People & Ideas

Sally Kornbluth: Nature's incredible contraptions

Kornbluth's laboratory studies cell cycle and apoptosis in cancer cells and *Xenopus* egg extracts.

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Sally Kornbluth

In multicellular organisms, many factors—including the condition of the cell's DNA and cellular metabolic status—are read into the decision of whether to proceed with the cell cycle or divert down the path toward apoptotic cell death. These considerations are all linked together through a labyrinthine network of interconnected signaling pathways and checkpoints whose operation is still being disentangled by researchers.

Sally Kornbluth revels in puzzling out the secrets of cell signaling pathways. Using *Xenopus* egg extracts (1, 2) and mammalian tumor cells (3, 4), her laboratory has investigated the regulatory mechanisms that usher cells through critical transitions in the cell cycle (5) and apoptosis (1–4). Now Kornbluth's career is about to undergo an important transition of its own. We called her at her laboratory at Duke University to talk about her work and her future plans.

WELL SUITED

Did you start out as a science major?

One of my teachers in high school was so fabulous in the area of political science that I actually became a political science major in college at Williams. A really good teacher can have a profound effect; you can't underestimate the impact of someone getting you excited about a subject.

I remember going through the science quad as a freshman tour guide and saying, "Well, this is the science quad, and I will never take a class here." It goes to show I was not completely self-aware as an 18-year-old. [Laughs] But I had to take a science course as part of my distribution requirements to graduate, and I took a class from Bill DeWitt, who was a fantastic professor for a class on human biology and social issues. I thought it was really interesting, and, once I saw what science was really about, I found it very exciting. I just hadn't had that opportunity in high school. I received a scholarship from Williams to go to Emmanuel College in Cambridge, England, for two years, and I did a second bachelor's degree there in genetics.

And then you continued with a PhD…

I had met my husband, who's also a scientist, and begun dating him at Cambridge. We were both applying for graduate schools in the United States, and we both wound up getting into Rockefeller University and decided to go there. I joined Hidesaburo Hanafusa's laboratory, and I have to say it was just an incredibly stimulating, fun place to be. There were so many great people there that are now prominent scientists.

Saburo's laboratory worked on the Src kinase, so by the time I was done with my degree there I was really interested in applying the study of cell signaling to physiological processes. But I was also really tired of doing tissue culture and wanted to learn another experimental system. When I went to interview with John Newport at UC San Diego, I was just really taken with the work he was doing in *Xenopus*.

> "The *Xenopus* system was perfectly suited to my personality."

The *Xenopus* system was perfectly suited to my personality. You have to think carefully about how you set up the experiments, but the extract you can make from *Xenopus* eggs is pretty forgiving. You can almost do bucket biochemistry with it. You make lysates, and you can throw things in or take things out and do a lot of experiments in pretty quick succession. It's the perfect biochemical system to elucidate very detailed signaling mechanisms.

It was also good for me to work in John's laboratory because, although Saburo and John were both outstanding scientists, the way they thought about science was very different. Saburo was very systematic. He had a really deep knowledge of his field that allowed him to synthesize novel combinations. John was a brilliant, out-of-the-box thinker who would come up with novel ideas that other people just wouldn't have thought of. My own approach is somewhere in between the two.

CHANGE IN PLANS

Early on, your work concerned the control of cell cycle progression…

When I first started my own laboratory at Duke, I was planning to work on mitotic entry and DNA checkpoints, very similar to what I had done in John's laboratory. One of the things I was trying to do was to purify a membrane-associated kinase that regulated CDC2, but within several months of getting my job at Duke Bill Dunphy identified and published the relevant kinase, Myt1. At that point I decided I had better rethink what I was going to work on, because Bill and I had both trained in John's laboratory, we both had access to the *Xenopus* system, and Bill is a really outstanding biochemist.

At the time, I had a rotation student in the laboratory who was trying to block nuclear export to see how this affected mitotic entry. Of course if you block nuclear transport, you don't get DNA replication and therefore trigger the DNA replication checkpoint. So in order to do the

Text and Interview by Caitlin Sedwick
csedwick@gmail.com

IMAGE COURTESY OF SALLY KORNBLUTH

***Xenopus laevis* eggs are an exceptionally versatile experimental system.**

experiment we had to replicate the DNA to completion and then transfer those fully replicated nuclei to another extract. The problem was, every time we transferred the nuclei along with some cytoplasm, they would fragment. When my student showed me this, I realized that this looked just like what another colleague, Don Newmeyer, had just reported about reconstituting apoptosis in *Xenopus* egg extracts.

