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Interview

Early work on apoptosis, an interview with Richard Lockshin

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RA Lockshin
Richard Lockshin was born in Ohio, USA in 1937. He was educated in Ohio and Massachusetts, and received his B.A., M.S., and Ph.D. from Harvard University. His doctoral thesis, 'Programmed Cell Death in an Insect,' became the basis of five papers published in 1964 and 1965. After spending his postdoctoral years at the University of Edinburgh with CH Waddington, he joined the faculty at the University of Rochester School of Medicine and Dentistry in Rochester, New York, where he spent 10 years before moving to St. John's University in 1975, where he was rapidly promoted to Professor and served 9 years as Chair of the Department of Biological Sciences. He was one of the founders of the Gordon Conferences on Cell Death and the International Cell Death Society. Among his approximately 150 publications are *Cell Death in Biology and Pathology*, edited with Ivor Bowen, 1981; *When Cells Die* (1998 and 2004); and *The Joy of Science* (2007). He has won several awards for both teaching and research. He lives happily with his wife and partner Zahra Zakeri, and is very proud of his two daughters Miriam and Nora.

Richard Lockshin was one of the first scientists working on apoptotic mechanisms. In particular, his early work was dedicated to the role of cell death in insect development, originating from his Ph.D. thesis in Harvard in 1963. But how did it all begin? What triggered his scientific interest in the field of cell death from his previous work? Now a large family of death-related proteins exists, with extremely promising clinical applications. Before the current era, the perception of homeostasis was 'Cells die, and they are replaced through mitosis,' reflecting the perception that death was an uninteresting, incidental, and stochastic event that was corrected by the organized correction of cell division. Today we realize that the emphasis should be on the first phrase, meaning that the death is organized and controlled. Here, *Cell Death and Differentiation* asks Rick, on the occasion of his 70th birthday, about the early work on apoptosis.

CDD: What was your scientific interest before working on cell death?

I once won a fellowship with an essay that began to answer the question, 'How did you become interested in science?' with the statement, 'All children are biologists. Some of us never outgrow it.' I don't recall not being interested in how things worked. Many years later, I joked that I planned to go to medical school to become a pathologist, because I thought that 'pathologist' was synonymous with 'medical researcher'. I am bemused by the thought that, although I later decided to get a Ph.D. rather than an M.D., my research ultimately ended up fitting well into the heart of pathology.

I was an undergraduate student who liked most things about biology, particularly development and physiology. I had, for many years, been very interested in insects, and, as I recall, my acceptance to college was, at least in part, based on an essay that I had written describing that interest. It also got me an introduction to the man who would be my future Ph.D. advisor, Carroll M Williams, and the opportunity to hang out in his laboratory originally as a dishwasher and laboratory technician during my undergraduate years. Williams was one of the world's experts on insect hormones, and he had brought the custom of afternoon tea back from a sabbatical in England. I, therefore, spent many afternoons listening in fascination to discussions about the mechanisms of insect metamorphosis.

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CDD: When did you first hear about apoptosis?

If you mean apoptosis *sensu strictu*, then my recollection is that sometime in the late 1960's and I paid a return visit to the Williams lab and he said, 'Rick, I'd like you to meet someone who is also interested in cell death.' My recollection is that it was John Kerr, who was returning from his sabbatical in England. However, John does not recall the incident. Perhaps it was another location, someone else from that group, or simply someone who had heard about the work. Cell death is another matter. There were a small number of us. There was Harold Fox in England and Rolf Weber in Switzerland, looking at tadpole metamorphosis, and Jan Ericsson in Sweden, looking at mammary gland involution, together with lots of people using prostate after castration as a model. Michael Locke in Canada did some elegant microscopy of metamorphosing insect tissues, and Ernst Gutmann in Czechoslovakia looked at the atrophy of hormone-dependent muscles. Most of these studies looked more at lysosomes as a causative agent, and the assumption was that activation of lysosomes was a cause of cell death. The topic was relatively modest. People tended to think that death was accidental and that mitosis was the active homeostatic process – not a concept, except perhaps for developmental biologists, that cell death was a biological process. I do remember that when I first saw the *Caenorhabditis* story of cell death genes, I muttered to myself, '#@$*, that's nice'. Kerr's original pictures looked similar enough to what I was seeing that I had no problem comparing our two efforts, and I even once gave a talk asking how it was possible for a cell to shrink. Thankfully, John Cidlowski has helped to explain that. We, finally, all met at a conference held in 1989 in Sardinia, organized by Amedeo Columbano and Vanna Ledda-Columbano. I can't speak for everyone, but I was very comfortable with the idea that we were talking about two aspects of very similar things.

