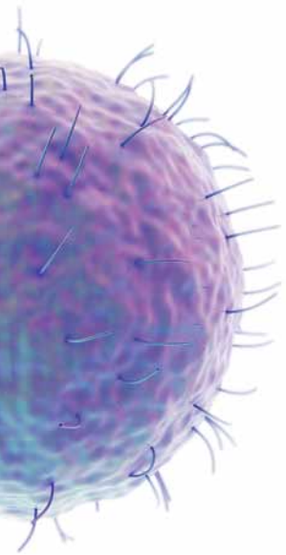
DR. DAVID ANDREWS

IN DEATH, life

One scientist's quest to reveal and master the body's most complex processes, in aid of developing more effective treatments for cancer and other diseases

BY STEPHANIE ROBERTS

ILLUSTRATION: MARC DRYER; PHOTOGRAPHY: CURTIS LANTINGA



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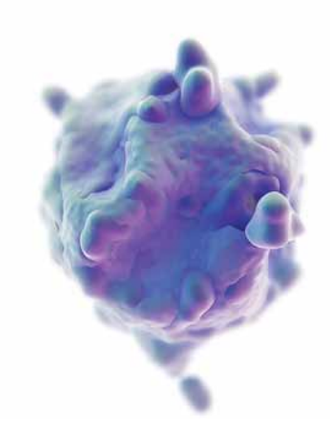
new capacity to study protein-protein interactions where they take place, in the membranes of live cells, opens up myriad possibilities for identifying drug candidates.



He calls himself a cancer researcher. Some might call him a masochist.

"I tell people who want to come and work in my lab that it's not good enough to be able to tolerate banging your head against the wall. You have to like it."

Like it with the same eagerness as he does: to be captivated by the shock of a setback, enraptured by a riddle. "One of my favourite things is when somebody brings me data and you look at it and think, 'What the heck is this? What's going on? This is completely not what I expected!' That's fantastic, 'cause now you know there's something really interesting to find out. I love working on those kinds of puzzles."

Dr. David Andrews is the newly appointed director of biological sciences at Sunnybrook Research Institute. The objects responsible for his happy suffering are proteins: he is in thrall to understanding how proteins interact to control the behaviour of cells, especially during apoptosis, or programmed cell death.

What is apoptosis, and why study it?

First, let's deal with pronunciation. The second p is silent. That's the point on which most agree. Sparking more lively discussion, however, is how to pronounce the "a": long or short? Ape or app? In general, south of the border, it's ape. In Europe, it's app. Here in Canada, as usual, we play it both ways. Andrews, however, goes short, backed by an authoritative source: a footnote in the publication that first described apoptosis. "These Scottish guys went to the classics department in a university in Scotland and asked them for a word that would be kind of like mitosis, but would have to do with cell death," he explains. After giving it some thought, professor James Cormack coined apoptosis, which in the original Greek means "the falling of petals from flowers, or of leaves from trees," and which, the authors noted, is pronounced with a short a, and a silent second p, with the stress on the second syllable; thus, we have: apo-*ptosis.*

Apoptosis refers to the biological process by which cells are genetically programmed to commit suicide. It is critical for healthy functioning. It tells cells to kill themselves if they are no longer needed, for example, during tissue development to get rid of unwanted bits, like webbing between our fingers, or if they are damaged, as in precancerous cells. It differs from necrosis, the other kind of cell death, in that with necrosis cells die owing to an acute, usually traumatic, injury.

It was first described in 1972 by scientists from the University of Aberdeen in Scotland, in the *British Journal of Cancer*, in an 18-page article that has been cited more than 11,000 times.[1] In that article, the features and function of this newly characterized process were laid out in as much detail as could be had with the technology of the time. The authors speculated that decreased apoptosis might be responsible for the runaway growth of tumour cells, and not increased mitosis (cell reproduction), as was the prevailing opinion, but noted, "We know of no definitive studies to support such a hypothesis."[2] They concluded that more research remained to be done to understand the phenomenon.

Four decades later, more work certainly has been done, most within the last 20 years. At last count, 1.6 million articles on apoptosis have been published.[3] Do researchers now understand it? Sort of. It is now known, as the Scottish team posited, that too little apoptosis does indeed cause tumour cells to proliferate and cancer to dig in—and not only cancer. It also is implicated in other diseases, such as autoimmune and viral diseases. Moreover, the problem can swing the other way, as in when apoptosis runs amok and kills too many cells, which can cause tissue damage, human immunodeficiency virus, and brain-wrecking disorders like dementia and stroke.

