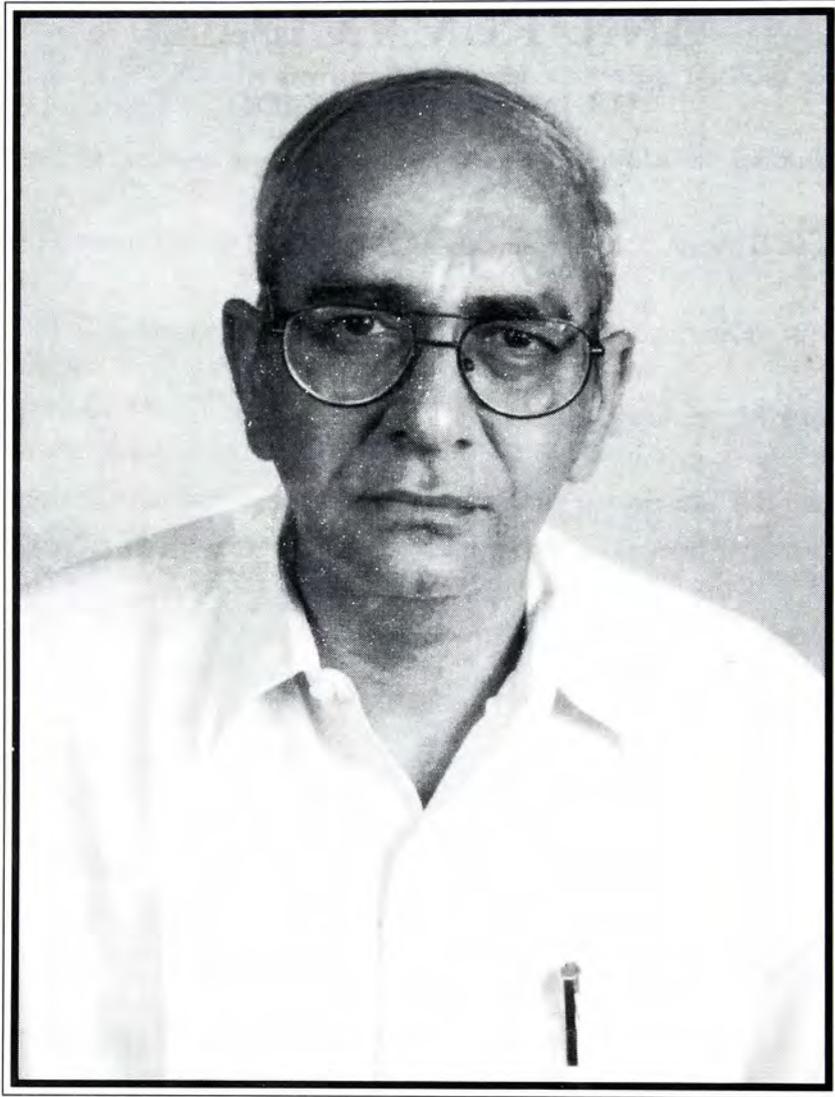


ANIL KUMAR LALA

(13 January 1950 – 2004)

Biog. Mem. Fell. INSA, N. Delhi **29** 115-128 (2006)





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ANIL KUMAR LALA

(1950 – 2004)

Elected Fellow 1993

EARLY LIFE AND EDUCATION

ANIL KUMAR LALA was born in New Delhi on January 13, 1950. He had his early education at AES Higher Secondary School, New Delhi. He graduated from the Kirori Mal College, University of Delhi. He joined the Department of Chemistry, University of Bombay, Maharashtra for M.Sc. degree. Later, he did his doctoral work in steroid chemistry in the same department under the guidance of Professor AB Kulkarni. After finishing his doctoral work in 1974, he spent a year at the Central Drug Research Institute, Lucknow. Subsequently, he took a postdoctoral position in the laboratory of Professor Marc Anteunis, at the State University of Ghent, Belgium. In his postdoctoral research, he studied the conformation of a neurotransmitter peptide Met⁵-enkephalin by high field PMR spectroscopy. His work on Met-enkephalin was considered as a milestone achievement in understanding the structure of small peptides (Roques, BP Garbay-Jaureguiberry, C, Oberlin, R, Anteunis, M & Lala, AK (1976) *Nature (Lond.)* 262, 778-779; Anteunis, M., Lala, AK, Garbay-Jaureguiberry, C & Roques, BP (1977) *Biochemistry*, 16, 1462-1466). Having been trained as a conventional chemist, Dr Lala was keen on joining Professor EJ Corey. But fate had something else in store for him. Dr Corey forwarded Dr Lala's application to Professor Konrad Bloch, a Nobel laureate, who at that time was looking for an organic chemist, who would synthesize alkyl cholestane derivatives to study sterol structure and function in various biological membranes. Taking this opportunity, Dr Lala joined the laboratory of Prof. Bloch at the Harvard University. It was in this laboratory that he became acquainted with membrane chemistry and was exposed to the various aspects of membrane biology especially lipid-protein interactions. He also had the opportunity to interact with various scientists working on the interface of biology and chemistry and these interactions further helped him to pursue his career in Chemical Biology.