CDD: So tell us about your early work?

The story of programmed cell death was my doctoral thesis. I went as a postdoctoral fellow to Scotland to work with CH Waddington on embryological and developmental problems. The work that I did there was also very interesting to me. I was intrigued with information transfer in eggs and succeeded in injecting isotopes and inhibitors into insect eggs to confirm that Paul Gross's then recent findings that messenger RNA was not required during the cleavage of sea urchin eggs was valid also for insects. I also collected some data to demonstrate that new messenger RNA was not required for the conversion of *Naegleria gruberi* from amoeboid to flagellate form.

I returned to the US to accept a position as an assistant professor at the University of Rochester School of Medicine in the Department of Physiology. I also wrote a sort of breathless first grant application in which I indicated that I would continue the programmed cell death project as well is the insect embryo and the *Naegleria* project. The National Science Foundation was at that time sufficiently relaxed and personal, in that they asked me which project I really intended to do. By that point, I had begun to understand that a Department of Physiology really had no interest in insect embryology or protozoan differentiation and that my best chances to collaborate, attract students, and survive in the department lay in the programmed cell death story.

CDD: How did the work on cell death start?

In the Harvard Graduate School and in Williams' laboratory, a common approach was for the professor to mention a few phenomena that might be worth exploring. In my case, one of the phenomena that he mentioned was the observation by Zoichi Kuwana, later followed up by LH Finlayson, that larval muscles persisted into the pupae of Lepidoptera and were finally destroyed shortly after the adult insect emerged. One reason that this project was appealing was that one could store pupae over the winter and that, therefore, one could avoid being restricted by season. Another reason was that Berthet, Wattiaux, and De Duve had begun to describe lysosomes and – even by the very crude and insensitive methods of those days – I could gather enough tissue to attempt to measure lysosomal enzymes. Thus, though I started to explore some of the other projects, this one began to move much more rapidly. Later, I got another huge break. Williams had gone to Japan and, captivated by the extremely low price at which cocoons were selling, he ordered 20 000 cocoons to be shipped to Harvard. When they arrived, he was horrified to realize that they had all initiated metamorphosis during the voyage. They were going to be nearly useless to almost everybody but me. As long as I was willing to work nonstop – and I was – for a brief time I had more material than I could have ever dreamed of having.

Of course, we were far from the only laboratory to be thinking about cell death. Dame Honor Fell had made quite a point of the differentiating death of keratinocytes and chondrocytes in culture, and Victor Hamburger and Rita Levi-Montalcini were very aware that, in the absence of what would prove to be nerve growth factor, differentiating neurons would die. Abraham White was interested in the death of thymocytes. The great embryologist John Saunders had established that the cells in the posterior necrotic zone of chicken wings could survive if they were transplanted elsewhere in the chick egg. I met Saunders for the first time near the time that I defended my thesis. We agreed that our arguments were very similar, and we have maintained warm and amicable relations since then. He deserves a lot more credit for his work.

And then there was the mysterious (to me) A. Glücksmann, who so carefully categorized and described the many situations in which cell death occurred as a biological process. I tried several times to locate him, to find a picture, or to learn more about him. If any of your readers have information, I would love to see it.

Most people don't think about it, but the bulk of plant differentiation, such as the differentiation of the xylem, the wilting of flowers, or the death of leaves, when stressed or in the fall, is programmed cell death. In fact, cells frequently commit suicide to sequester and block the spread of pathogens.

CDD: What other work was being done in the early time on apoptosis?

On apoptosis, nothing other than John Kerr's beginning to wonder how 'shrinkage necrosis' was brought about and how, physiologically, it might work. On the other hand, lots of people had started to reflect on cell death. As the Clarkes pointed out, many 19th century researchers had been aware of cell death in development and pathology. Researchers into metamorphosis were very aware that larval tissues died; it was well known that sexual tissues atrophied if the gonads were removed, and that muscles atrophied if they were immobilized or denervated. Saunders was emphasizing the normality of cell death in embryos. By the early 1960s, sex differentiation was understood, and there were initial investigations into the disappearance of the Wolffian and Mullerian ducts. Of course, the discovery of lysosomes turned attention to the possibility that lysosomes killed cells. (The organelles were named because they ruptured in the carbon tetrachloride-damaged liver, and it was assumed that this was a typical situation). Interest in the topic tended to be sporadic and scattered, and many researchers had 'day jobs': cell death was not their primary interest.