Harnessing this process, therefore, holds untold promise in designing pro- and anti-cell-death therapies.

KA-BOOM!

During apoptosis the suicidal cell appears to pull into itself, a function of its shrinking cytoskeleton, which lives in the cytoplasm, the jelly-like environment inside the two-layered plasma membrane where much of cell life takes place. Then, the chromatin, which comprises the proteins and DNA that make up the cell's nucleus, condenses. The nucleus cracks under the pressure and fragments. The shrinking cytoplasm causes the membrane to bulge, forming stubby finger-like objects called blebs. The blebs grow rounder and fall away from the membrane to form bubble-shaped apoptotic bodies. I imagine this is what a planet exploding in slow motion would resemble. At this point, phagocytes, white blood cells that help protect the body, engulf

the debris. The process takes hours to days, but once done, it cannot be undone.

The powerhouses behind this dramatic process are protein-protein interactions that take place in the outer mitochondrial membrane. Signals to and from proteins direct every aspect of cell function. Proteins enable the cell to receive and respond to messages about what to do and how to do it. Without them, the cell might as well exist in a blacker-than-night, soundproof box. Understanding how proteins interact, therefore, is the key to mastering the life-and-death processes they control. This, as one might imagine, is no easy feat—apart from proteins' complexity, the number of them staggers: a database of proteins has catalogued almost 24,000 proteins, and more than 73,000 interactions.

Andrews has honed in on a family of membrane proteins called the Bcl-2 family, which is made up of proteins that activate cell death and those that block it. As a class, membrane proteins, which comprise about 30% of the body's proteins, are hot drug targets. That's because most drugs that work do so because they target membrane proteins.

"The beauty of the Bcl-2 family, from my point of view, is that there are two proteins, Bax and Bak, that are the ultimate decision-makers, and then lots of other proteins funnel down to those two. That gives the cell a way to monitor many different events, and integrate a whole bunch of signals to make a decision. From the point of view of somebody like me who's a biochemist or [someone] in the pharmaceutical sciences, it means that is the point you can regulate and have the most effect, because it's as close to that decision point as possible," he says.

Bax and Bak are the executioner proteins. Think of them as akin to the Queen of Hearts, without the nasty temper. They control the final decision as to whether a cell that should die, like a precancerous cell, does so. They take their cue from other family members, which sense when a cell is damaged and needs to be snuffed out. Sometimes, though, their activation is blocked by yet another branch of the family, the anti-cell-death relatives, like Bcl-2 and Bcl-xl. This blocking effect not only allows cancerous cells to grow; it also helps them resist chemotherapy. While there is much jockeying for dominance within the family, the essential event in apoptosis is the binding of the executioner proteins to the membrane. Here, they make it porous, and in doing so launch the irreversible chain of events that commits a cell to self-destruction.

"My interest in those proteins is that they regulate the response of cancer cells to chemotherapy. The ultimate goal is to be able to make chemotherapy more effective and more selective, and these proteins, most of the time, they are the decision-makers as to whether or not the cells will die," says Andrews.

Ensuring that Bax and Bak can do their job, either by making sure they get activated, or that nothing blocks them, is thus the focus of scientists looking to design anticancer drugs that would target Bcl family members. Of course, the anti-cell-death proteins are not always a menace; activating them could offer a way to treat diseases like stroke or heart attack, in which too many cells die, by halting the process long enough to minimize damage or enhance the effects of treatment. As one might imagine, the precise mechanisms by which this warring family of proteins achieves its ends are complex and a matter of much study.

A TALE OF DISCOVERY

Back when this family of proteins was first discovered, in the mid-1980s, Andrews was a graduate student in medical biophysics at the Ontario Cancer Institute in Toronto. It would be a good few years before he would fall under their spell, but the work he was doing would prove to be instrumental to his later research.

