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K. S. KRISHNAN OBITUARY

Remembering K. S. Krishnan (1946–2014)

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Abstract

Dr. K. S. Krishnan was on the faculty of the Division of Biological Sciences at the Tata Institute of Fundamental Research (TIFR) in Mumbai, India, and later emeritus professor at the National Center for Biological Sciences (NCBS) in Bangalore, India. His research using fruit flies has contributed richly to our understanding of synaptic function and mechanisms of anesthetic action. Dr. Krishnan passed away suddenly of a heart attack on the 24th of May, 2014. Below a few of his students fondly recall how it was to work in his group.

Keywords

Krishnan, obituary, Tata Institute, TIFR

History

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Professor K.S. Krishnan was the first Principal Investigator (PI) invited by Obaid Siddiqi to work in Neurogenetics in the Molecular Biology Unit (MBU) of the Tata Institute of Fundamental Research, Mumbai, now universally recognized for its distinguished and pioneering contributions to *Drosophila* Neurogenetics (Krishnan et al., 2012). Here we remember Krishnan, our thesis advisor, an incomparably creative scientist, a rare mentor, and uniquely inspiring human being.

If you were a newly recruited graduate student in the Molecular Biology Unit during the 80s and 90s, chances are that the person you would first meet in the corridors would be Dr. Krishnan—a bright eyed, intensely alive, and highly conversational individual. It is also highly likely that you would end up having a free-running, sometimes rambling conversation over the next hour over many things scientific and otherwise. As you stepped out of his office (which was essentially a desk and a chair within the laboratory; see Figure 1), you realized that you still did not quite know who he was, except that now your eagerly receptive mind was buzzing with the seeds of myriad ideas, one more fantastical than the other. Several days later, you would learn that Dr. Krishnan was a Professor working on the cell biology of the synapse, using *Drosophila* as a model organism. Although graduate students were encouraged to be on a first name basis with everyone, Krishnan was always an exception—although he was the least officious, least intimidating person around, he was always "Dr. Krishnan" to us students. Not because he demanded it, far from it, but mostly because we had no idea what the "K. S." stood for, and well, he always looked like he would be a "Dr. Krishnan". The closest we got to not calling him Dr. Krishnan was to call him "KSK." Through our entire tenure Ph.D. students took KSK's presence and accessibility for granted, which, in after sight, represented both a remarkable sense of entitlement and a rare gift to aspiring young scientists.

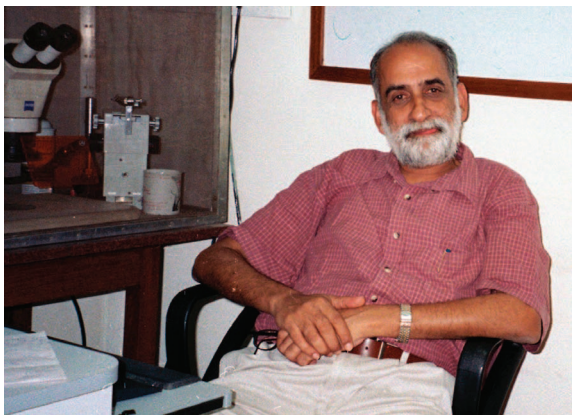

Figure 1. Dr. K. S. Krishnan in his "office" within the laboratory next to the electrophysiology rig in the Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, India (*circa* 2004).

As we have each embarked on our respective careers, we realize now how lucky we were to be allowed to operate in an environment that was so free and devoid of pressures that typify many a workplace. Research can be incredibly challenging and self-esteem sapping on a good day, but KSK's natural gift was that he made the bad days seem par, even productive—admittedly sometimes to our inexperienced indignation as we thought he was making light of a serious matter! Every failed experiment, to us a depressing setback, was to him the chance to speculate, sometimes wildly. Experiments that did not work as expected were a particular joy to him ("most fraud in science comes from hypothesis-driven experiments," Krishnan would cheerfully state, summarily dismissing the conventional

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wisdom of NIH study sections; "seeing new things is the best part of science"). Thus, "failures" invariably led to the formulation of "crazy" experiments (we recount a few below) and even exasperating arguments that were somehow never lastingly burdensome. This was an experience not unique to his own students, but across the board, be that at MBU/DBS or later on at NCBS in Bangalore. KSK passed away on the 24th of May, 2014 of a sudden heart attack and with his demise disappeared a presence that we all drew upon without giving it much thought. KSK was simply always there, always ready to listen, always ready to give freely of his time and always ready to offer his out-of-the-box ideas. With his passing this softer, gentler side of scientific research, appreciated by many scientists from around the world who visited TIFR/NCBS, stands significantly diminished.

