I. A. Niazi (1928–2022)

Iqbal Ahmed Niazi, who passed away on 24 February 2022, made an extraordinary finding bearing on multicellular development, particularly on the issue of how embryonic tissue spontaneously acquires form and structure. A handful of discoveries made in the 20th century qualify for a similar description. Two of them originated from Indian laboratories; the first, which was Niazi's, paved the way for the second. Both involved the curious phenomenon of regeneration, which mimics embryonic development. At the time he made it, differentiation-inducing chemicals, termed morphogens, had long been hypothesized to give rise to the spatial patterns that characterize multicellular development. By virtue of being present at varying concentrations within a tissue, morphogens were considered to cause initially identical cells to acquire distinct fates; in other words, to play a determining role in patterning the embryo. Morphogens had been long searched for, but had proven elusive. The crucial clue regarding the first morphogen identified in a multicellular organism came from Niazi's laboratory. It turned out to be a retinoid, generally retinoic acid and retinol palmitate, both of them derivatives of vitamin A (vitamin A is the generic term for several functionally equivalent retinoic acid derivatives. Distinctions between them are meaningful, because species vary with regard to which derivative is functional; we use the terms interchangeably). The observations, of how amphibian limb regeneration proceeds in the presence of vitamin A, changed developmental biology. The rest of this account outlines the discovery, describes how Niazi almost lost the credit for making it, and ends with a short sketch of his life.

At the time, Niazi (Figure 1) was studying regeneration in tadpoles from which a limb had been amputated at various levels and various stages of development. Regeneration involves the replacement of a damaged tissue or lost body part with a freshly minted replica. It is ubiquitous in plants, common in 'lower' animals and sporadic in most animal phyla, but rare in present-day vertebrates[1]. Going by fossil evidence, it may have been common in ancient tetrapods[2]. The phenomenon has excited common wonder as well as scientific attention, much of it fuelled by the hope that one day it might be made to work in humans. That has been impossible to achieve so far (apart from the normal turnover of tissues that goes on throughout life, wound healing with scar tissue and genuine replacement without scarring in fingertips, the liver and endometrium, to which one should add 3D printing of organs today). Unlike urodeles (newts and salamanders, which retain their tailed larval forms throughout life), mature anurans (frogs, which metamorphose from tailed larvae into tailless adults) cannot normally regenerate their limbs, though rare instances are documented[3]. Anuran tadpoles can regenerate limbs, at least for a short time after they develop, but as metamorphosis draws near, they can no longer do so. Niazi wanted to see whether, by prolonging the onset of metamorphosis by applying vitamin A, it might be possible to also maintain the ability to regenerate for longer than usual[4].

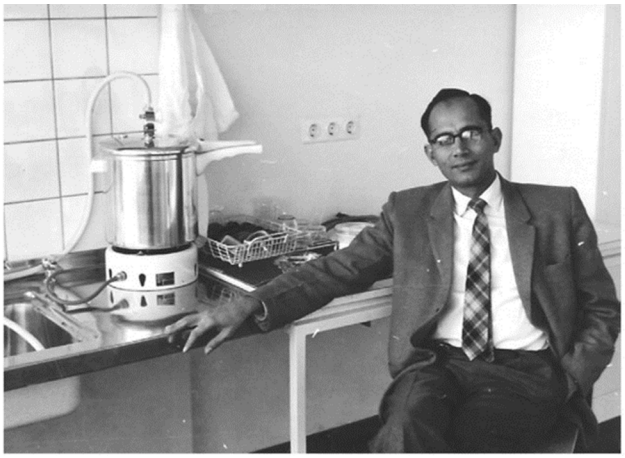

Figure 1. Niazi in his laboratory at the University of Rajasthan, Jaipur (1965).

