Remembering Obaid Siddiqi, a pioneer in the study of temperature-sensitive paralytic mutants in *Drosophila*

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Although Obaid Siddiqi's major research focus in neurogenetics was on chemosensation and olfaction in *Drosophila*, he made seminal contributions to the study of temperature-sensitive paralytic mutants that paved the way for research that we and many other investigators have continued to pursue. Here we recount Siddiqi's investigation and the impact it had on our own studies especially at a formative stage of our careers. We acknowledge our debt to Obaid Siddiqi and remember him fondly as an inspired and inspiring scientist, mentor, role model and human being.

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1. *Drosophila* neurogenetics at the beginning

Professor Obaid Siddiqi, whom we honour here, is appropriately remembered as an outstanding mentor who inspired and catalysed the work of his students and junior associates. This sentiment is widely shared and vividly portrayed in the fascinating personal accounts of Obaid's friends and colleagues published in a recent special issue of the *Journal of Neurogenetics* in honour of Obaid's 80th birthday (Carlson 2012; Hasan 2012; Singh 2012; Vijayraghavan 2012). But even Obaid may not have fully realized that his mentorship and influence extended well beyond his immediate colleagues and included those with whom he did not interact on any regular or formal basis. Such is the case for us and for the stimulus that Obaid provided that guided our work on temperature-sensitive (ts) paralytic mutants in *Drosophila* at a seminal point in our careers and which has remained an integral component of our research ever since.

It is now almost 40 years since we embarked on our postdoctoral studies with Seymour Benzer, and the field of neurogenetics and neurobiological studies in *Drosophila* has grown enormously in size and sophistication over that time. It is probably safe to say that today almost no knowledgeable investigator outside the field would question the goals, value or significance of such studies in *Drosophila*. The landscape was quite different back then when the entire community of investigators working in this field could be easily fit in a small room. It was both exciting and somewhat frightening to be entering a field that was essentially brand new and to be working at the edge. Seymour's dictum, 'from gene to behaviour', was an inspiring slogan, and Seymour's clout and genius were sufficient to lend it considerable weight and to instill belief in the possibilities. But it was still a slogan, not a road map or a carefully charted plan for how to get from point A to point B. In Seymour's lab and in a few other labs around the world, the cohort of believers was still drawing up the map as they went along.

In those days, before the availability of the vast bulk of tools that everyone uses routinely in their work today – cloning, sequencing, PCR, transposon mutagenesis, germline transformation, spatially and temporally regulated gene expression, etc., the driving force, as Seymour had recognized from the beginning, was the ability to isolate single-gene mutants affecting various behaviours in *Drosophila* (Benzer 1967). Mutants were king. Simply finding a new mutant that altered phototaxis, circadian rhythm, courtship, or learning and memory was cause

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for great excitement and the basis of a publication even though it was not at all clear how we would ever figure out precisely, using the tools then at hand, what protein the mutation affected or what the exact consequence was of a defect in that protein.

When we joined Seymour's lab in 1976, we were among the second wave of postdoctoral fellows to do so, about 10 years after Seymour got the field started. Work being done by other investigators elsewhere was beginning to focus more on the actual neural signalling mechanisms that underlay behaviour using electrophysiological techniques. Bill Pak was carrying out increasingly sophisticated analysis of phototransduction in various mutants (Alawi and Pak 1971); Kazuo Ikeda in a technical tour de force managed to obtain intracellular recordings from neurons in the thoracic ganglion (Ikeda and Kaplan 1970); and Bob Wyman was characterizing flight motor pathways (Levine and Wyman 1973). But aside from Pak's work on phototransduction, there were no systematic efforts to apply electrophysiological analysis to a broad collection of mutants. What type of mutants would be most appropriate for such an approach?