His attention then was held by trying to solve the "oil and water" conundrum of membrane proteins. "All proteins get made in the cytoplasm, which is essentially a water-type environment," he explains. "The cytoplasm is hydrophilic [water-loving]. It's a different environment from the membrane, which is lipid." The lipid environment is the opposite of the cytoplasm milieu, in that it's hydrophobic, or water-repelling. "The easiest way to think

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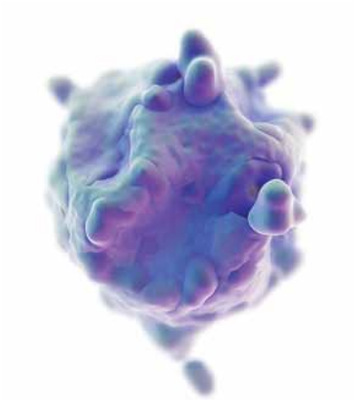

of it is as oil and water. Oil and water don't mix—but you can only make proteins in the water. So if you can only make proteins in the water, what kind of machinery do you need to keep them from aggregating in the water and still be able to put them into oil?"

While wrapping up his postdoctoral degree in cell physiology at the University of California, San Francisco, in the late '80s, he made a discovery that not only solved this mystery, but was to help solve another puzzle he would face when he turned his mind to apoptosis a few years later. "I rediscovered something that people already had known about, but at this time were conveniently forgetting, because it didn't fit anything [that was known]: that some proteins have only one hydrophobic piece on the protein and that's right at the c terminus, right at the end of the protein."

It was this "rediscovery" that enabled him to figure out how some proteins could get targeted to the membrane: basically (an abridged explanation), via a series of signals and with the help of a "tail anchor" that allowed it to bind to the membrane. His finding of the unexpected role of this tail-anchored protein made the oil-and-water issue moot, and was critical at a time when almost nothing was known about membrane protein targeting.

A few years on, now an associate professor in biochemistry at McMaster University, he and his colleagues were working on the newly discovered Bcl-2 protein when he had another brainwave. "I realized many of the Bcl-2 family proteins have that hydrophobic sequence at the extreme c terminus," he says. Turns out, they, too, were tail-anchored proteins—which explained how they got targeted to the membrane. The realization hit him with a gratifying thud. "The original paper on Bcl-2 was all wrong—everything about the membrane targeting and assembly of the protein was wrong. It was a full article in *Nature*. So I thought, there's an opportunity here," he says, his glee evident.

He seized the opportunity—despite hostile reaction from the scientific community that delayed publication of these findings for three years—and hasn't let go since. Some 15 years later, he and others studying apoptosis have made real headway in understanding how it works. He says his eye now is trained on making that knowledge clinically useful. "The most important thing is that it impacts the patient. As my career has progressed, it's become more and more important.

"When you're intellectually curious about a certain biological phenomenon and you figure that out, the next logical thing is, 'OK. What can I do with it?'"

He is not saying that all the puzzle pieces for protein-protein interactions and cell death are in place—far from it; it's clear that the table holding them is as big as one's imagination can build—but he is saying there is enough knowledge to be working toward getting it to patients, while continuing to study the fundamentals.

JOURNEY TO THE MEMBRANE

Drug discovery: high risk; high stakes. There are many steps that go into identifying a potential drug target and taking it all the way to where it gets the regulatory thumbs up. Once a drug is discovered, it will be 10 to 15 years before a patient can benefit from it—if it gets past preclinical testing showing that it doesn't have nasty side effects, and then if something doesn't go belly up during the last stage of clinical trials, as happens more often than not, because trials are the only way to know for certain if a drug works in people. It costs about $1.8 billion to develop one drug; with rewards of annual sales in the billions of dollars, however, it's not hard to see why companies make the effort.

As we've already seen, membrane proteins are the most promising and the most difficult of drug targets. One of the greatest challenges is being able to see how the proteins work and interact with each other where the action takes place, in the membrane.

Traditional methods have been inadequate. "Because these protein-protein interactions take place in a membrane, you can't

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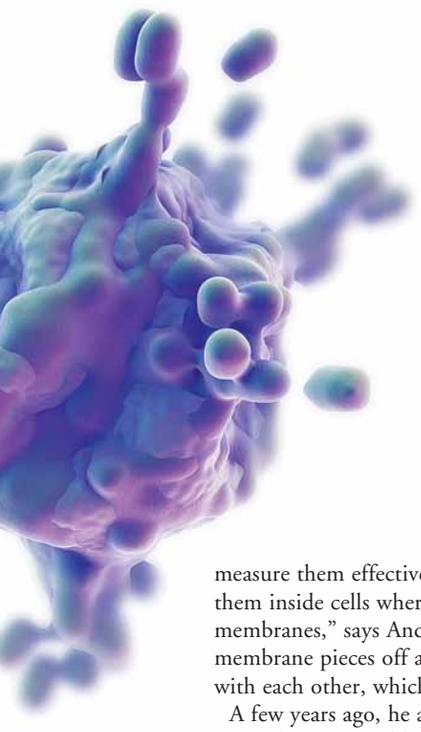

measure them effectively outside of cells. You can only measure them inside cells where you have the normal architecture of the membranes," says Andrews. An early method was to cut all the membrane pieces off and look at the rest of the pieces interacting with each other, which, Andrews notes, "was very unsatisfactory."

