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Interview

Early work on the role of mitochondria, an interview with Doug Green

DR Green*,[1]

[1] La Jolla Institute for Allergy and Immunology, 10355 Science Center Dr, San Diego, CA 92121, USA
* Corresponding author: DR Green, La Jolla Institute for Allergy and Immunology, 10355 Science Center Dr, San Diego, CA 92121, USA. E-mail: doug@liai.org

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Doug Green received his Ph.D. from Yale University. Before coming to San Diego, he was a faculty member in the Department of Immunology at the University of Alberta at Edmonton. He is Head of the Division of Cellular Immunology at the La Jolla Institute for Allergy and Immunology. Doug is best known for wearing Hawaiian shirts, singing off key, and sustaining a passionate interest in the biology of cell death. He has published over 300 papers, not including essays he writes under a pseudonym (which of course he won't divulge).

The mitochondrion was first shown to act at the center of the apoptotic mechanism in the early 1990s. The identified role of mitochondria and of the apoptosome in human cells showed that it had relevance to human diseases such as cancer, neurodegeneration and autoimmunity; now, a large body of evidence exists. Here, *Cell Death and Differentiation* asks Doug Green about the early work on mitochondria.

CDD: What was your scientific interest in Edmonton?

Between 1985 and 1990, I was working in the area of immune regulation, and I became interested in the mechanism of clonal deletion – the way in which cells of the immune system 'learn' to discriminate self from nonself. The initial hypothesis was that developing lymphocytes die if they are activated by exposure to their specific antigen. I wondered how, and we found ways to explore it. We basically stumbled over the process of activation-induced cell death (AICD), and showed that it was by apoptosis.[1] I was strongly advised by my more senior colleagues to find something 'more interesting' to work on.

CDD: Would you write a book on 'the collapse of immunology'?, and why?

Sneaky! You KNOW I'd write a book called 'The Collapse of Modern Immunology', but it's a fair question anyway. Here's the deal, and here's why we're talking about it.

In the 1980s, immunology went through a stunning revolution, when the principles and techniques of molecular biology were applied to understanding the immune system. By the early 1990s, the central mechanisms of the functions of the immune system were solved. In essence, every major problem facing modern immunologists was basically wrapped up.

Except for autoimmunity. The nagging problem was that people (and animals) could suddenly get a disease (or be given one) that occurred because the immune system attacked the body. As immunologists picked at this problem in the 1990s, the lovely construct that was the tidily wrapped up field of immunology started to unravel. Suddenly, other phenomena that couldn't be easily explained within the paradigm came to the fore. In the last couple of years, immunologists have gone back to first principles and found that many of our cherished notions were just that – notions – and the principles and paradigms are being redefined. It's an exciting time.

So why are we talking about that? Well, the field of apoptosis is widely thought to be solved (at least, the big questions have been put into a perspective that many people are satisfied with) and we seem to be working around the details. However, those of us thinking about the field are all too aware that there are holes in our knowledge that continue to defy us: things that might ultimately force us to re-evaluate what we know. The paradigms will collapse and will be rebuilt in slightly (or completely!) different ways. Mitochondria may represent one of those black boxes. However, there are others (and I'm not going to tell you what they are!).

CDD: When did you first hear about apoptosis?

Back in Edmonton, we had observed AICD, and I had the idea that we had to just *LOOK* at it. The cell biology department had hired a new electron microscopy (EM) technician, Margot Szalay, and she offered to do some transmission EM on our dying cells. When we got the images, we simply had no idea what we were looking at – very strange! The talented young graduate student who was doing the work, Yufang Shi (now a very talented but *not* so young professor!!!), literally browsed through books on cytology, looking for anything that looked like our cells.[2] He found a review by Andrew Wiley on apoptosis, and that wonderful review (published years before, in 1980) introduced us to the field. A few months later, coincidentally, Andrew visited Edmonton and we showed him our work. His wonderful enthusiasm was an inspiration.

CDD: How was the role of mitochondria in cell death first identified?

I believe that the first paper (at least the first I was aware of) suggesting a role for mitochondria came from Guido Kroemer, who used our system of AICD in T-cell hybridomas. He said that mitochondria lose function before other apoptotic events could be observed.[3] We had done the same experiments, but didn't get those results. However, Guido's work certainly alerted me to the possibility. A short time later I met Don Newmeyer, who developed a cell-free system for apoptosis, in which mitochondria have an essential role.[4] Don and I have worked together ever since.

CDD: What other early work was being done at the time on mitochondria?