2. A seminal paper on temperature-sensitive paralytic mutants

In September 1976 – the month we joined Seymour's lab – a paper by Siddiqi and Benzer appeared in *PNAS* that pointed the way (Siddiqi and Benzer 1976). They described the isolation and electrophysiological characterization of X-linked ts-paralytic mutants. David Suzuki gets the credit for being the one who first isolated such mutants (Suzuki *et al.* 1971). Obaid heard Suzuki talk about these mutants at the time that he was preparing to move from molecular biology to behaviour genetics and decided to pursue the analysis of these mutants when he joined Seymour's lab in 1972 (Siddiqi 1975). The PNAS paper with Seymour was the culmination of the three years he spent working on this project. Although several of Obaid's own associates including Shankar Kulkarni (Kulkarni and Padhye 1982) and Satpal Singh (Singh and Siddiqi 1981) would also go on to work on ts-paralytic mutants, Obaid's own research shifted to studies of olfaction (Ayyub *et al.* 1990), leaving ample ground still to be explored in studies of ts-paralytic mutants.

We read and re-read this paper. Several general conclusions that could be drawn from the work were particularly noteworthy. First, their results suggested that the total number of genes in the *Drosophila* genome capable of being mutated to produce a ts-paralytic phenotype was a target of reasonable size. In addition to more alleles of *shi* (*shibire*) and *para* (*paralytic*) previously identified by Suzuki on the X chromosome, Obaid and Seymour discovered *comt* (*comatose*), a new X-linked ts-paralytic locus. Thus, assuming a random distribution of such genes among the chromosomes of the *Drosophila* genome, one could argue that minimally the autosomes should harbour another 12 such genes. Second, because they had obtained multiple alleles of *para*, *shi* and *comt*, it appeared unlikely that many more such genes on the X chromosome remained to be found. Third, by screening at a higher restrictive temperature (37°C instead of 29°C), Obaid and Seymour found mutations of the same genes as Suzuki but at a frequency that was orders of magnitude higher. Suzuki screened about 250,000 mutagenized X chromosomes to find the first allele of *para*. In contrast, Obaid and Seymour obtained ts-paralytic mutations at a frequency of about one in a thousand mutagenized chromosomes. This result was especially important because it suggested that it would be feasible to embark on subsequent screens for autosomal ts-paralytic mutants. Finally, as a true pioneer, Obaid, who was trained as a molecular biologist, carried out one of the first systematic electrophysiological analyses of a set of mutants in *Drosophila* by recording from adult flight muscles to assay electrical activity in the flight motor pathway. He thus demonstrated that each of the three paralytic mutants had a distinct electrophysiological defect, 'indicating that mutants of this kind will indeed be a rich source for neurophysiology' (Siddiqi and Benzer 1976). Earlier in 1976, Ikeda published a paper in *Nature* on the electrophysiological analysis of *shi* in a leg muscle preparation where he demonstrated a block in synaptic transmission at the neuromuscular junction (Ikeda *et al.* 1976) in complete accord with Obaid's results with the flight muscle preparation.

3. Following in Siddiqi's footsteps

These studies were extremely encouraging and provided us with strong incentive to believe that the isolation and analysis of additional ts-paralytic mutants would be of considerable value and that conducting a screen of the autosomes would be very worthwhile. Indeed, just prior to our arrival in Seymour's lab, Lily and Yuh Nung Jan had initiated such a screen. As a newly minted PhD in *Drosophila* genetics, BG was delighted to take charge of this screen when the opportunity arose. Further impetus was provided by another extremely important development made by the Jans shortly before our arrival. Working with *Drosophila* larvae, a preparation on which Obaid had also made some early forays (figure 1), they found that the neuromuscular junction (NMJ) of body wall muscles was an excellent system for electrophysiological studies (Jan and Jan 1976). It was fairly straightforward to record intracellularly from the large, easily accessible muscles while stimulating the long nerve bundle containing the motor axons with a fine suction electrode. This preparation enabled many different aspects of the motor pathway including nerve excitation, synaptic transmission, and muscle response to be examined and was thus ideal for the analysis of a mutant collection. The power of this system was demonstrated when Lily and Yuh Nung discovered that *Shaker* mutants caused