It was known that an excess of vitamin A had a teratogenic effect. For instance, in rats, it caused abnormal embryonic differentiation that is recognized as a birth defect in humans[5]. The first step of regeneration in frog tadpole limbs is the reversion of cells in the neighbourhood of the wound to an undifferentiated state (the formation of a blastema), followed by growth and re-differentiation, reminiscent of what happens routinely during embryogenesis. Therefore, it seemed a reasonable hypothesis that pattern re-specification during limb regeneration and ab initio pattern formation during embryonic development shared similar mechanisms. Together with his students Saroj Saxena and Om Prakash Jangir, Niazi decided to explore the consequence of adding vitamin A to the regeneration medium. To their surprise, two blastemas appeared on the same stump, instead of one; and each mass went on to form a limb. In other words, a duplicated structure formed on the same stump.

In due course, two dramatic outcomes were reported in a series of publications[6]. When the level of retinoic acid in the culture bath was sufficiently high, the two blastemas formed a pair of bilaterally symmetric limbs; and the duplicate limb was a mirror image of the normal limb (occasionally a triplicate limb had also developed). The experimental treatment appeared to have made the pattern-forming mechanism indifferent to the distinction between left and right, reminiscent of the original state (the frog egg is cylindrically symmetric before it is fertilized). That was not all. Until then, it had been thought that the polarity of the regenerate had to be the same as that of the remaining portion of the limb. Thus, regenerated elements were arranged in the normal sense, proximal-to-distal with respect to the main body. For example, if an arm was cut at the level of the elbow, the new outgrowth contained only the forearm and digits in that order, never the upper arm structures. Known as the 'law of distal transformation', it had been rationalized as the expression of an inherent vectorial property ('gradient') of the growing or regenerating limbs. To their surprise, it turned out that high levels of vitamin A also caused regenerating limbs to flout the law of distal transformation: sometimes, the newly formed limb included proximal elements. Both observations had

the element of novelty and surprise that is the hallmark of fundamental discoveries.

The short-term aftermath was interesting. Niazi had sent a paper containing the exciting findings to the *Journal of Embryology and Experimental Morphology* (now *Development*), one of the premier journals in the field. It was rejected. Wanting to get the results out quickly, he sent it next to *Folia Biologica* (*Krakow*), and a second paper to the *Indian Journal of Experimental Biology*. Outside a restricted circle of zoologists, largely in the respective countries, neither publication was likely to come to the notice of the general community of biologists. Consequently, at first the dramatic effects of vitamin A on regenerating limbs had little impact. It is not that the content of those two journals was entirely inaccessible, but one had to search hard. In a personal reminiscence of the history of retinoids in limb regeneration, Maden[7] states that he came to know of them only because he carried out a meticulous literature survey.

It may not be wrong to state that it took ten years for the community of biologists to realize that retinoids could have a central role in multicellular development. It happened when Thaller and Eichele[8] showed an endogenous posterior-to-anterior gradient of retinoic acid in the chick limb bud. Why did they try retinoic acid? The publication cites Niazi and Saxena (1978)[6] along with the words 'RA and some of its congeners have dramatic effects on the pattern of regenerating amphibian limb blastemas'. But there is no indication of the nature of those effects, nor is it stated that the motivation came from there. They also cite four publications dealing with the effect of retinoic acid on inducing pattern duplications, but none of them is to Niazi's work. The earliest of the four is a study by Tickle *et al.*[9] which reported that focally applied all-trans retinoic acid could make the chick limb develop a mirror-imaged pattern of digits. Tickle *et al.*[9] had taken off from a well-known discovery by Saunders and Gasseling[10]: reminiscent of the classical embryonic organizer, a zone within the posterior margin of the developing chick limb bud possessed 'polarizing activity'. This activity could be demonstrated by transplanting a mass of cells from the posterior zone to the anterior margin of another limb bud. When that was done, two sets of digits developed; in addition to the usual set, a second mirror-imaged set was induced. And why did Tickle *et al.*[9] try retinoic acid? Because, they said, a colleague who had known of the effects of retinoic acid on cell communication and cell differentiation, had suggested it. Niazi's work was not cited there either. The fact that he had worked on amphibian regeneration, while their object of study was chick development, may have also been behind the omission. Maden[11] confirmed the findings of Niazi and co-workers by observing the effect of limb regeneration in the axolotl (a urodele). Much later, Maden and Okada made efforts to set the record straight[12], and by then, Niazi's centrality to the field was established[13]. Niazi[14] himself provided a comprehensive view of the observations made by his group in a retrospective. One hopes that the significance of his achievement will be properly acknowledged in the future.