Don had done a kind of Sigma catalog experiment where he threw a bunch of things into his extracts to see if any of them affected apoptosis, and he observed that phosphotyrosine blocked the process. Now, I had come from Saburo's laboratory, so the first thing I thought of was that phosphotyrosine is interacting with some SH2-containing protein and disrupting signaling. Ann Marie Pendergast's laboratory was downstairs at Duke, and she had recombinant expression vectors containing the SH2 domains of several signaling proteins in her freezer. So I looked to see if one of them could block apoptotic signaling. It turned out that the SH2 domain from the oncoprotein CRK was the one that worked best. Interestingly, in those experiments we had found that membrane was required in that initial incubation. I now realize that what we were probably doing was reconstituting ER stress in vitro.

Your laboratory has had a strong interest in the role of caspase-2 in apoptosis…

At first I was interested in caspase-2 as a sort of orphan caspase that didn't have any well-defined function. My postdoc Leta Nutt was looking at it in egg extracts to see if we could figure out what it was doing. But Leta came to my laboratory with an interest in both metabolism and cell death, so she started throwing different metabolites into the extract. It turned out that glucose-6-phosphate could potently inhibit apoptotic events in the extract. Leta then collaborated with Seth Margolis in the laboratory on a long series of biochemical experiments to tease apart the mechanism by which glucose-6-phosphate was inhibiting apoptosis. I think we would have gotten there eventually anyway, but Leta's two projects converged very quickly when she found out that caspase-2 was central for apoptosis in the egg extract and that the effects of metabolism in inhibiting apoptosis were exerted at the level of caspase-2: Glucose-6-phosphate prevents apoptosis by promoting inhibitory phosphorylation of caspase-2. Caspase-2 is maintained in this inhibited state by binding to 14-3-3, a protein that many others had shown earlier also regulates the activity of CDC25 in the cell cycle.

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FINDING CONNECTIONS

Is this pathway recapitulated in mammalian cells?

We've shown that key points are extrapolated to mammalian cells, but most of our mammalian work recently has been done in the context of cancer. I was interested in whether there might be control of apoptosis not only at the premitochondrial phase but also in the postmitochondrial phase, at the level of the apoptosome. We started in the egg extract system and found that a mammalian leukemic protein, BCR-ABL, blocked cytochrome c–induced apoptosis. That got us into thinking about whether something similar happens in leukemias, and obviously we had to study that in mammalian cells. As we were working on that, we became interested in other ways that cancer cells might control apoptosis, and we uncovered a complex pathway wherein a failure to degrade the E3 ubiquitin ligase MDM2 in lapatinib-resistant, HER2-expressing breast cancer cells helps to prevent apoptosis. That's something that's become a central theme of my work and the thing that I have found is the most fun to do: we wind up dissecting some byzantine signaling pathways that you never would have invented if you were trying to think of a logical way to do things.

What are you up to now?

I've been an administrator for eight years already, helping to administer the biomedical graduate programs and enhance the research infrastructure at Duke, so I've done a lot of running back and forth doing administrative tasks and interacting intensively with people in the laboratory. But I've recently been appointed provost at Duke, and that is a bigger job, there's no doubt about that. The provost oversees all the academic activities of the university, the schools of the university, student life, just a huge range of things. So, going forward my laboratory is going to be much smaller. I'm trying to arrange things so that everybody in the laboratory also has a co-mentor so that they don't only have to interact with me on projects and can move their projects through to completion. I'd never say "never" to taking on new students, but for now I am focusing on my new responsibilities.

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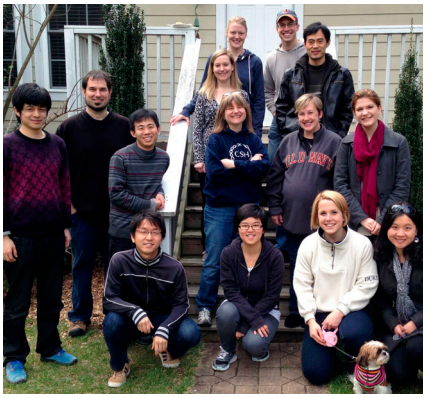

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The Kornbluth lab poses for a group photo on the Duke campus.