Crossing Boundaries

A groundbreaker in the study of *Listeria monocytogenes,* Pascale Cossart continues to build her research tool kit to understand how to fight such intracellular human pathogens.

By Anna Azvolinsky | September 1, 2014

PASCALE F. COSSART
Professor
Unit Head: Bacteria-Cell Interactions
Institut Pasteur, Paris, France
Senior International Research Scholar,
Howard Hughes Medical Institute
COURTESY OF PASCALE COSSART

In the early 1980s, Pascale Cossart was a researcher at the Institut Pasteur, working on the interaction of *E. coli* proteins with DNA, when the Pasteur's scientific director encouraged her to switch to the study of infectious diseases so as not to have to compete with bigger laboratories working on *E. coli* in the U.S. A biochemist with no biology training, Cossart chose to work on the food-borne bacterium *Listeria monocytogenes,* a little-studied and intriguing intracellular pathogen that can have serious consequences if it infects a pregnant woman or travels to the brain. Those with a compromised immune system are particularly at risk from complications of a *Listeria* infection. "?This was a time when researchers were only just beginning to use molecular biology to study organisms other than *E. coli*," says Cossart.

Cossart says she chose *Listeria* because a friend and colleague who worked with the species advised her that it would be a good choice for conducting infection-related molecular-biology experiments: "I really wanted to understand how the organism is able to penetrate and invade human cells." Since then, Cossart has extended her reach to encompass a number of biology disciplines in addition to bacteriology: fundamental microbiology, cell and molecular biology, and epigenetics. Her research, illuminating many aspects of how *Listeria* infects humans and evades the immune system, has established the bacterium as a model for studying bacterial intracellular behavior and bacterial regulation of host-pathogen interactions. (See Cossart's article "The Maverick Bacterium," *The Scientist*, January 2010.)

Here, Cossart reflects on her career at the Pasteur, how she became interested in biology relatively late, and how time spent thinking through research projects before taking them on can yield rewarding results.

Cossart Commences

A world of molecules. "I was not particularly interested in science when I was a child," says Cossart, but at the start of high school in Arras, a small town in northern France, she brought home her first chemistry textbook, whose array of structures mesmerized her. "It was a big discovery for me. I was fascinated because I had never thought of anything like this before. I was not from a scientific family, so I had never heard of these type of [concepts]." Cossart requested to be switched from her classics- and literature-heavy curriculum to a science-based one.

Early success. As an undergraduate, Cossart studied chemistry at the nearby University of Lille. She had begun a master's program in chemistry there when she took a biochemistry course offered by the biology department. "I loved it right away, and so much that I went to the professor, telling him I had made a mistake in choosing chemistry as a major and that I wanted to switch to work in a biochemistry laboratory." Cossart completed a certification in biochemistry before earning a master's degree in the field. She was offered a permanent teaching position at the University of Lille, where she remained for two more years.

A new world. When her research supervisor moved from the University of Lille to another university, Cossart, then just 22 years old, decided to apply for a research fellowship in the U.S. "I had colleagues

saying that I was crazy to leave a stable teaching position, but I had vacationed in the U.S. and really wanted to go there. This was a time when the job market for scientists was good, and I was ready for something new." In 1970 Cossart was accepted into a one-year master's program in the chemistry department of Georgetown University, working on lipid-protein interactions. "I loved it there! The research itself was not that exciting, but I learned how to be very precise in my experiments." Most importantly, Cossart valued the style of teaching, which was done in small groups with great attention paid to each student. "It was absolutely amazing and very different from the French system I was used to."

Back to the future. "My advisors at Georgetown told me that if I were to go back to France, I had to go to the Pasteur Institute." Pasteur was prominent on scientists' radars—a very active institution following the awarding of the 1965 Nobel Prize to André Lwoff, François Jacob, and Jacques Monod for their work on bacterial gene regulation and bacteriophages. During her Easter vacation, Cossart lined up her next move—a PhD with Georges Cohen at the Pasteur Institute working on protein biochemistry in *E. coli.* "I knew what I wanted to do—to sequence a protein. My project was very interesting but it was a bit unrealistic because the protein I was trying to sequence was huge. Nevertheless, the advantage of this project was that there was not much competition."

A lifelong home. After six months, Cossart was offered a one-year fellowship and then a permanent position at the Pasteur, even though she had yet to complete her PhD. "I got the position after a two-hour interview with Jacques Monod, who was the director at the time. I knew then I could stay there all my life. I have never wanted to leave."

Fearless transition. Cossart had been working solely on protein sequencing at the Pasteur. "It was very, very slow work that required growing huge cultures and then purifying a lot of protein. One day, Moshe Yaniv, a colleague who had just come back from visiting Harvard University, told Georges Cohen that they had set up a technique to sequence DNA and that it would be interesting to sequence the gene encoding the protein I was sequencing. I realized I would probably never finish my protein sequence anyway, so I rapidly wrote and defended my thesis and agreed to try to clone and sequence the *E. coli* thrA gene. This was really the beginning of my postdoc. I went to work in Moshe Yaniv's laboratory, which was just across the street. I had never worked with DNA, but it was lots of fun but also a really big, big job because the technique had not been published yet. We even had to even make our own ATP-gamma P32 to label the pieces of DNA. We were the first at the Pasteur to sequence DNA!"