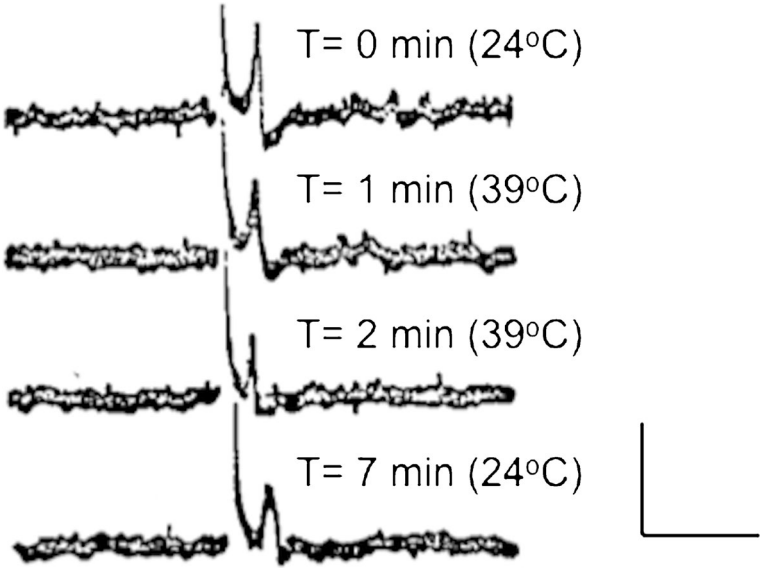

Figure 1. Examples of Obaid Siddiqi's extracellular recordings of compound action potentials from larval nerve bundles. These recordings are from wild-type larvae. Scale: 400 μV; 2 ms. Adapted from Siddiqi (1975) Figure 7.

abnormally prolonged release of neurotransmitter at the NMJ, owing to failure of the presynaptic terminal to repolarize properly, a phenotype that led them to correctly infer that *Shaker* mutants encoded defective potassium channels (Jan *et al.* 1977).

All the pieces were now in place. We wanted to use this new, powerful larval NMJ preparation and duplicate what Obaid had accomplished with studies of ts-paralytics in the adult preparation but with better resolution. Although he was not present, Obaid's previous example and success were constant reminders of what we should hope to achieve and were always stimulating us to strive to attain similar accomplishment. Fortunately, our work progressed well and it did not take long before we had 5 or 6 good paralytic mutants, most of which we have continued to work on. From the early studies on *shi* and *comt*, it was clear that not all paralytic mutants are paralysed in the same way. The mutants differed in the precise nature of their locomotor defect – some were completely immobile at the bottom of the vial, others had seizures, others become progressively more debilitated over time, etc. They also differed in kinetics of onset of paralysis and kinetics of recovery. *para* passed out quickly and recovered quickly, whereas *shi* passed out quickly but recovered slowly.

4. A mutant with impaired action potentials

The mutant we chose to work on first was *nap* (*no action potential*), which was interesting for several reasons (Wu *et al.* 1978). At 37°C *nap* homozygotes became completely paralysed within seconds and recovered equally quickly when returned to room temperature regardless of the length of time they were exposed to the restrictive temperature. In addition, larvae as well as adults exhibited strong paralysis at elevated temperature. These features made *nap* an ideal candidate for electrophysiological analysis. We quickly discovered that although the excitatory junctional potential (EJP) at the larval NMJ appeared normal in *nap* larvae at 20°C, it was completely lost at elevated temperatures. In principle, loss of the EJP could have been due to loss of the propagating action potential, a defect at the presynaptic terminal, or a failure in muscle response. In collaboration with Lily and Yuh-Nung Jan, we were able to demonstrate that release of neurotransmitter at the presynaptic terminal and the postsynaptic responses were normal even at restrictive temperatures. Thus, we inferred that *nap* caused a block in the propagation of action potentials in larval nerves.