FAMILY BACKGROUND

Anil Lala married Krishna whom he met while working in the Department of Chemistry at Bombay. Krishna was then a Master's student in the same department. They have a son Vishal, who has followed his father's legacy and taken up academics as his career only in a different discipline, Business Management. He is pursuing his career in the USA. Dr Lala was very committed to his family.



successfully overcame adverse situations with the support at home from his wife and son. Together they had a wonderful thirty years of marriage – one that many young couples would like to emulate.

PROFESSIONAL CAREER

Dr Lala joined Department of Chemistry, Indian Institute of Technology Bombay in December 1978. A young man full of new ideas and enthusiasm started his career with a lot of hope and expectation of working in frontier areas of chemistry. He entered a department that had a stronghold in synthetic and natural product chemistry. Dr Lala was a determined man with a strong belief that he would make a meaningful contribution to Indian Science. He infused a new research culture in the department. He was among the first in the department to write research projects and bring in major grants to the institute. In February 1985, he was selected by the "Gentleman" magazine as one of the outstanding persons less than 40 years of age, who could lead Indian science into the 21st century. He was cited as a powerful example against the "brain drain". He became a professor of chemistry in 1986.

Dr Lala recognized very early in his career that a good foundation of chemistry could be successfully applied to a better understanding of the biological systems. He was among the first of a new generation of scientists to have christened themselves as "Bioorganic Chemists". He was instrumental in establishing a biology program at IIT, Bombay. He served as the Convener of Biosciences and Bioengineering group from 1983 to 1987. Few years later, he became the Head of the Regional Sophisticated Instrumentation Centre, at IIT, Bombay. He also served as the Head of the Biotechnology Centre for the period 2001 to 2002.

Teaching: Dr Lala was a passionate teacher. He introduced several bio-oriented courses including Enzymes and Coenzymes and Bioorganic Chemistry in the chemistry curriculum. In early eighties, the Government of India took a timely decision to set up a Biotechnology Board to boost research and development activities in various interface areas of chemical, physical and biological sciences. Dr Lala grabbed this opportunity and with the help of some colleagues, started a M.Sc. program in Biotechnology at IIT, Bombay. He taught several courses to the M.Sc. students of Biotechnology including Molecular Enzymology, Introduction to Biomolecules, Analytical Biochemistry, Molecular Immunology, General Organic Chemistry and Computers in Biology. He had a strong belief that a good training in the laboratory techniques would give students the required edge to undertake research involving intense experimentation. He brought in new ideas and significant changes in running laboratory courses. One of his dearest teaching interests was to provide computer lessons to young biologists.