Someone once said that if you wanted to learn about nature, all you had to do was look. And KSK's unique talent lay in looking very closely, in observing details that often escaped the casual, or even the informed glance. In the 90s there was intense excitement in the field of synapse biology. Various molecular players were being identified, genes encoding these proteins were being cloned and the tapestry of synaptic vesicle release, recycling and trafficking was being woven rapidly from this new information. During this time, together with Mani Ramaswami, a life-long friend and collaborator, Krishnan embarked on a journey to identify mechanisms underlying synaptic vesicle recycling by screening for genetic modifiers of *shibire,* a temperature-sensitive paralytic mutant in Dynamin. Typically, he designed the "*sushi*" (suppressor of shibire) cooker, a double-walled glass contraption attached to a water bath that allowed him to control temperature with an accuracy of 0.5°C—something he excitedly demonstrated to all who came into the laboratory. Interestingly, it never seemed to bother him that Sushi is not actually cooked. He also went on to fabricate the Immersion Device for Larval Immobilization (IDLI) cooker, but that story for another day. These mutagenesis screens resulted in a rich crop of suppressors and enhancers of *shibire* paralysis, but the fun part was that each mutant underwent a celebratory naming ceremony. While some were named with initials of people in KSK's life and laboratory, other names were drawn from several "difficult to pronounce" Tamil, Telugu, and Malayalam (languages in South India) words for lazy, slow, paralytic, and stunned, such as *orangi, vizhichai*, and *atal*. Indeed, we recall with fondness his search for synonyms in different languages to better name new mutants with. The screen for suppressors gave multiple interesting alleles of *shibire* itself, which later provided fundamental insights into functional interactions between the subdomains of the Dynamin protein (Ramaswami et al., 1993; Grant et al., 1998; Narayanan et al., 2005). The screen for enhancers yielded several different alleles of *shibire* in addition to two enhancers, which affected nucleotide (ATP and GTP) levels at the synapse and in turn specifically affected synaptic vesicle recycling (Krishnan et al., 2001; Rikhy et al., 2003).

In this fast-changing landscape, the role of yet another synaptic protein called *N*-ethylmaleimide sensitive fusion factor (NSF) remained enigmatic. After endless discussions on what NSF might be doing at the synapse (the fly homolog had recently been cloned by Leo Pallanck, Richard Ordway, and Barry Ganetzky showing that temperature-sensitive (ts) paralytic mutants called *comatose*, were NSF alleles; [Pallanck et al., 1995]), we devised an experiment to test what would happen if action potentials in motor neurons were blocked at the same time that we inhibited NSF function in *comatose* mutants. While the details of the study can be found elsewhere (Sanyal et al., 1999), what was really unexpected was the odd behavior of *paralytic* and *comatose* double mutants. When returned to a permissive temperature from a shift to restrictive temperatures, these flies seemingly recovered from paralysis instantaneously only to immediately paralyze at *permissive* temperatures! This was a subtle and fleeting phenotype that we must surely have missed had it not been for KSK's keen powers of observation. Importantly, this one result told us that NSF was involved in the resolution of SNARE complexes rather than solely in its predicted function as a fusogen—a view that was much less widely held at that time, but one that these behavioral experiments bolstered substantially.

If catching flies paralyze oddly was striking, KSK really outdid himself in the identification of the mutant *Kumbhakarna* (*Ca-P60A*^{Kum}) (Sanyal et al., 2005). *Kum*, later identified as a mutation in the fly homolog of sarco-endoplasmic reticulum calcium ATPase (SERCA), was discovered in a screen for autosomal dominant paralytic mutants. But its discovery was serendipitous and it is safe to say that except for KSK's playful yet keen approach to doing science, it would never have been isolated. The extraordinary aspect of *Kum*'s phenotype is that paralysis induced at restrictive temperatures lasts 24–48 *hours* after being brought back to permissive temperatures. During the course of a genetic screen for reversible paralytics, on several occasions flies simply die and are discarded. But in this case, with regard to a fly lying at the bottom of the vial, an argument ensued with KSK over whether the fly was dead/dying or reversibly paralyzed! KSK insisted on the latter (for reasons known only to him) and asked for the fly to be kept under observation. A day passed and still the fly had not recovered. But KSK had not lost his faith. Sure enough, a day and half after the heat shock the fly recovered fully. Such a phenotype was completely unknown at that time. And the prolonged paralysis later turned out to be important in identifying the role played by SERCA in regulating membrane excitability. Always on the lookout for the strange and the unusual, KSK was ideally primed to discover such a mutant, and of course to yet again come up with a fitting name for it—*Kumbhakarna*—after a character from the Indian epic Ramayana who sleeps for six months at a time.