Iqbal Ahmed Niazi was born on 22 August 1926 (officially in 1928) in Amroha, Uttar Pradesh, India. He was one of four siblings. As a child he is said to have been obsessively tidy, a trait that persisted into adulthood. After 1934, when his father lost his job, he lived in Amroha with his maternal grandfather, who got him enrolled in a school right away in the sixth class. After four years he was shifted to a high school, from where he joined Christ Church College in Kanpur to study physics, chemistry and mathematics. Wanting to enter medicine, Niazi found that he was deficient in biology. To make up for lost time, he joined an intermediate college for two years before enrolling in the B.Sc. programme at Aligarh Muslim University with zoology, botany and chemistry as electives. Having admitted him, the University discovered that he did not have the required background in botany, but agreed to let him stay on if he managed to go through the prerequisites on his own and, within one month, pass a special test. But the professor who was supposed to test him forgot and Niazi stayed on. Lectures on the comparative anatomy of vertebrates, which were framed around the theme of an evolutionary process, fascinated him. That made him decide to stay on after graduation in 1946 to do a Master's in zoology. Much of his time was spent on political activity; by then he had become an active member of the Communist Party. India became independent in 1947, but his engagement in politics continued, and he had to go underground during an all-India railway strike. All this meant that he could not complete the Master's course as planned. Not knowing what to do, at the end of 1950 he thought of studying law. He became disappointed with it soon and went back to Aligarh where Professor Babar Mirza, then Head of the Zoology Department, agreed to give him a seat to study and take his M.Sc. finals in four months. He had no job after that, and he survived for two years on a temporary position. Finally, a lectureship in DAV College, Kanpur opened up, and he taught zoology there from 1953 to 1957. His mind was on doing research, especially on a problem that had to do with changes in animal form. A visit to a Canadian exhibition that mentioned zoological work in universities there made him try for a Ph.D. position. He chose McGill University, and after some adventures, landed there and began his research career in September 1957.

Niazi's initial task was to study of the population dynamics of competition between two species of the flour beetle *Tribolium*. It hit a roadblock when, after two years, his professor informed him that though the initial findings were interesting, they had to be interpreted mathematically.

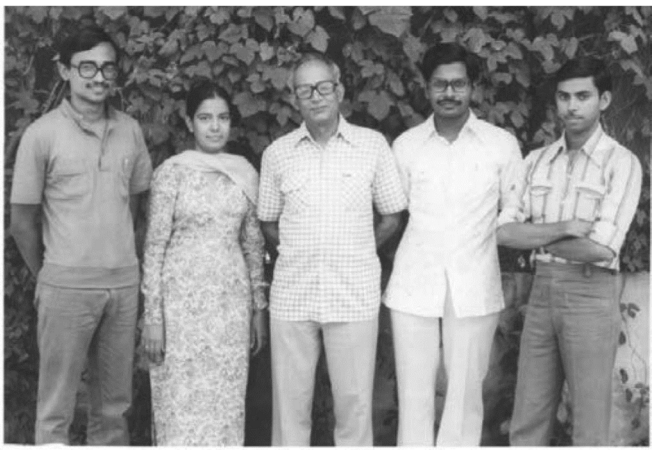

Figure 2. Niazi with his students (1983). L to R: Sagar Mal Lahiri, Shaheen Alam, Iqbal Ahmed Niazi, Charles Ratnasamy and Krishan Kumar Sharma.

Box 1.