RESEARCH CONTRIBUTIONS

In the beginning of his scientific career at the Indian Institute of Technology Bombay, Dr Lala studied sterol-phospholipid interactions in model membranes. He obtained data indicating that the double bond at C5-C6 in cholesterol plays an important role in cholesterol-phospholipid interaction (Ranadive and Lala 1987). His group also modified cholesterol and monitored the effects of the sterol oxygen function on the sterol-phospholipid interaction. One of Dr Anil Kumar Lala's outstanding contributions was the elucidation of the structure-function properties of some important membrane-interacting proteins using the technique of hydrophobic photolabeling. The three-dimensional structures of a very few membrane-interacting proteins are available till today. Further, the conformations of these proteins in the membrane are likely to be different from those in solution. Their orientation in the membrane would involve regions of high hydrophobicity and the hydrophobic surfaces should be exposed for interaction with the membrane, which is not likely to be the case in solution. In addition, the identification of membrane-inserted segments of pore-forming soluble toxin is important for understanding the action of these toxins at the molecular level. The limited progress with conventional structure determination techniques in the case of membrane-bound proteins calls for the use of other techniques. Dr Lala's work involving hydrophobic photolabeling was a useful approach in the elucidation of the structure-function of membrane-interacting proteins. In this technique, a hydrophobic photoactivable reagent that partitions into the membrane is used, which upon photoactivation, labels the membrane-spanning domains of transmembrane proteins. After fragmentation, the photolabeled region is separated by chromatography or electrophoresis and sequenced for identification. Hydrophobic photolabeling enables mapping of the regions of proteins that are interacting with the membrane during its entry through the cell membrane. He also synthesized fluorescence based photolabeling probe, which could replace radio-labeled probes used in photoactivation purposes. His group successfully used fluorenyl fatty acids as depth dependent probes for probing membrane hydrophobic core (Lala *et al.*, 1988). He also extended the use of photo-activate probes in understanding the pathways of protein unfolding (Lala and Kaul 1992, D'Silva and Lala 1998, 1999 & 2000). A hydrophobic photoactivable reagent $^3\text{[H]}$ diazofluorene was used to detect intermediates involved in the unfolding pathway of diphtheria toxin (D'Silva and Lala 1998). In recent times, he was interested in drug development studies.

The existence of a protein toxin, diphtheria toxin (DT), in *Corynebacterium diphtheriae*, has been known for almost a century. The crystal structure of its soluble form is available; but it can give only a speculation of its molecular mechanism of membrane insertion. Because of the very limited availability of information on the structure of membrane-bound toxin, the mechanism of entry of DT into cells was not understood. With the use of the hydrophobic photolabeling reagent diazofluorene



(DAF), Dr Lala reported a clear-cut identification of the regions of DT that interact with the membrane, which has taken us further from knowing the solution structure of DT to knowing something about the mechanism of entry of DT through the cell membrane into the cell (D'Silva and Lala 2000). DT consists of two chains, A and B, that are joined by a disulfide link and is composed of three structural domains: the N-terminal catalytic domain C, transmembrane domain T, and receptor-binding domain R. The diphtheria toxin (DT) inserts into the membrane and after disulfide reduction, the catalytic domain translocates into the cytosol where its ADP-ribosylates the elongation factor-2, leading to inhibition of protein synthesis and eventual cell death. Solution studies had implicated only the TH8 and TH9 helices of the T domain to be mainly responsible for the toxin's interaction with the membrane. Using hydrophobic photolabeling of DT when it is bound to membranes with diazofluorene (DAF), Dr Lala, along with Dr D'Silva, showed that apart from the TH8 and TH9 helices other parts of the T and C domains, namely, the TH1 α -helix of the T-domain and the CB6 β -strand of the C-domain, are also associated with the membrane. Further, their work shows that when DT is inserted in the membrane it adopts a molten globule-like state, which has been observed as a stable intermediate state in the folding pathways of many proteins. This gave further support to the hypothesis that soluble proteins might be entering into the membrane via a molten globule-like state.

The nicotinic acetylcholine receptor (nAChR) is the prototype for a protein super family that includes the receptors for the excitatory amino acids glutamate and aspartate, the inhibitory amino acids gamma-aminobutyric acid (GABA) and glycine, as well as the serotonin 5-HT₃ receptor. The neuronal nAChR is a target against a variety of diseases, including cognitive and attention deficits, Parkinson's disease, anxiety, and pain management (Holladay MW, Dart MJ, Lynch JK: Neuronal nicotinic acetylcholine receptors as targets for drug discovery. *J. Med. Chem.* 1997, **40**: 4169-94; Lloyd GK, Williams M: Neuronal nicotinic acetylcholine receptors as novel drug targets. *J Pharmacol Exp Ther* 2000, **292**: 461-7). The experimental structure of nAChR consists of 4.6 Å data from electron microscopy (Miyazawa A, Fujiyoshi Y, Stowell M, Unwin N: Nicotinic acetylcholine receptor at 4.6 Å resolution: transverse tunnels in the channel wall. *J Mol Biol* 1999, **288**: 765-86), a resolution that does not allow for structure-based design approaches. In a study that was published in 1998 in the *Journal of Biological Chemistry*, Dr Lala, in collaboration with Dr Cohen's group, examined the structural changes in the *Torpedo californica* nicotinic acetylcholine receptor (nAChR) ion channel induced by agonists. Based on the results obtained from the DAF-labeling experiments, a model hypothesizing agonist-induced conformational change in the transmembrane helices arranged around the central axis of the pore in response to agonist activation was proposed. The *Staphylococcus aureus* α -toxin, a 33-kDa soluble exotoxin, oligomerizes to form pores in the erythrocyte membrane to effect hemolysis. The three-dimensional structure



the *S. aureus* α -toxin-toxin monomer is not available. Dr Lala's elaborate elucidation of the orientation of this toxin into the membrane using the same technique again assumes great significance.