CDD: So what happened?

If you mean, how did the field suddenly take off in approximately 1990, I think that there was a sort of syzygy of events: Wyllie and his group, who in 1972 had defined apoptosis, published a simple and inexpensive means of looking for apoptosis (DNA laddering) that made it possible to look for apoptosis and demonstrate that it was far more common than perceived; Horvitz and his group identified genes controlling cell death and established that they were conserved; and the recognition by (in alphabetical order) Korsmeyer, Krammer, Nagata, Vaux, and others that a lymphoma gene (bcl-2) regulated apoptosis and the Fas-Fas ligand interaction could destroy tumors, and the recognition that p53 could be a cell death gene as well as a mitosis-inhibiting gene, made apoptosis interesting to clinicians.

CDD: What does it mean when you say type I, type II, or type III cell death?

These terms come from a paper by Schweichel and Merker in 1973. Unfortunately, the paper did not attract very much attention at the time, and, as I gather, they stopped pursuing their very interesting analyses. To me, the terms refer to what are called today (by most researchers) apoptosis, autophagic cell death, and necrosis. There are a lot of terms and subclassifications in the literature today but I love Claude Bernard's observations: first, words change meaning over time, and it is a mistake to insist on a definition when the word means different things to different people. As he says, in that situation the word is only a source of confusion. Second, he scorns those who define a word and think that they, therefore, have understood a phenomenon. Finally, I would note that one does not really need an instruction manual to die. It is quite possible, for instance, for a cell to initiate apoptosis, exhaust its energy supply, and ultimately terminate in a necrotic state. Nevertheless, the pure types are not difficult to find, and we can learn much about the mechanisms from them.

CDD: Were there any developmental implications of this work?

The genetics of cell death were of course brilliantly defined by Bob Horvitz, Charles Sulston, Michael Hengartner, Junying Yuan and several others. The idea of programmed cell death was from its origin inherently developmental. I don't remember when I first explicitly made the comment, but it was obvious that, if the death of a cell could be predicted in an embryo or a metamorphosing insect, then that death was as obviously a genetic trait of the species as its color, shape, structure of hair, or any other commonly accepted genetic characteristic. Although I was working with insect metamorphosis, which was accessible, I considered my work to relate to anything that I found in embryology and pathology.

CDD: If apoptosis not only helps cell–cell interactions, how does it affect cancer?

I'm not sure what you mean by this, but I can make an observation: the first big disappointment in apoptosis research was the realization that to control the mechanics of cell death did not seriously impact either cancer or diseases of cell attrition. There are some obvious and some subtler reasons for this. The more obvious includes the realization that in most antisocial cells – cells that are not dying when they are supposed to, or cells that are dying when they are not supposed to – the machinery of cell death (typically a caspase cascade) is fully operational. What has changed is the sensitivity of these cells to signaling mechanisms, or the threshold at which they respond. We know something but not everything about these signaling mechanisms, and we need to know more about the metabolism of the affected cells. Getting back to your question, if the cell fails to respond properly to these 'cell–cell interactions' then it is heading toward pathology.

The subtler reasons are related: if a cell is truly sick or stressed, then preventing its immediate death by, for instance, intercepting the pathway at any point before the activation of caspase 3, will not in the long run save it. The analogy that I use is that if a person has a heart attack while swimming, merely getting him out of the water will not guarantee his survival. Many laboratory experiments miss this point. Blockage or inhibition of caspases in a cell lacking a vital growth factor will delay cell death, but this is not survival. A worse mistake is to inhibit a caspase cascade and then to define survival by failure of the appearance of caspase-dependent changes, such as margination of chromatin or exteriorization of phosphatidylcholine. Apoptosis is efficient and rapid, but a very sick and improperly nourished or protected cell will eventually die, although using a lot of autophagy or becoming necrotic.