Don observed that mitochondria produce something that activated apoptotic events, such as DNA fragmentation (and ultimately he figured out that mitochondria activated caspases). Ruth Kluck worked to purify the key factor, and found that its production was blocked by Bcl-2. Then Xiaodong Wang published his first brilliant paper (of many!), showing that cytochrome *c* activates caspases.[5] Within a few minutes, Ruth informed us that, yes, the factor she had enriched was cytochrome *c*. As she already had all the data in place showing that the release of this factor was controlled by Bcl-2, it was a very simple step to the realization that mitochondrial outer membrane permeabilization is controlled by the Bcl-2 family members.[6]

CDD: So cytochrome *c* is crucial, but how is it released and what are the consequences?

Well, we were certainly aware of Guido Kroemer's emerging story on the mitochondrial permeability transition (PT), and now we thought we knew what it did – that is, caused the release of cytochrome *c*. However, when Ella Bossy tried to correlate cytochrome *c* release with loss of $\Delta\Psi$m (an indication of the PT), she noticed that this was sustained if caspases were blocked. Subsequently, Josh Goldstein and Nigel Waterhouse rigorously confirmed this.[7] Nigel went on to show that after cytochrome *c* release (we now call this mitochondrial outer membrane permeabilization, or MOMP), the electron transport chain remains functional unless caspases are activated.[8]

CDD: What did this mean?

It meant that caspases disrupt mitochondrial function. Jean-Ehrland Ricci went on to show that caspases enter the mitochondria through the (now) permeable outer membranes, and disrupt complexes I and II of electron transport.[9]

CDD: So how did you come across p75?

Knowing this, Jean-Ehrland went on a search for a mitochondrial caspase substrate that could account for the effect of caspases on electron transport. One of the first ones he found was p75 – a key subunit of complex I.[10] Mutation of the cleavage site of this protein profoundly altered the effects of caspase activation on mitochondrial function during apoptosis.

CDD: Does apoptosis influence cell metabolism?

Absolutely. Upon caspase activation, ATP levels in the cell crash. When noncleavable p75 is present, this crash doesn't occur.

CDD: Were there any clinical implications of this work?

One of the major functions of ATP in the cell is the maintenance of the plasma membrane – the many ion pumps require ATP, and if this is lacking the cell rapidly loses integrity. Another effect is phosphatidylserine (PS) externalization: there is a well-characterized translocase that maintains lipid asymmetry, provided ATP is available. In cells with noncleavable p75, PS externalization is slowed. One consequence of this might be on the immunological effects of the dying cells.

CDD: If apoptosis helps immune cell interactions, how does it affect cancer? Why did you look at apoptosis and cancer?

In terms of p75? Scott Lowe has some very intriguing findings that beg to be analyzed in these terms: we would like to know if blocking p75 cleavage can have an effect on the efficacy of cancer therapy, but it's much too early to say one way or the other.

CDD: So you and Don Newmeyer overlapped for several years? What did he do with this work?

Don and Tomomi Kuwana have done brilliant work on the mechanisms of MOMP, and how the activation of the proapoptotic members of the Bcl-2 family can permeabilize membranes.

CDD: While Don was continuing to study the mito-apoptosome axis, you went to proteomics. What did you do there?

Identified p75 as a caspase substrate. We're working on others!

CDD: How was it that you started to work on the 'cell death' movie?

When Josh Goldstein was a graduate student in my lab, he decided to develop tools to analyze the process of apoptosis at the single cell level. He and Nigel Waterhouse chose cytochrome *c*-GFP release as their first goal.

CDD: So what happened?

Against all expectations, Josh discovered that cytochrome *c*-GFP is released suddenly and unpredictably during the process, and that every mitochondrion releases at about the same time. It was startling, and forced us to completely rethink apoptosis. The work that Josh started, and is being continued by Cristina Munoz and Lisa Boucher-Hayes, remains one of the most powerful approaches to understanding apoptosis that we use.

CDD: What movie do you like? Would you act?

Please tell Spielberg that I'm available.

CDD: What music do you like to listen to?

I have tremendous respect for jazz and classical musicians. But I listen to rock.

CDD: What is your preference in term of meetings, and why?

My ideal meeting is one with just a few select scientists, no slides or powerpoint presentations, only discussion (and, of course, cocktails). And, while I'm delighted to speak at larger meetings (held in very nice places), I no longer find them as useful as they were when I was starting out and learning lots of new things. I think most of us would agree that the smaller meeting is more likely to be interesting and useful.

CDD: Now tell us more about your sailing project

Yes, it's true, I have a life away from science. I love to play music, perform and be creative. One hobby I really enjoy is sailing with a few of my craziest friends. In June, five of us participated in a race called Vic-Maui, which started in Victoria, British Columbia, and ended in (Yes, you guessed it!) Maui. The Scaurend completed the 2500 mile trip in a record 22 days (a record for the Scaurend, not for the race). We hoped that my wonderful friend Gerry Melino would have a party organized for our arrival, but as it turned out, while the party was wonderful, Gerry didn't make it!

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