Seymour became pretty interested in *nap* and was eager to join in on the fun of doing the electrophysiological analysis. The only problem was that Seymour was a night owl (unlike us) and we often did experiments with him after dinner until 2 or 3 in the morning. He was enjoying playing around with larval neuromuscular junction electrophysiology. Our data strongly suggested a block in axonal conduction but we still lack direct evidence from actual nerve recordings. CFW made a few initial attempts to obtain extracellular recordings from larval nerves that were unsuccessful. Seymour insisted that we keep trying and emphasized that 'Somehow, Obaid has done so. You know, he was trained as a molecular biologist. In general, they are not expected to be technically sophisticated'. The implied challenge was that CFW, trained as an electrophysiologist, should be able to do at least as well, if not better.

CFW continued to work on the design of the suction electrodes used for nerve recording, and late one evening, we succeeded in obtaining good recordings of the compound action potential. We were then able to show that as predicted, conduction of action potentials failed in *nap* at the restrictive temperature (Wu *et al.* 1978). Once again, Obaid had served as a model and as a stimulus to push our experiments forward.

It was rather exciting that the first ts-paralytic mutant we characterized had a defect in the propagation of action potentials. That seemed like the best phenotype we could ever hope to find among the mutant collection. We anticipated that it could provide a tool that would enable investigators to block neural signalling in specific parts of the nervous system as a way to dissect circuits underlying specific behaviours and that molecular identification of the gene could lead us to key proteins required to generate action potentials. Our colleagues were quick to point out that there were many possible ways in which conduction of action potentials could be impaired and some would describe our approach as 'shooting in the dark'. However, from the beginning, we were focused on voltage-gated sodium channels as being the most likely target and the most consistent with other aspects of the mutant phenotype, including the rapidity of onset and recovery for paralysis and loss of action potentials, the sharp temperature threshold for paralysis and nerve conduction block, the all-or-nothing failure of action potentials, and the absence of a cumulative effect on recovery following longer exposures to the restrictive temperature. We were rather confident in asserting that *nap* was somehow affecting sodium channels – in retrospect, perhaps more confident than the data warranted at that time.

5. *nap*, *para* and sodium channels

If *nap* did affect sodium channels, we wondered whether it might be possible to identify other ts-paralytic mutants among the collection that also affected sodium channels on the basis of phenotypic similarity with *nap*. Our attention was immediately drawn not to the other autosomal paralytics we had isolated, which did not resemble *nap* at all, but to one of the X-linked paralytic that Obaid had worked on – *para*. In fact, other than their location on different chromosomes and the lower restrictive temperature for most mutant *para* alleles (especially $para^{ts1}$), *nap* and *para* were almost indistinguishable with respect to their behavioural phenotypes. Moreover, Obaid had previously concluded from his electrophysiological analysis of *para* in the flight motor pathway that it was likely to cause a conduction failure.

Lacking the tools for positional cloning in those days, we sought to investigate the relationship between *nap* and *para* further by examining their interaction in double mutants. We were surprised and pleased to discover that the double mutant was nonviable at all temperatures (Wu *et al.* 1978; Wu and Ganetzky 1980). There were many implications to this observation, the most important of which was that both mutants were impairing the same pathway and the combined deficit was worse than either of the individual mutants. We concluded that both mutants were affecting sodium channels in some way with a conditional block in active conduction in either single mutant and unconditional failure in the double mutant.