A few years ago, he and colleagues devised a better method. It involves reassembling proteins in a sort of artificial membrane using liposomes, which are like soap bubbles, but composed of two layers of lipid molecules, and then using an imaging tool called fluorescence resonance energy transfer, FRET, to observe the proteins interact. This tool, commonly used in drug discovery research, gives information about how molecules associate with each other by measuring the energy transfer between them, which appears as the movement of fluorescent light from one molecule to another. Because the transfer happens only if the molecules are close enough together, it can be used to track where and when molecules interact.

Using this system, Andrews and colleagues undertook to witness the steps by which Bcl-2 family members regulate apoptosis. They inserted purified proteins into the liposomes and used FRET to see what happened next.

They weren't disappointed. They saw precisely how Bcl-2 family members interact, resulting in the executioner protein Bax binding to the membrane, and the membrane becoming permeable and kick-starting apoptosis. They were also able to see how the effects of a meddling relative prevented Bax from binding to the membrane. Perhaps most importantly, they were able to see that another protein interaction reversed the effects of the meddling relative, so that Bax could once again bind to and make porous the membrane. The results were a big deal. They showed that the process was orderly, step-wise and reversible, and that the membrane was not only essential, it was also an active participant. The findings profoundly changed researchers' understanding of how Bcl-2 proteins regulate apoptosis.

"This was a big step forward, which is why it came out in *Cell*, because we could see interactions that people had completely missed, and just recently there was a review article which pointed out that that paper still is the only demonstration that Bid actually binds to Bax directly, and that's in the membrane," he says.

Although a major advance, Andrews is quick to note the technique is a stopgap. "It's in an artificial membrane. To study these things in real membranes, we have to be able to make all those spectroscopic measurements in live cells." Alas, such a highly specialized tool doesn't exist. Correction: it didn't, until Andrews set to thinking about it.

Bax

and Bak are the executioner proteins. Think of them as akin to the Queen of Hearts, without the nasty temper.

PRETTY AS A PICTURE

Spectroscopic tools analyze properties of light to provide information about a molecule. For Andrews, three properties are of interest: intensity, wavelength and something called fluorescence lifetime, which measures how long a molecule that has been stained with fluorescence dye is excited when light hits it. Lifetime is the property in which drug companies are most interested, because it tells them if a drug is working.

Confocal microscopes are a mainstay of the biologist's toolkit. They are miles away from the widefield microscopes of our high-school science classes. There are two kinds of confocals, the raster and the spinning disc. Each excels at measuring the intensity of light, and, with software, produces crisp, high-resolution images that can be reconstructed as 3-D multicoloured structures on a screen.

The raster is typically used for fixed specimens, in part because it takes a while to capture an image. It can deal with very thin slices of specimens, enabling scientists to peer deep inside cells. It may sound complicated, but as Andrews explains, it works much like a regular TV does. "You scan a beam of light across the sample; at each point in the sample you measure the fluorescence that is given off, and that creates your image." The spinning disc is a bit different. It can do time-lapse imaging of live cells and is much faster than the raster, but at the expense of crispness of images. Neither is good at measuring wavelength, and neither can measure fluorescence lifetime.

Confronted by these limitations, Andrews began to think about building a more powerful microscope. "If I need an answer and there isn't any equipment or tool to get the answer, then I make one myself," he says. He had done that before, work that resulted in two patents and the launch of a spinoff company. This would be something else entirely.

It began with the Opera—a type of microscope, not a stage drama headlined by Pavarotti. The Opera is a spinning disc confocal microscope that produces ultra-high-resolution images.

It's unique among confocals in that it is fully automated. "It will take 100,000 pictures a day without you being there," says Andrews. This capacity to do high-content screening was perfect as a starting point for his reinvention of the tool, because he knew that to get the kind of data he wanted he would need truckloads of images.

