

## Remembering Uno Lindberg

Uno Lindberg, a biochemist whose excellence at purifying proteins carried him to the frontiers of modern cell biology, died on June 30, 2017, almost exactly 40 years to the day since we had begun our collaboration to investigate the structure and function of profilin, a small but remarkable protein he discovered in 1974. How we came to meet and establish a durable partnership that endured many setbacks, and a few triumphs, is a story that mirrors the many changes in the conduct of international scientific communication and joint discovery that have occurred during that time. Quite apart from our own joint efforts, Lindberg trained and worked for years with a small group of Swedish scientists who established important connections between the molecules on the outside of a cell that call forth for action and growth, the essence of life, and those interior structures, what he referred to as the microfilament system, that create movement and force in response to those signals.

It is often said that fortune favors the prepared mind, but I have never figured out how Lindberg consistently found new mother lodes in Nature's vast storehouse of secrets to pursue, nor did I discover how it was that he could consistently exploit the particular to illuminate the general case. Of course (a phrase that he hated), we talked about the scientific process often, and the closest he ever came to explaining his method was his frequent refrain, following one of my own flights of fantasy, "I am 'dumb' from Varmland, please explain more clearly what you mean". Before the invention of the Internet we would run up enormous international calling charges discussing our experiments each morning, and what I remember most fondly was his use of 'digging', by which he meant the close reading of the scientific literature, and 'chopping wood', dealing with the bureaucratic requirements of all of the thankless roles he took on in the academic life.

I have known many scientists. They all declare themselves to be hard workers. But Uno Lindberg really was a hard worker. He had these deep reserves of physical and emotional strength which he could draw upon in the midst of a scientific controversy, even those

seemingly conducted in slow motion at a laboratory bench far removed from one's scientific adversaries. I am reminded of a car trip between Uppsala and Stockholm where he explained the origins of the piles of massive rocks and stones deposited by glaciers and cleared from pastures by stout Swedes behind rope and horse centuries before. To 'I am dumb from Varmland' I would add 'and we work until we fall'.

In 1966 Lindberg reported the isolation of a potent inhibitor of DNase I, a cellular enzyme that breaks down DNA, setting the stage for a mechanistic understanding of a molecule that would eventually become a critical tool in biotechnology. Nearly a decade later, Elias Lazarides and Klaus Weber used fluorescent antibodies to reveal the incredible architectural diversity of actin-containing structures in eukaryotic cells. These two apparently unrelated discoveries were joined at a meeting at Cold Spring Harbor Laboratory in 1974 when Jim Watson wondered why DNase I inhibitor was one of the most abundant proteins in the cell. Perhaps it was actin? This put Lindberg and Lazarides on a trail that soon led to the discovery that actin itself was the inhibitor, raising questions about this mysterious role for non-muscle actin that is the subject of active investigation to this day.

Without crystals, no higher resolution structure could be obtained. The gold standard of protein purification is crystallization. Uno saw himself as carrying on the traditions of several generations of Swedish biochemists who developed techniques for this purpose, including Svedberg, the inventor of the ultracentrifuge, whose photograph occupied a place of honor in Uno's office. When Lindberg revealed that he had obtained beautiful crystals of DNase I inhibitor, now established as being actin, as far back as 1966, it meant that the major obstacle to crystallizing actin, namely inevitable polymerization into helical filaments whenever the ionic strength was increased, had been surmounted.

Why was DNase I inhibitor monomeric? Lindberg and his students quickly established in a tour de force of protein chemistry that DNase I inhibitor is a complex of actin bound to a small protein, profilin,

named in response to Tilney's suggestion that monomeric actin in cells could be a complex with auxiliary factors which he termed 'profilactin'.

Now that the inhibitor was known to be actin, one of the most conserved and abundant proteins, and a principal component of thin filaments in striated muscle fibers, Lindberg made a bold career-defining choice to lead the effort to use x-ray crystallography to obtain a three-dimensional structure of actin. Sarah Hitchcock, an expert on muscle proteins, catalyzed a collaboration with Hugh Huxley at the MRC-LMB, the world's leading expert on muscle structure, who then asked Richard Henderson to teach Lindberg's graduate student, Lars Carlsson, how to apply x-ray crystallography to obtain the structure.

'Uno will be so happy!' declared Lars-Erik Nystrom, a graduate student dispatched to the MRC Laboratory of Molecular Biology in Cambridge in order to obtain the amino acid sequence of profilin, when he heard that Henderson, had recruited me to the problem. At the time I had just completed my Ph.D. work at Harvard on the structure of tomato bushy stunt virus and was very interested in higher order structure. I had heard previously about 'Big Lars' and 'Little Lars', but I wasn't sure which one had discovered the unusual property of Lindberg's profilin:actin crystals that interested me (more about below). "I sure hope you're 'Big Lars'" I remarked as I looked across the table at a very large man with a bushy beard. "When Uno comes off his mountain, he will come immediately to England to meet you," he declared. I conjured up a mighty Viking, club in hand, shaking the land around him as he advanced, clothed in leather and fur, to the North Sea coast.