At the time, we were unaware that Obaid himself had recently done more larval nerve recordings on various *para* alleles (as well as *comt* and *shi*) and had also concluded from these studies that *para* caused a conduction block. We learned of these data when Seymour sent Obaid an early version of our *nap* manuscript seeking his comments. He replied with a number of helpful comments but also included a beautiful, hand-drawn figure summarizing his recent data (figure 2) and pointing out the similarity between *nap* and *para*. We went on to do more electrophysiological analysis of *para* ourselves using our improved suction electrodes for nerve recording and found, in agreement with Obaid, that the compound action potential failed when temperature was raised, although the critical temperature was higher than required for adult paralysis.

In any case, we were sufficiently convinced of our conclusion that *para* and *nap* were affecting sodium channels to devote subsequent efforts to figuring out how to clone these genes with the techniques available at the time. It took another 10 years but we succeeded in cloning *para* (Loughney *et al.* 1989) and then *nap* (Kernan *et al.* 1991). *para* turned out to be the structural gene for voltage-activated sodium channels – the first invertebrate sodium channel to be cloned, and *nap* turned out to be an RNA helicase, required for the proper editing and splicing of the *para* transcript (Reenan *et al.* 2000). It was extremely gratifying to obtain molecular validation of all the inferences we had made many years before based solely on genetic and phenotypic analysis. In a broader sense, these results were a validation of Obaid's inspired pursuit of ts-paralytic mutants because of their potential for dissecting the molecular machinery required for neural signalling. Similar molecular validation was obtained for the other paralytic mutants Obaid studied: *shi* was found to encode dynamin (van der Bliek and Meyerowitz 1991; Chen *et al.* 1991) and *comt* was found to encode NSF (N-ethylmalemide-sensitive fusion protein) (Pallanck *et al.* 1995). Both mutants have subsequently been widely used in *Drosophila* to block specific steps in synaptic vesicle exocytosis and endocytosis and have provided many seminal insights into the molecular mechanisms of synaptic transmission (cf. Kim and Wu 1990; Masur *et al.* 1990; Sanyal and Krishnan 2012).