Dr Lala has contributed immensely towards the development of a depth-dependent photolabeling technique, which has been widely used to gain information at different depths in the membrane hydrophobic core. These probes were found to be extremely useful in understanding membrane organization and structures. Particularly, they were used to unravel the roles of membrane lipid rafts. Lipid rafts, which are specialized membrane domains enriched in certain lipids, cholesterol and proteins, are implicated to have roles in cholesterol transport, endocytosis and signal transduction. Further, depth-dependent membrane analysis gains importance considering the recent reports on the functional significance of intra-membrane proteolysis of proteins at different depths as a regulatory mechanism, which seems conserved from bacterial to animal systems (MS Brown and JL Goldstein, A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proc Natl Acad Sci USA* 96 (1999), pp. 11041–11048; MS Brown, J Ye, RB Rawson and JL Goldstein, Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans (*Cell* 100 (2000), pp. 391–398). Thus, Dr Lala was a visionary in that he contributed towards the development of a method that helps to answer some important questions in membrane biology.

Both fluorescent probes and photoactive probes have been used for the depth-dependent analyses of membranes. Not only did Dr Lala design new fluorescent fatty acids as probes (*Biochemistry* 1988), but he also showed how careful designing of these probes is necessary in order to minimize membrane perturbation due to the probe and thereby achieve a more optimal depth-dependent assessment of the membranes (*Biochemistry* 1988; *Chem Phys Lipids* 2002). He worked extensively in designing fluorenyl fatty acids and showed their efficaciousness, in terms of their high fluorescence quantum yield, polarization values and proper alignment with the membrane, in a number of reported studies (*Chem Phys Lipids* 2002). He also showed that fluorenyl fatty acids could be used to differentiate between normal and a malaria-infected red blood cell membrane, both in terms of the membrane function and its composition (V Koppaka, R Sharma and AK Lala, Fluorescence studies on erythrocyte membrane isolated from *Plasmodium berghei* infected mice. *Mol Cell Biochem* 91 (1989), pp. 167–172). Further, fluorenyl fatty acids were used to investigate the interaction and localization of the anti-psychotic drug chlorpromazine (CPZ) in membranes. He evidenced the formation of chlorpromazine-rich domains towards the middle of the bilayer in both artificial and natural membranes at physiologically relevant concentrations of chlorpromazine, which gave further credence to the hypothesis that the mechanism of transport of chlorpromazine to the brain involves its getting sequestered in the erythrocyte membrane.



Photoactivable fatty acids or phospholipids derived from them, on incorporation in membrane followed by photolysis give rise to cross-linked products which are further analyzed. The high reactivity of carbenes and nitrene-based photoactivable probes leads to very low insertion yields in the membrane. Dr Lala achieved a high insertion yield (35-40 %) by suitably designing phospholipids prepared from benzophenone-based fatty acids for depth-dependent labeling and reasoned that benzophenones may be the reagents of choice in the future. Furthermore, he reported on different benzophenone-based probes that labeled the membrane at different depths, revealing their usability (Depth-dependent analysis of membranes using benzophenone-based phospholipids. John B, Kumar ER, Lala AK (*Biophys Chem* 2000 Sep 15; **87(1)**: 37-42).

In the past few years, Dr Lala's contribution to science was the discovery of and studies on peptide toxins from the Indian scorpion *Buthus tamulus* and the Indian marine snail *Conus amadis*. Scorpion venom is rich in peptide toxins that perturb ion channels and cause neurotoxicity. Because these small peptide inhibitors modulate ion-channel activity by binding to them, they can be effective tools to understand the structure and function of ion channels (R MacKinnon, SL Cohen, A Kuo, A Lee and BT (*Chait Science* **280** (1998), pp. 106-109); ML Garcia, M Hanner, HG Knaus, R Koch, W Schmalhofer, RS Slaughter and GJ Kaczorowski (*Adv. Pharmacol* **39** (1997), pp. 425-471). Interestingly, as these toxins are found to have stringent ion-channel specificities, they are immensely useful in the individual structure-function characterization of ion channels.