He brokered a deal with the company that made the Opera, later acquired by PerkinElmer, to modify the system so that it could do fluorescence lifetime imaging microscopy, FLIM, the only way to measure protein-protein interactions in live cells. His first attempts fell short, though not fatally. "It didn't work very well. We realized there were problems with it, but that it showed tremendous promise," he says.

He also realized he needed some way to make sense of what he was seeing.

"You can ask all kinds of physiological questions [with this microscope] that you can't ask with a normal microscope, because you can get huge numbers of images. The problem is that you get huge numbers of images, and so you can't interpret them," he says.

For example, they did one genome screen that produced four million images, which was thrilling—except that no one could look at them. "So we had to write software that would interpret the images," he says.

Others had written software to interpret images automatically, but the programs were "philosophically different" from what Andrews had in mind. "All of that software depends on the observer being as close to perfect as he or she can be," he explains. "So the person whose data it is becomes the gold standard, and they decide, 'These are what I'm looking for, and these are what I'm not looking for.' And then the computer learns how to do that.

"Our approach was to say, 'I don't know what I'm looking for—if I'm going to knock out all the genes in the genome, I don't know what's going to happen—so I'm not the right person to ask which are the ones I'm interested in and which are the ones I'm not interested in. We had to teach the computer to learn for itself to find anything that wasn't normal. And if it found things that were not normal often enough, [then] it would group those together and say, 'When you knock out this gene, a whole bunch of cells all do something abnormal similarly, and here are those cells.'"

Needing to write this software "distracted" him from retooling the microscope, he says, laughing, and would swallow two of the five years he spent working on it. (The effort, still ongoing as his lab refines the program, was not for naught: in addition to giving him the capability he needed, it has led to a provisional patent for diagnosis of primary brain tumours with colleagues at the Sanford-Burnham Medical Research Institute in California.)

Distraction notwithstanding, reimagine the Opera they did, producing the first fully automated high-content screening microscope that can do FLIM. "It works," says Andrews. "And we can get what I was interested in getting all along, which was binding curves, because it's biochemical binding curves that the pharmaceutical industry uses to say whether or not a drug is working, and how well a drug is working and how it works—and we can do that now at high speed."

As significant an advance as the retooling of the Opera is, he notes it's not perfect. It cannot provide the level of detail he wants. And, it's terribly expensive. So, naturally, he's been building another microscope, one that he says will have much higher resolution, but operate at the same high speed and cost much less.

It will enable researchers to shut off genes or proteins associated with a particular disease or process, and see what happens next, snap!, just like that.

His invention is called the multiplexing streak camera. It solves the problem of how to measure the intensity, wavelength and fluorescence lifetime of light at once. Working with collaborators Dr. Qiyin Fang at McMaster University, INO in Quebec and Spectral Applied Research in Ontario (which helped build Canada's first space telescope), he cobbled together a prototype device. The team set up different microscopes using mostly off-the-shelf components and assembled them in such a way that they could take all three measurements by scanning a single beam across the sample. They then amplified the effort so that they could scan 100 beams at once. The next step involves a neat bit of physics that rearranges the data, which come out in a square array, into a tidy line of points.

"Once you have the line of points, you can take them and put them into a glass prism, and now all the colours separate from each other, and you can also put the line of points into something called a streak camera, and then you can measure all the fluorescence lifetimes. We can measure the spectra, the intensity and the lifetime 100 times faster than any other microscope," says Andrews.

They have secured two provisional patents and are setting up a company to commercialize the device. They're building a production prototype, which Andrews predicts will be functional within two years. It won't supplant the retooled Opera, however. He'll use the streak camera to analyze a relatively small number of samples in detail, whereas he'll use the Opera to look at lots of samples in less detail.

ON TARGET

This new capacity to study protein-protein interactions where they take place, in the membranes of live cells, opens up myriad possibilities for identifying drug candidates. It will enable researchers to shut off genes or proteins associated with a particular disease or process, and see what happens next, snap!, just like that. Together, the microscopes are part of a system that uses robots to store a massive library of DNA, pull samples out upon command and organize them in just the way a researcher needs for automated analysis. "It allows anyone who is looking at a disease to find and validate targets for treatment," says Andrews. "You can look at the effect of knocking down hundreds of genes, instead of looking at them one at a time, and then use the robot and the software and the automated microscopy to say, 'Of these hundreds or maybe thousands of genes, which are the ones I really need to go back and look at in detail one at a time?' You can also use the equipment to screen small molecules [drug-like chemicals]."