Incidentally, although we have talked about autophagic cell death for a long time, I never was able to find a clear-cut distinction between autophagic cell death and autophagy – that is, atrophy of a cell that is lacking something vital or has

damaged organelles, but to a quiescent state, from which it can recover. When I was working on muscles, I compared the situation I was looking at to denervation and starvation atrophy. Even in the insect muscle and labial gland 'autophagic cell death', after the cytoplasm is almost completely eliminated, one encounters characteristics of apoptosis, such as margination of chromatin, DNA laddering, exteriorization of phosphatidylserine, and perhaps activation of insect caspases. It is very much as if the cell, even in a rich hemolymph, is starving or is unable to acquire a vital constituent. It, therefore, undergoes autophagy but, like a lost explorer who tries to preserve his resources but eventually consumes them, ultimately fails to outlast the stress and commits suicide. Autophagy is real, but, as Guido Kroemer and others have emphasized, it is not clear that autophagy represents a linear path to death as opposed to an indication of cell agony, which ultimately leads to apoptosis or other (necrotic) failure of the cell.

CDD: What were the wider clinical implications for the work?

The clinical relevance has always been present, but the wider implications in teratology, virology, oncology, neurology, and even cardiology have been very eloquently expressed by many others, including in many of the reviews published in *Cell Death and Differentiation*, and even when intended for a non-professional audience, such as in Jean-Claude Ameisen's wonderful *La Sculpture du Vivant*. It is more reasonable to give them the stage.

CDD: Where do you see the new challenge for apoptosis?

I think that one of the issues has always been the question of what a cell, that is, contemplating suicide is feeling. Much of our research creates a situation in which we know what will happen: a regulatory component, such as bcl-2 or bcl-XL, is upregulated or downregulated, and then the cell is exposed to a very toxic situation, such as exposure to staurosporine or cycloheximide coupled with less-than-physiological serum concentrations. We learn a great deal from these highly controlled situations, but life does not operate in this fashion. In most situations, neither the regulatory molecules nor the effector molecules are drastically altered, and cells individually commit to death because of other intervening metabolic or ancestral reasons. Likewise, for a given apoptotic stimulus, a population of presumptively homogeneous cells shows a substantial range of variation in response, and highly stressed cells will still die even when apoptosis is prevented. It is basically the same situation as if we knew what makes a car run and where the brakes are, but had no idea about traffic rules or red and green lights, or even where the roads were, left-side or right-side rules, or whether they were bidirectional or one way. Take, for example, the situation of non-familial Alzheimer's Disease: there must be something wrong with the metabolism of the neuron throughout its life, but for 50 or 60 or 70 or 80 years, the neuron seems to function, until it finally dies. The explanation of a suicide is not that the victim died of a skull fracture when he hit the pavement, but what went on in his life and his mind that led him to jump. That is another of Bernard's arguments: 'The anatomical point of view is therefore completely insufficient and the alterations that one observes in cadavers after death more accurately give the characteristics to recognize and classify diseases than they do to understand the lesions capable of explaining the death.' We have a great deal to learn about the triggers and balances that cause cells, with normal levels of proapoptotic and antiapoptotic regulators and caspase proteases, to tip over into apoptosis. This will be a difficult task, as the entry into a positive feedback loop such as caspase activation is always very vigorously and redundantly defended.

CDD: What about your pupils?

When I was a student, I don't think that I would have considered a position that was not academic, but times have changed. None of my students really stayed in the field of cell death, though some remain peripherally connected to it. Some have stayed in academia, but others, including some of the best, have gone on to positions in companies and governmental organizations. I probably should not say too much more, because what I say, forget to say, or don't say will suggest favoritisms or judgments that I don't want to make. I deeply regret that an excellent, promising, and personally lovely postdoctoral fellow, Jana Jochová, became ill and died shortly after leaving my laboratory.

CDD: Can we dare asking about your private life, that is the personal and scientific relationship with Zahra?