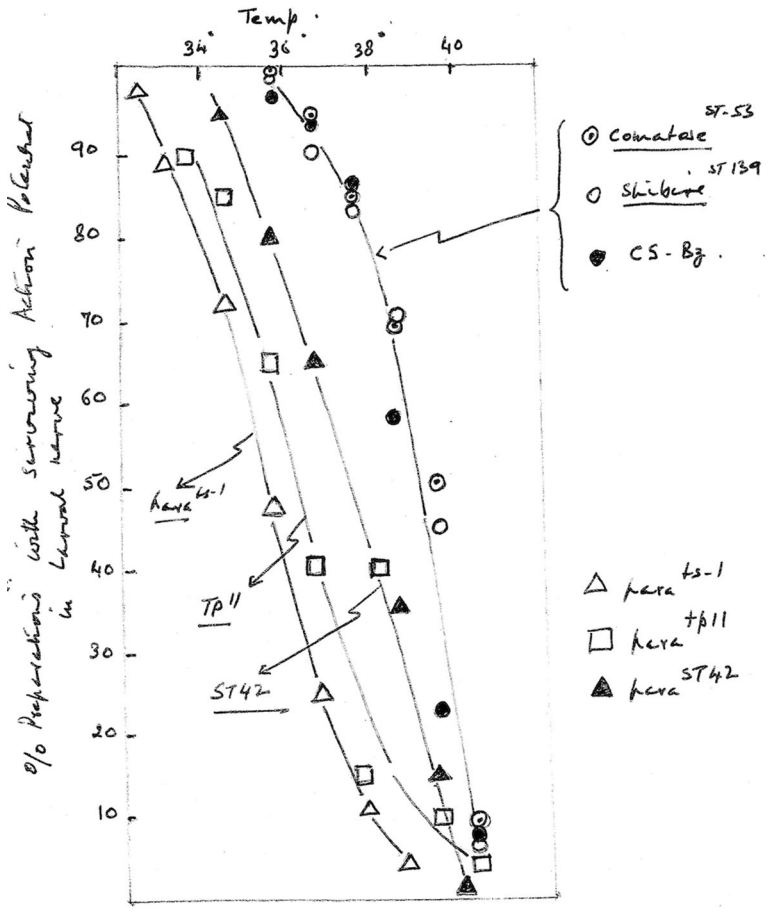

Figure 2. Hand-drawn figure in a letter from Obaid Siddiqi to Seymour Benzer, dated February 14 1978, with comments on an early draft of the *nap* manuscript sent to Obaid for his evaluation. The figure summarizes his results on extracellular recordings of compound action potentials in larval nerve bundles in various ts-paralytic mutants as a function of temperature. The X-axis is labelled, '% preparations with surviving action potential in larval nerve'. The genotypes examined were $para^{ts1}$, $para^{TP11}$, $para^{ST42}$, $comt^{ST53}$, shi^{ST139} and *CS*. The note on the bottom reads, 'Based on N=30 preparations for each strain'.

6. Some personal reminiscences

6.1 *'The Siddiqi paradox'*

During those days when our recordings of compound action potentials from larval nerves seemed to be in good agreement with our working hypothesis that *nap* and *para* caused a conduction failure at high temperatures, we encountered a very perplexing result. When we did a simultaneous nerve recording in *para* larvae along with an intracellular recording of the EJP from the postsynaptic muscle, we observed a failure of the compound action potential while we were still able to evoke an EJP by stimulating the nerve. How was that possible? We began referring to this curious puzzle as the 'Siddiqi paradox'. In fact, Obaid had made similar observations in his previous work. For example, he reported that even though spontaneous leg movements in *para* mutants cease at elevated temperature, cervical stimulation could evoke leg jerks. Similarly, Kaplan and colleagues had reported that at elevated temperatures, a light flash still triggered a leaping response in apparently paralysed *Hk* (*hyperkinetic*) *para* double mutants (Williamson *et al.* 1974). Siddiqi and Benzer concluded, 'at least some neural and neuromuscular pathways are intact in *para* at high temperature' (Siddiqi and Benzer 1976). We reached a similar conclusion and argued that the likeliest explanation of our 'Siddiqi paradox' was that although the full compound action potential appeared to fail at elevated temperatures, there were probably individual motor axons that were more resistant and continued to propagate action potentials under those conditions. As in other nervous systems, neurons with larger diameter axons, such as the larval motor neurons and the adult giant fiber interneuron (the giant axon that mediates

the jump-and-flight escape reflex), not only propagate action potentials more rapidly but also have a larger safety margin, enabling them to continue to fire under conditions in which impaired sodium conductance causes smaller axons to fail. Eventually, when our nerve recordings became even more refined, we were able to distinguish the activity in single motor axons and confirm this interpretation.

6.2 *BG*

I first met Obaid in person when he visited Seymour's lab toward the end of our tenure there. But he came to visit Seymour, not us, and I have little recollection of that meeting other than my impression of him as soft spoken, unassuming and kind. Because of the influence Obaid exerted on my career even from a distance, I felt as if I had known him for a long time even though we only interacted in person on some occasions. Whenever I did encounter Obaid at meetings such as the Cold Spring Harbor meeting on Drosophila Neurobiology, my earlier impressions were always reinforced – always modest, encouraging, generous and genuinely enthusiastic about any new results I described to him. We shared the bond of being Benzer alumni and working in what was then still a small field, and it felt like family.