Krishnan's natural curiosity to know what the fly genome project was going to offer us was evident after the *Drosophila* genome sequence was out in the public domain. His excitement for data mining of the fly genome to identify genes required for vesicular trafficking was coupled to powerful reverse genetics approaches, such as that applied to *endophilin*. Cloning and expression analysis of this gene itself was exciting, especially under Krishnan's guidance. Since transposon insertions at the *endophilin* locus were lethal, KSK suggested that we make eye clones for *endophilin* and measure synaptic activity from mutant eyes using electro-retinograms. It was a gratifying experiment indeed since we observed a distinct activity-dependent decline in synaptic transmission (Rikhy et al., 2002). This was also a

time when KSK's laboratory had started combining classical genetics with molecular biology and biochemistry and making antibodies and transgenic animals became routine (though it must be admitted, he was not a particular fan of "bind-and-grind" experiments). These tools were used to study *syndapin*, once more in collaboration with Mani Ramaswami. Syndapin was interesting in several ways. For instance, although it physically interacted with Shibire, Syndapin did not localize to the presynapse but caused massive expansion of the sub-synaptic reticulum when over-expressed in the muscle (Kumar et al., 2008, 2009). This overturned the simplistic notion that all interactors of Dynamin must play a role in endocytosis at the nerve terminal. Interestingly, one of the offshoots of generating additional *syndapin* alleles was the isolation of *angur*—(a Hindi word meaning grapes)—in which synaptic boutons appeared to grow like a bunch of grapes.

Krishnan had diverse interests. He pursued many different topics through his life with almost equal attachment, protein biochemistry in the plasma membrane, mechanisms of action of anesthetics in the nervous system, synaptic function and observations of birds, chameleons, snails, and wasps. In collaboration with Howard Nash, he had made built an apparatus, which he proudly called the "inebriometer". It was a long glass tube and KSK would be seen perched precariously on a high chair introducing flies into this apparatus to watch them "elute" depending on their differential sensitivity to anesthetics. He used this effectively to understand the neural locus of anesthetic action in flies and also generated mutants which have altered sensitivity (Nash et al., 1991; Mir et al., 1997). Sometime around 2002, Krishnan and Mani, together with Krishnan's Ph.D. advisor (and close friend of more than 40 years), Dr. Balaram, started experimenting with conotoxins—with the idea of isolating, identifying, and ultimately synthesizing toxins that could potentially be used as drugs (Gowd et al., 2005). In the initial stages, Krishnan's fervor—the main driver of this project—was quite apparent because he would assiduously collect and maintain various snails from the Arabian Sea coastline abutting the TIFR campus in Mumbai. At times, as students we were bit embarrassed when we used to see him reaching the laboratory before us and starting his experiments on conotoxins. By 2003, Krishnan had built up a remarkable collection of beautiful and diverse cone snail shells, each of which he could describe with great enthusiasm. In fact his collection had become so famous that any visitor to MBU/DBS would first go to his office and KSK made it a point that she/he did not leave without appreciating his collection!

Krishnan's passion for science and his knowledge of diverse fields was natural. He impressed upon us that if there was a phenotype, there must be an underlying reason and all we had to do was to search for it. In particular, an appreciation for the incredible worth of temperature-sensitive paralytics as a tool for discovery, first pioneered by Seymour Benzer, was his intellectual gift to us. The other, more human, more enduring lesson was the realization that being a good person and a good thinker is not mutually exclusive. Krishnan, and his unique persona and scientific outlook, will be deeply missed by all his students and by all those who came in contact with him (Ramaswami et al., 2014; VijayRaghavan, 2014; Srinivasan, 2014).

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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