As you and many people are aware, Zahra Zakeri and I have been married since 1989. As is obvious to everyone who knows us, she provides the energy, and I try to keep up. With her gregariousness, optimism, and sociability, she keeps me visible in the world. She also taught me molecular biology. She loves the field and her curiosity and questioning make me also have to think about alternative interpretations. She has a quick mind and questions everything. (From the realization that both she and I had the same childhood nickname, I did an informal survey. Do you know how many future scientists have the nickname 'Questions'?) She also has the patience to teach what she knows to others. Her eagerness to share her knowledge is the origin of Scientists Without Borders (http://www.scientistswithoutfrontiers.org/), and is the source of her ability to find ways to get to many different countries to teach about the field of cell death and to work with the scientists and students that she finds there. Apart from the times in which we disagree about the meaning of a result, she brings in the perfumes of the East, and I have enough romanticism that she picks up on it. We work well together and I hope, as she says all the time, we can always look at life now and make a better tomorrow for others. Those characteristics are visible to others. She managed to put together a party – more of a Festschrift, actually – for my 70th birthday, and it went way beyond anything that I imagined, even with long experience, that she could do. But everyone's attendance really was far more a tribute to and an expression of love for her. In addition to being a terrific scientist, she has this incredible ability to network and to bring scientists together. This year she will get

a special award, 'Ambassador of Science,' from the International Cell Death Society. That about sums it up. It is also typical that she chose the title for my last book. I did this book about how scientists think, an effort to make science comprehensible to non-scientists – we can use that a bit in the US! – but my projected title, 'How Science Works,' turned out to be a previously-used title. We were casting around for a new name. *The Joy of Science* was her prompt suggestion, and it gives a good indication of her personality and attitude. Also, she took the cover picture.

CDD: What is your preference in term of non-scientific interests?

I'm not sure what you would expect, but Americans would probably expect me to describe a hobby: whether I like fishing, or reading, or going to the opera. I don't really do it that way. I can't say that I really focus on a particular personal interest. Aside from getting a chance to taste all the wonderful cuisines of the world, I suppose that it's fair to say that I once defined success as being able to feel comfortable in any culture. I really enjoy encountering the world's variety, and I feel delighted that a scientific career has given me that opportunity. Besides, meeting Zahra put that whole package into one.

Acknowledgements. Oscar-type thank-you's are really not a graceful style, but of course it all started with my parents (Samuel D Lockshin and Florence Levin Lockshin), who were remarkably tolerant of all the bugs, frogs, and rodents that my brother and I brought into the house. They were always pleased that both of us were drawn to biomedical sciences. (My brother, Michael D Lockshin, is currently Professor of Medicine and OB-GYN at Weill Medical College of Cornell University and editor of *Arthritis and Rheumatism*.) My children, Miriam and Nora, also were very willing to put up with my nonsense (usually, until some of their friends called my painted cicada molted skins, which I stuck to their dresses and called recycada jewelry, geeky) and even were the patient proofreaders for the first cell death book. There are many, many predecessors and successors to whatever work that I did. I tried to mention as many of them as I could in a review that Zahra and I did a few years ago, and I hope that I have not again forgotten anyone. It was a wonderful opportunity for me to meet my future doctoral mentor, Carroll M Williams, and to have the opportunity to work as a lab tech in his laboratory during my undergraduate days. He had many gifts of which we were deeply envious: a wild and uninhibited imagination, in which he would spout 100 ideas per minute, of which only 99 were completely ridiculous; and a facility with language that could stop conversations. We all worked very hard to emulate that style, however feebly. In my immediate era, of course, there were John Saunders, John Kerr, Andrew Wyllie, Jamshid Tata, and Bob Horvitz, and many others who sensed the inherent interest in cell death but were more inchoate. There was Jacques Beaulaton, with whom I collaborated in the 1970's. I have been very fortunate in developing some very deep friendships as a result of the many meetings and conversations, including Amedeo Columbano and Vanna Ledda-Columbano, who put together one of the first meetings to bring us all together; you (Gerry Melino), Mauro Piacentini, and Felix Bursch, whom I met at that meeting; Marie-Lise Gougeon, whom I met at the first Gordon Conference on Cell Death; Jean-Claude Ameisen, whom I met a few years later, Guido Kroemer, Boris Zhivotovsky, Seamus Martin, Aviva Tolkovsky, Francesco Cecconi, Marianna Sikorska, Roy Walker, Soraya Smaili, Rafael Linden, Roya Khosravi-Far, Afshin Samali, and many others and the spouses of my friends, too many to list – many dear friends from many countries. I have already mentioned Zahra. If I have omitted anyone, I hope that they will forgive me. I have been fortunate to meet many young investigators, who are now the heart and soul of this enterprise, and who carry it well beyond anything that I did. I also have been inspired by the eagerness and energy of scientists from third-world countries, who also bring a fresh and medically important perspective to what we do. In brief, I got a lot of very lucky breaks in my life. I hope that I can begin to return the favor.