In the second semester of my Master's degree programme, in February 1976, at the Department of Zoology, University of Rajasthan, Jaipur, I came in contact with the great teacher that Prof. I. A. Niazi was. His presentation skills were such that no one ever missed his class. Always smiling, he used to enliven his teaching with stories. I recall one involving the famous Theodor Boveri, who used to collect eggs from the Marine Zoological Station at Naples, Italy, and bring them to the laboratory while riding on a horse; most of the time the eggs were damaged, but using those that survived, Boveri solved fundamental questions of development. His manner of teaching used to hypnotize us. I learnt to do many classical embryological experiments with the tools that he had designed.

It was a turning point in my career when Niazi agreed to take me on as a Research Fellow in his laboratory in 1977; he was looking for a good student to confirm and extend the studies on retinoids and regeneration. I started working on early post-amputational developmental changes under the influence of vitamin A on the spadefoot frog, *Rana breviceps*. The laboratory was my sleeping place, and a 3×7 foot wooden table my bed. A huge collection of books and literature was around. Hundreds of experiments were carried out to verify that whole-limb regeneration at all the three levels (thigh, shank and ankle) takes place in frog and toad tadpoles. In view of my national merit, soon I got direct JRF of CSIR. More parameters were added to the existing research plan and a synopsis was submitted for my doctoral research to the University of Rajasthan. Prof. Niazi was also interested in other developmental problems dealing with the eye (Sultana Niazi, Pratibha Chaudhary), the thyroid (Saroj Saxena, Pramila Gupta) and the gonads (K. L. Bohra). Four of us belonged to the vitamin A and regeneration group (Saroj Saxena, Om Prakash Jangir, K. K. Sharma, Shaheen Alam). Later I joined the faculty myself, shared the developmental biology laboratory with Prof. Niazi, and continued our research collaboration until he retired in 1988. He used to inspire younger members of the group to attend and organize training programmes, seminars and conferences. In 1985, I was part of a training programme at the Hubrecht Laboratory in Utrecht, The Netherlands. J. Faber, the Director, arranged a bicycle for my use with the comment 'your doctor–father Dr Niazi used the same bicycle when he was a trainee at this Institute'. Discussions with Prof. Niazi, irrespective of whether they concerned religion, politics or social relations, were invariably frank and open.

K. K. Sharma

Box 2.

When I returned to India in 1975 after completing my Ph.D. in a leading amphibian laboratory in the US, I decided to work on the amphibians of India. While setting up a laboratory at Utkal University, I discovered a variety of frogs and toads on the campus. With my previous training, I had no trouble in raising any of them up to metamorphosis. As far as I knew then, Dr Niazi and his group in Rajasthan were the only others in the country working on amphibian development (I have been informed that Leela Mulherkar used embryos of *Microhyla ornata* and *Bufo melanostictus*, mostly in teaching and occasionally for research, but have no idea about publications). The Indian Society for Developmental Biology (ISDB) was established during the Indian Science Congress in 1976 in Bhubaneswar. The moving force behind it was the first Secretary, S. K. Goel of Pune University; P. N. Srivastava was chosen to be the President. Prof. Niazi was well known for his pioneering work on regeneration in tadpoles under the influence of vitamin A. We met during a conference in Jaipur in the late 1970s. He took me to his laboratory and discussed the work they were doing. The first time I saw a dissecting microscope with photographic attachment was in his laboratory. He lamented that Rajasthan being a desert state, had very few species of amphibians and also a short breeding season, restricting his research. He was happy that we had several species which was an advantage. In the late 1980s, ISDB held another meeting in Bhubaneswar. Niazi was the President and attended the meeting which was critical for us. My colleague S. K. Dutta presented a consolidated paper on regeneration of tails in anuran tadpoles under the influence of vitamin A. Among other findings, he showed that in some species, in addition to promoting regeneration of the tail, it caused a limb to form in place of the tail, i.e. regeneration occurred along with a homeotic transformation. I asked Dr Niazi if he had observed anything like that in his work. After staying silent for a while he replied 'Dr. Hejmadi, I have never seen this and I don't think anyone in the world has seen it'. On his insistence I sent the paper to *Nature* and learnt from the reviewers that this was the first-ever homeotic transformation in vertebrates. He was happy when the paper was accepted and was delighted that our discovery of homeotic transformation was covered on national television.