To wit, he has already identified two small molecules that will shut down the pro-cell-death proteins Bax and Bak in live cells. He plans to use these molecules in a stroke model, where just briefly knocking down these executioners reduces the impact of stroke by preventing the death of oxygen-starved cells, results he hopes will convince a drug company the targets are viable.

Moreover, he's identified 18 new Bcl-xl inhibitors (retrieved with robotic aplomb from a library of 35,000 compounds), an especially relevant finding in light of the promise being shown by other such molecules now in patient trials, including navitoclax, a small molecule in phase 2 clinical trials for chronic lymphocytic leukemia. So far, results show that navitoclax prevents some of the anti-cell-death proteins from thwarting the executioner proteins, thereby permitting cancer cells to fulfil their mandate of self-destruction, boosted by chemotherapy. As Andrews explains, because cancer cells are trying to die, their capacity to deal with stress is lower than is that of healthy cells. "What the Bcl inhibitors do is reduce that reserve capacity even more, so the tumours will start dying of their own accord. Plus, because the Bcl-2 family is already engaged, when you come in with chemotherapy, the cancer cells should have no reserve capacity left, and they should just explode."

The makers of navitoclax, Abbott and Genentech, have worked with Andrews to analyze their drug and sent people to his lab for training.

Andrews is also looking at if activating Bax can selectively kill cancer cells, sleuthing for drug targets within the cell death pathway for hormonally triggered breast cancer, and examining the links between the oncogene myc and apoptosis, all on the basis of early promising results from his lab.

The technologies Andrews and his team have developed are game-changers for the drug discovery process. There are many examples of drug targets that looked intriguing but that couldn't be studied outside their live cell environment. Perhaps more importantly, these devices will be able to impart information about a compound's toxic effects much earlier in the discovery process, saving time and money.

We won't wake up to a headline tomorrow proclaiming that new therapies for cancer based on controlling cell death are here. Although several such therapies are in clinical trials, it could be 10 or more years before we see such reports—if all goes well. But if it does, then the clinical implications are as headline-worthy as it gets. It means doctors would be able to stop giving treatments that poison the whole body, and that may or may not work, determined only by time, to giving those that work selectively by targetting genes and proteins, where the outcome is known, and without harming healthy cells.

THE GREATEST "TRICK" OF ALL

He calls himself a cancer researcher, but the labels biochemist, engineer and medical biophysicist fit as easily, though perhaps not all on one business card. There's one more title that could be added: magician. Not for his science—though one could say that whoever succeeds in revealing these life-and-death mechanisms and designing therapies to control them will in effect become a wizard of apoptosis, able to switch on and off vital cell processes at will, no sleight of hand involved—but for his avid interest in performing stage magic, one he has held for twice as long, 40 years, as he's been studying apoptosis.

It's the psychology that grabs him the most, he says. "There are all the different kinds of people that will come and look at you and watch what you're doing. Some are fun. Some are not. But the people who enjoy it and don't mind suspending disbelief for a few minutes so that we can have fun and interact with each other, that I really like. I like it to be fun."

Fun like running smack into the wall of one science stumper after another, solving only some, but always pressing on, eye on the prize.

"I would desperately like to have enough money so that I would not have to write grants, not have to worry about all that stuff, and take on something high-risk, high-gain, and really plough all of my resources into it for 10 years to see if we can really accomplish something.

"I don't know exactly what it would be, but it would be something in the cancer therapy direction, with the Bcl-2 family of proteins. I would love to be able to map all of the interactions between them in such a way that you could really determine how they're regulating the physiology of the cell. Because if you understood exactly what it is that they're doing, then you would know how to manipulate them to have an impact on patients."

Andrews' research is funded by the Canadian Cancer Society Research Institute, Canadian Institutes of Health Research, Canadian Stroke Network and the Ontario Institute for Cancer Research. Infrastructure support was provided by the Canada Foundation for Innovation, Natural Sciences and Engineering Research Council, and Ontario Ministry of Economic Development and Innovation.

1. Google Scholar. Accessed January 31, 2012.
2. Kerr, J. F. R., Wyllie, A. H., & Currie, A. R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *British Journal of Cancer*, 26, 239–257.
3. Google Scholar. Accessed January 31, 2012.