Depending on whether the peptide inhibits the sodium, potassium, chloride or calcium channels, the peptide toxins are classified into four families, which are then further categorized into subfamilies based on other properties. From the venom of the red scorpion *Buthus tamulus*, Dr Lala's group purified BTK-2, a novel inhibitor of the human voltage-gated potassium channel Kv1.1. An elaborate characterization of this peptide by sequencing and multiple sequence alignment led to its classification into the ninth subfamily of scorpion toxins (J Tytgat, KG Chandy, ML Garcia, GA Gutman, MF Martin-Eauclaire, JJ van der Walt and LD Possani. *Trends Pharmacol. Sci* **20** (1999), pp. 444-447). Electrophysiological studies revealed that BTK-2 had all the characteristics of a pore blocker like other scorpion toxins. Further, they linked subtle variations in sequence, the type of secondary structural element and homology-modeled the structure of BTK-2 to its ion-channel specificity thereby providing insights into its structure-function properties. The specificity of this toxin suggests why the scorpion venom may be effective in very small amounts. Also, BTK-2 may be considered as a valuable tool in the neuro-physiological dissection of physiological processes on account of its channel specificity and it may also serve as a primary template for the design of specific blockers of this ion channel (BTK-2, a new inhibitor of the Kv1.1 potassium channel purified from Indian scorpion *Buthus tamulus*).



tamulus; Dhawan R, Varshney A, Mathew MK, Lala AK (*FEBS Lett* 2003 Mar 27; **539(1-3)**: 7-13).

Dr Lala purified another toxin, BtITx3, from the venom of the same Indian scorpion *Buthus tamulus*. It was found to block the chloride channel of the insects belonging to the Lepidopteran species *Helicoverpa armigera* and was extremely lethal to the insect larvae (Purification and characterization of a short insect toxin from the venom of the scorpion *Buthus tamulus* (*FEBS Lett.* 2002 Sep 25; **528(1-3)**: 261-6). Not only did his careful and elaborate analyses of the multiple sequence alignment with other scorpion toxins and the three-dimensional model of its structure support its classification as a chloride-channel blocker, but they also led to an interpretation of the structure-function properties of this novel peptide (Purification and characterization of a short insect toxin from the venom of the scorpion *Buthus tamulus*. *FEBS Lett* 2002 Sep 25; **528(1-3)**: 261-6). Based on them, the mode of binding of BtITx3 was suggested to be different from that of other chloride-channel blockers, which pointed towards the necessity for further structural characterization.

The marine *Conus* snails are venomous predators that have some 40-200 distinct biologically active peptide toxins in their venom, which they use to immobilize their prey. The toxins in cone shell venoms possess pharmacological qualities that make them valuable tools in medical research. Their targets are ion channels and receptors in the neuromuscular system. Like the scorpion toxins, conus peptides are proving to be chemical probes of great resolving power owing to their target specificity (RA Myers, LJ Cruz, JE Rivier and BM Olivera (*Chem Rev* **93** (1993), pp. 1923-1936). Dr Lala and his group at IIT Bombay participated in a collaborative study aimed at exploring the diversity of conotoxins produced by the conus snail species found off the Indian coast. They reported the isolation and characterization of a delta-conotoxin, named Am 2766, from the molluscivorous snail *Conus amadis*, a hitherto uninvestigated species of snail collected in the Bay of Bengal. Electrophysiological studies revealed that Am 2766 inhibits the inactivation phase of the mammalian voltage-gated sodium channels.

Apart from these three main areas, namely, hydrophobic photolabeling of membrane-bound proteins, depth-dependent labeling of membranes and peptide toxins from scorpion and snail venoms, Dr Lala also contributed in the study of membrane components as well as protein folding (*J Biol Chem.* 1992; *Mol Cell Biochem* 1989; *Biochemistry* 1987; *J Biol Chem* 1979; *PNAS* 1977).

