, varying by a few bases. The spacer length is typically similar to the repeat length. Three distinct functional stages can be distinguished in the CRISPR–Cas mechanism: CRISPR adaptation, the recognition of alien DNA by dedicated Cas proteins and/ or host proteins, as well as the subsequent processing and integration into the chromosomal CRISPR locus. CRISPR expression, that is transcription of the poly-spacer precursor crRNA (pre-crRNA), is followed by binding to a complex of Cas proteins known as Cascade [Cas-complex for anti-virus defense] and processing to mono-spacer crRNAs that serve as the guide sequences. CRISPR interference involves the binding and/or

degradation of the target nucleic acid. CRISPRFinder is a web service that offers fundamental tools for CRISPR detection and analysis of CRISPR loci. This tool is freely accessible at http://crispr.u-psud.fr/Server/CRISPRfinder.php.

Session chaired by Prof. Edathil Vijayan FNASc

Lecture VIII : Dr. Jackson James on '**FLUORESCENT PROTEINS AS TOOLS TO TRACK SIGNALING PATHWAYS INVOLVED IN NEURAL PROGENITOR MAINTENANCE *IN VITRO* AND *IN VIVO***'

Fluorescent proteins have numerous applications in biological research, especially in elucidating cell signaling pathways. Reporter genes confer certain easily selectable or identifiable or measurable characteristics. Cell signaling through various pathways can be studied by monitoring the activation or expression of reporter genes attached to cell signaling components. Fluorescent proteins are members of a structurally homologous class of proteins that share the unique property of being self-sufficient to form a visible wavelength chromophore from their own polypeptide sequence. Green fluorescent protein is a fluorescent protein present in the bioluminescent organs of *Aequorea victoria.* It was discovered and developed by Martin Chalfie, Osamu Shimomura and Roger Y. Tsien. Another fluorescent protein similar to green fluorescent protein is red fluorescent protein (DsRed), isolated from *Discosoma spp.* In life science research, fluorescent proteins are used as biological markers and reporter genes. Random expression of different fluorescent proteins in different neurons flags each neuron with a distinctive color to create a Brainbow. It is used as an important tool in connectomics and neuroanatomy. Dr. Jackson James used fluorescent proteins for elucidate the Notch mediated cell signaling in nerve cell differentiation.

Lecture IX : Dr. Sarita G. Bhat on '**CRISPR SYSTEM - ROLE IN EVOLUTION OF THE HOST**'

Horizontal gene transfer (HGT) is the major source of genetic variability for bacterial evolution. The ability of CRISPR systems to limit phage infection and plasmid conjugation is well established. Though CRISPRs have been shown to prevent electroporation of plasmid DNA, it remains to be determined they constitute an effective barrier against natural DNA transformation. Thus, CRISPR systems interfere with at least two major routes of HGT and have an important role in bacterial evolution. HGT is the major mechanism for the acquisition of antimicrobial resistance genes and the pathogen genes that encode virulence factors. A crucial healthcare issue is the emergence of methicillin resistant *Staphylococcus aureus* (mRSA) and vancomycin resistant *S. aureus* (vRSA) strains, the genesis of which is directly linked to the transfer of antibiotic resistance genes by plasmid conjugation. Sequencing of the highly virulent mRSA strain USA300 indicated that HGT has allowed acquisition of resistance encoding elements and virulence determinants that enhance fitness and pathogenicity. *S.*

aureus and *S. epidermidis* strains are the most common causes of nosocomial infections. CRISPR interference has been found to limit conjugation of the pG0400 plasmid from *S. aureus* to *S. epidermidis* in the laboratory and possibly constitutes a natural barrier to the spread of antimicrobial resistance. CRISPR immunity against lysogenic bacteriophages interfere with the spread of virulence factors among pathogens. For example, many bacterial toxins reside in prophages found in the genomes of *Corynebacterium diphteriae*, *Clostridium botulinum*, *Vibrio cholerae*, *E. coli*, *Streptococcus pyogenes* and *S. aureus*. Long before the elucidation of CRISPR function, the variability in the spacer content of the cluster was used to detect and identify strains of *Mycobacterium tuberculosis* for diagnostic purposes and epidemiological studies. This genotyping method was named spacer oligotyping or 'spoligotyping'. It is widely used for the identification of *M. tuberculosis* strains and has been applied to other organisms as well.

Valedictory Function

Dr. Kavitha O, Department of Biotechnology delivered the Welcome address. The impression about the Science Academies' Lecture Workshop was shared by Dr. Sr. Rosa K. D., Vice Principal, Resource persons and Participants. Prof. Edathil Vijayan FNASc, Convener of the workshop expressed his appreciation for the smooth conduct of the program. Mr. Naijil George, Co-ordinator of the workshop expressed his gratitude to Dr. Vijayan, Convenor of the workshop, all the speakers and participants for their co-operation. The workshop ended on a high note with National Anthem.

Convener	Co-ordinator	Head of the institution
Prof. Edathil Vijayan FNASc	Naijil George	