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That was something Niazi felt unable to do, and both of them decided that he must join another group. A chance encounter in the corridor with the legendary Norman Berrill helped Niazi find the advisor he was looking for. Berrill asked him to choose his problem and Niazi decided on the area of regeneration, which remained his focus thereafter. In his doctoral work, he made the significant finding that the spinal cord was essential for tail regeneration in the tadpole larva of lampreys (jawless primitive fishes), while the notochord (the cartilaginous precursor of the backbone) was not[15]. However, because it stretched the tail, the notochord had a mechanical influence on the shape of the regenerate, and simultaneously on the distribution of cell types in it. The work foreshadowed a major focus of contemporary research, namely the role of mechanical forces, and more generally, geometry, in morphogenesis.

Next, Niazi spent a postdoctoral year in Toronto, Canada, where he learnt the technique of culturing chick embryos and studied regeneration in the chick limb bud. Meanwhile, a friend who had finished his Ph.D.

in physics at McGill University wrote to him about available positions in a new university in Rajasthan. The Vice-Chancellor of Rajasthan University happened to be in Toronto for an international meeting on education; Niazi met him and was told he could join as Lecturer, which he did in 1962. He lived in Jodhpur, where the University Departments were temporarily located, moved to Jaipur in 1963, and remained there until he retired as Professor in 1988. He was cherished there as a teacher and mentor (Figure 2; Box 1). In 1963, he married Sultana Salaruddin, a fellow developmental biologist, who later also became a colleague in Jaipur.

Niazi's research ranged over several aspects of the biology of amphibian regeneration. Over the years, in addition to the striking findings that have already been described, other effects of vitamin A treatment were discovered. Some of them remain to be explained. For example, vitamin A normally enhances regeneration and occasionally delays normal development[16,17]; makes the regeneration blastema completely equivalent to the original limb bud[18]; and in urodeles, its ability to cause duplications varies in a stage-specific fashion[19]. A nagging puzzle, already referred to, is that a vitamin A variant that has a striking effect on one species may not affect another. Indeed, retinoic acid *inhibits* regeneration in the popular laboratory organism *Xenopus laevis*[20]. As it took a while for this to be recognized, some people doubted the claim of pattern duplication initially. Well after he had retired, Niazi had a role to play in connection with the second extraordinary finding on regeneration to come from India (Box 2). Both discoveries were outcomes of curiosity-driven research carried out by individuals working in an unfashionable area, with relatively small setups and modest funding.

At various times Niazi held visiting professorships in the Universities of Mosul, Montreal and Illinois. He was one of the founders of the Indian Society of Developmental Biologists in 1982 (later he was to receive their Swami Pranavanand Science Award) and served on the board of the International Society for Developmental Biology from 1986 to 1989. He was elected to the fellowships of the National Academy of Sciences, Prayagraj and the Indian Academy of Sciences, Bengaluru. He represented India at the inauguration of the National Institute of Developmental Biology in Beijing, China, in 1986. The recognition that made him most happy came in 2004, when at the initiative of the well-known developmental biologist David Stocum, Indiana University, USA, awarded him a medal for lifetime contributions to developmental biology. Not long after retiring from the University of Rajasthan, Niazi moved to New Delhi and served as a regular examiner for the Indira Gandhi National Open University for some years. His daughter Zeenat describes him as a humanist, stoic, socialist and communist (though he was not politically active after his student days). Curiosity, science, logic and intuition were his characteristics. Despite the fact that he treated religion with irreverence, he accepted that some people had a need for it. Iqbal Ahmed Niazi is survived by his wife Sultana and three children, Zeba, Zeenat and Asad.

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