In addition to his excellent scientific achievements, he helped scientists across the nation by sequencing proteins and peptides for them. He was the project coordinator of the National Protein Sequencing Facility at IIT, Bombay. He also organized the First Indian Symposium of the Protein Society, which was a highly successful meeting. This article is by no means, an exhaustive account of all of his



work, but it is a reflection on the work of an untiring and impassioned experimental scientist and an ingenious thinker.

AWARDS AND HONOURS

In 1981 he won the Indian National Science Academy Young Scientist Award and later became an Associate Fellow of Indian Academy of Sciences between 1983 – 1986. He was elected member of Guha Research Conference in 1988. He was an INSA Research Fellow between 1989 – 1991. He became a Fellow of Indian Academy of Sciences in 1993 and a Fellow of INSA in 2003. He was an Alberta Heritage Foundation Visiting Fellow, Canada, in 1987. He was a visiting Professor at Harvard Medical School and at the University of Ulm, Germany in 1992 and 2001, respectively. He also served in the Editorial Board of the journal *Protein Science* published by the Protein Society.

He served various Committee Assignments:

- Member, Program Advisory Committee for National NMR facility, TIFR. 1987 – 1993
- Member, DBT Task Force on Environmental Biotechnology. (1991 - 1997)
- Member, DST Management Advisory Committee for Young Scientists. (1992 -1997)
- Member, Research Council, NEERI Nagpur. (1992 - 1998)
- Member, Editorial Board, Proc. Indian Acad. Sci (Chemical Sciences) 1993 – 2000
- Member, Sectional Committee for General Biology, Indian Academy of Sciences (1999 – 2001)
- Member RAP-Scientific Advisory Committee, Centre for DNA Fingerprinting (CDFP). (2001)
- Member DST Program Advisory Committee on Biochemistry, Biophysics and Molecular Biology. (1998 – 2001, 2001 - 2004)
- Editorial Advisory Board, Protein Science (2002)
- Member, RAP - Scientific Advisory Committee, Bose Institute, Kolkata (2003)
- Member, Selection Committee – Advanced Centre for Treatment, Research & Education in Cancer (2003).
- Member, DBT task force on Basic Research in Modern Biology, New Delhi (2003)
- Member, Board of Biotechnology Education, AICTE, New Delhi. (2003)
- Member, National Committee for IUBMB and IUMS, INSA (2003)



- Member, Sectional Committee IX – Biochemistry and Biophysics, INSA (2003- 2006)
- Member, Selection Committee for Biochemistry, IISc Bangalore (2004)
- Member, RAP-Scientific Advisory Committee, National Institute of Immunology (NII), Delhi (2004)

He has been a Reviewer of projects submitted for funding to The Department of Science & Technology (DST), The Department of Biotechnology (DBT), Council of Scientific and Industrial Research (CSIR), Board of Research in Nuclear Sciences (BRNS). He was a reviewer for papers submitted to *Biophysical Journal*, *Current Science*, *Indian Journal of Biochemistry and Biophysics*, *Journal of Biosciences and Protein Science*

PERSON TO BE REMEMBERED

Anil Kumar Lala was a very committed scientist and a great teacher who had influenced the career choice of several students. It was his passion for teaching and his ability to express the complex concepts with simplicity that inspired students to take on research careers. The Indian Institute of Technology, Bombay has internationally reputed undergraduate engineering and science programs. But to lure students from engineering and other science disciplines to pursue research careers in biological sciences is always a difficult task. This was successfully achieved by Prof. Lala, which speaks volumes of the charismatic teacher that he was. As a mentor and friend, he certainly carved a niche for himself in the lives of many colleagues.

As a child, he was fond of reading especially English literature and the habit continued till his involvement in his scientific endeavours, which left him little time for other activities. He loved reading the plays of George Bernard Shaw and the philosophers like Jean-Paul Sartre. He had a natural flair for drawing and painting. He watched plays and movies very keenly as he would have loved directing them. Photography was one of his great passions. He believed in modern technology and computers became a passion. He was nothing short of being called a “Geek” by some.

Anil Lala was a meticulous person and a great organizer. Everything from his desk to the working table in his room was spotlessly clean and in place. He could pull out references and papers irrespective of its age, because it was so well organized and filed. One of his students coined a term for Dr Lala a “Filomaniac”, very much to his knowledge, which he fondly remembered. He can be remembered as a man who spoke his mind without mincing words. He was deeply committed to improving the system and the society at large. He will always be loved and remembered as “guru” by all his students.



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