Lars Carlsson had carried out meticulous experiments on the crystals, establishing that they were exquisitely sensitive to even the smallest perturbations of the chemical and physical milieu. This sensitivity implied that the problem would be much harder to solve with the usual method of 'soaking' the crystals in heavy-metal containing solutions, which requires that the unit cell dimensions of the crystal remain unchanged by derivatization. It was precisely this feature of

the crystals that excited me. If the profilin:actin crystals diffracted x-rays to high resolution through a range of structural states it should be possible to investigate the fine biophysical events underlying the role of profilin in controlling the entry of actin monomers into helical filaments.

We eventually obtained structures for crystalline profilin:actin in 'open' and 'closed' states that showed how hinge and shear movements between the major domains of actin accounted for Carlsson's original observations. The solution to the problem of crystal instability required the application of both heavy-atom approaches and molecular replacement using the structure of actin in the DNase:actin crystals obtained by Wolfgang Kabsch and his collaborators in Heidelberg, whose work was motivated by Lindberg's pioneering discoveries showing the identity of the inhibitor to actin.

One of Uno's most endearing English phrases was 'in the nearness'. He used it often, in many different contexts, sometimes meaning 'in apposition to', as when he referred to the thick layer of actin containing structures just below the plasma membrane in eukaryotic cells, but also in the realm of ideas when he was probing his mind for an apt metaphor to bring a scientific insight to the surface. I think that it is fundamental to understanding how and why he took on the incredibly difficult task of using electron microscopy to bridge the gap between Lazarides' and Weber's visual microscopic observations on actin stress fibers with the physical structures formed by helical actin filaments. With Anna-Stina Hoglund and Roger Karlsson, young researchers in his Uppsala laboratory, Lindberg developed a delicate biochemical procedure for removing lipid bilayers ('the membrane') from eukaryotic cells so as to expose and preserve the 'cortical actin weave' underneath. This enabled them to use negative staining with metals to visualize and describe the organization and polarity of microspikes, filopodia, and meshworks that form the basis for cell movement and motility. In his colorful lectures, Uno would use his fingers, arms, legs, and flexed torso to imitate the movements he envisioned and to make his case that the 'plasma membrane', portrayed in textbooks as a powerful barrier to outside influences, was nothing more than 'a thin layer of oil' protecting the thick,

powerful, and highly dynamic layer of actin and its associated proteins. In today's parlance, Uno was channeling Bob Marley, whose music he listened to on his long car trips between Uppsala and Stockholm in his early days.

But the best was yet to follow. Cell and molecular biologists in the 1980's were making rapid progress in addressing the greatest of existential questions in the life of cell: how do 'signals', such as growth factors, hormones, and small molecules, on the outside of a cell bring about changes on the inside that lead to big events like cell division or the firing of a neuronal axon? In Lindberg's landscape, the problem of signal transduction became "what triggers 'pools' of profilin:actin complexes to form filaments, bundles, and meshworks?". Perhaps I should have, could have, might have known what to expect. After all, my golf partner at the Gog Magog Golf Club in Cambridge was Michael Berridge, who had discovered recently the phospho-inositol 'second messenger' system, wherein a component of the plasma membrane itself is enzymatically processed into PIP2, a potent internal cellular signal. But I didn't. Instead, I learned on one of my many visits to Stockholm to pick up crystals that Ingrid Lassing and Uno had established unequivocally that PIP2 triggers the separation of profilin from actin, freeing the latter to polymerize, and drive motility.

This was the first of many seminal discoveries by Lindberg and his small team of collaborators on the factors influencing the *in vivo* role of actin. Using selected-site mutagenesis, especially with Roger Karlsson, Uno investigated the fine molecular details of profilin-catalyzed polymerization, and the role of ATP hydrolysis. At the time of his debilitating accident, Lindberg was devising experiments to classify the functional roles of the wide diversity of tropomyosin isoforms in cells, sensing 'in the nearness' that this might reveal the fine-tuning of the balletic movements at the cell edge.

We faced storms of controversy and outright rejection of our ideas for the role of actin in skeletal muscle contraction, where it had long been assumed that thin filaments were merely passive ropes that summed the forces developed by the myosin motors projecting from

thick filaments. Our views sprung from the way actin molecules are organized in 'ribbons' in the profilin:actin crystals held together by bonds resembling those seen in other biological fibers such as sickle cell hemoglobin and simple viruses. Furthermore, actin is an ATPase and it is not unreasonable that it could hydrolyze ATP and generate local forces in response to the action of myosin. After several years of 'digging' and vigorous engagement with experts in the field, we did manage to publish several papers showing that we could explain the principal facts of muscle contraction, as well as account for some serious discrepancies arising from *in vitro* studies of the isolated components of muscle. We say 'the jury is still out', nearly everyone else says the 'court has long since adjourned'.

'Onward and upwards' Uno would always say as we ended our conversations. As I conclude this piece, and the longest chapter in my scientific life, I finally realize that it is Uno who has been and still is 'in the nearness'.

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