Certain encounters with Obaid stand out vividly in my memory. In one instance, I was visiting the University of Cambridge at the same time that Obaid also happened to be there. We met for lunch and he took me to the Eagle Pub, where, according to legend, Crick had dashed in claiming to have discovered the secret of life the morning he and Watson cracked the double helix. I do not recall what I had for lunch, but I do remember having a great time with Obaid. The best memories are from the conference at the Tata Institute in 1993 organized in honour of Obaid's 60th birthday. Several other former Benzer postdocs, including Mark Tanouye and Alberto Ferrus, also attended the conference, and it was a most enjoyable meeting, reconnecting with old friends and interacting with all the talented and enthusiastic faculty and students at the Institute. Seeing firsthand what Obaid had helped to create left a lasting impression. Obaid hosted a wonderful dinner for a group of us at a lovely restaurant, after which we all returned to his apartment for more camaraderie. It was all a truly wonderful experience, and I felt a twinge of sadness when the conference ended, knowing that it would be a once in a lifetime event – as indeed it turned out to be.

6.3 *CFW*

One enduring impression of Obaid was his style and approach to science. I can recall him telling me once with some regret that, 'Nowadays, the young guys don't know how to play and don't have enough time and freedom to play'. That was typical of his love of exploration and his willingness to take risks versus the 'safe science' that funding agencies now prefer. Although this remark is very pertinent today, in fact, Obaid made this remark back in the late 1990s during a weekend excursion to New York from a summer 'workshop' in Cold Spring Harbor. In 1998 he managed to convince Jim Watson, then the direct of the Cold Spring Harbor Laboratory, to sponsor a small summer workshop that would bring together a small group of experts just to explore the electrophysiological and optical imaging techniques for probing functions in the *Drosophila* nervous system to gain insights into different behavioural phenotypes. He recruited Yi Zhong, who had recently set up a new group at the Beckman Center of the Cold Spring Harbor Laboratory, to host this summer activity. It was like summer camp for a few PIs who got to spend time playing in the lab themselves and follow up some new ideas. Obaid would go to the various subgroups just to try out different sorts of mutants and to satisfy his curiosity about the basic principles and applications of novel approaches.

Obaid's mild manner could be somewhat deceiving in giving the impression of a laidback attitude. In fact, he was extremely persistent in pursuing his scientific goals, not for the recognition or the number of publications, but for satisfying his own intellectual curiosity. This can be seen in his published work, often with long intervals between papers. However, in many instances he sent me 'preprints' on various aspects of his olfaction studies. These are carefully prepared, beautifully presented, lengthy papers, but he never published most of them.

I invited Obaid to visit Iowa City in 2006. We had long discussions about his views on the development of science in India and in the Third World. Obaid's quiet but deliberate and determined approach and his farsighted focus on building institutions rather than just padding his CV with many publications were instrumental in training new generations of outstanding scientists in India and the culture of excellence he helped establish at the Tata Institute and subsequently at the National Centre for Biological Sciences (NCBS).

My last visit with Obaid was at the symposium in Bangalore in celebration of his 80th birthday. I felt very honoured to be invited, together with Satpal Singh, to Obaid's home for afternoon tea with his family. I was amazed that his wife, Asiya, remembered our previous discussion from my first visit to the Tata Institute two decades earlier about why Buddhism originated in India but did not persist there and was later replaced by Hinduism and Islam. Since I was raised in Buddhist culture, this is a topic that fascinates me. Asiya explained to me her theories in our first discussion and two decades later described her further thoughts on the subject. She also gave me a copy of one of her papers on a related socio-historical subject. Obviously, Obaid's entire family was highly intellectual and cultivated

with keen interest in a variety of subjects. It is not surprising that their offspring have been extremely talented and successful in their own right.

7. Closing thoughts

It is said that good teachers teach and great teachers inspire. With the benefit of hindsight we can appreciate even more fully now than we could at the time how fortunate we were to have Obaid Siddiqi as an inspiring mentor even in absentia. The example he set in his approach to science, his devotion to pursuing where his curiosity led, his willingness to take chances and even to fail occasionally, his spirit of kindness, friendship, and generosity, his quiet pursuit of excellence, his steadfast determination to create outstanding opportunity for new generations of scientists, and above all his humanity are worthy of respect and emulation. Let us hope that Obaid Siddiqi will continue to serve as an inspiration for many new generations of students.

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