Cossart's Contributions

Starting from scratch. Even though she had never worked with *Listeria*, Cossart had some hints that the bacterium would be an interesting model for studying intracellular pathogenesis. *Listeria* was easy to manipulate genetically, grew relatively quickly—almost as quickly as *E. coli*—and was adaptable to a wide range of growing temperatures and environments, from 4 °C to 37 °C. "I knew that there were related species that were nonpathogenic, so that we could compare the genes of *Listeria monocytogenes* to those of these nonpathogenic species."

A serendipitous discovery. In 1991, Cossart, who was still working at the lab bench, although she now headed a laboratory, isolated *Listeria* mutants that lacked the enzyme phospholipase, a protein she thought might play a role in the virulence of the bacterium by facilitating lysis of mammalian cell membranes. A postdoc in Cossart's lab, Christine Kocks, was using the mutant to investigate whether the *Listeria* surface protein Internalin A—which Cossart and her postdoc Jean-Louis Gaillard had previously shown is necessary for the bacterium's entry into mammalian cells—was also involved in the organism's ability to spread from cell to cell. "[Kocks] came back from the microscope and showed me that this mutant was not able to move around the cytoplasm of the host cell or to move from cell to cell."

In 1989, Lewis and Mary Tilney and Daniel Portnoy had demonstrated that *Listeria* polymerized actin at its tail end to propel itself through the cytoplasm of its host, but the *Listeria* gene that controlled this actin polymerization had not yet been identified. "Our lab had not been working on this actin-based motility, but here was this mutant and it was not moving. We figured out that the gene encoding the phospholipase I wanted to work on was just downstream from the gene that allows actin polymerization. The mutant I isolated had a silenced gene that was responsible for actin polymerization and a silenced phospholipase gene that was also within the same operon. So by isolating a phospholipase mutant I had also isolated a mutant that could not spread from cell to cell." Cossart and her colleagues named the new gene *actin gene A (ActA)*. "The ActA protein is on the surface of the bacterium, and this is the protein that somehow recruits a cellular complex and allows actin polymerization, which allows *Listeria* to move within the host cell and from cell to cell." Both Internalin and ActA are present on the surface of *Listeria*. Internalin is involved in the ability of the bacterium to enter the host cell while ActA functions to spread the bacteria from cell to cell.

A unique mouse model. To learn how *Listeria* behaves in the different types of epithelial cells it infects, such as those of the liver and intestine, Marc Lecuit, a Pasteur colleague who was then a PhD student in Cossart's lab, was attempting to infect murine cells in vitro, but was having trouble. "We thought this was a bit strange until we finally realized that the protein needed for invasion into cells, Internalin A, is very special. This protein can react with the human E-cadherin, its receptor on the human cell surface, but not with the mouse E-cadherin protein." Cossart's laboratory discovered that a single amino acid difference in the murine E-cadherin prevents *Listeria* from entering murine cells. "We made a transgenic mouse which expressed the human E-cadherin protein. This allowed us to orally infect the mouse, making it highly susceptible to *Listeria*. This is a model of the natural route for a *Listeria* infection, and it was the first humanized mouse model for *Listeria* and for a bacterial disease." Other models use an intravenous route of infection, which bypasses the intestinal barrier, allowing the *Listeria* to reach its target organs directly through the bloodstream.

> "Important questions give important results and identify major mechanisms."

Temperature sensor. Cossart has also tackled other aspects of *Listeria* molecular biology. She and colleagues had noticed that *Listeria*'s virulence factors are only expressed at 37 °C—body temperature—but not at room temperature or lower. "Below 37° ActA, Internalin A, and other proteins are not expressed, and neither is the regulator protein that controls the expression of these virulence factors." Cossart's postdoc Jörgen Johansson discovered that this is because the mRNA transcript of the virulence regulator gene, *PrfA,* is folded into a stem-loop structure at lower temperatures, preventing its translation. At body temperature, the mRNA unfolds, allowing the translational machinery to access the transcript and produce PrfA, which then turns on the virulence genes. "We called this PrfA transcript a thermosensor."

New directions. "Since the thermosensor study in 2002, we have become interested in noncoding RNAs, and with Alejandro Toledo-Arana [of the Universidad Pública de Navarra in Spain] we have been discovering a lot of these at work in *Listeria*." Cossart's laboratory recently discovered a new type of noncoding RNA that regulates survival of the bacterium. "Noncoding RNAs are important regulators in bacteria, and we are using *Listeria* not only to study the bacteria-cell interactions, such as infection biology, but also to study cell biology by examining the mammalian cell and the bacterium itself. *Listeria* is really a model for different aspects of biology." Cossart is also exploring how *Listeria* is able to reprogram its host cell's epigenetic profile and analyzing posttranslational modifications and organelle targeting by the bacterium. Together with colleagues Hélène Bierne and Alice Lebreton, she showed that *Listeria* manipulates chromatin. More recently, along with lab member Mélanie Hamon, Cossart has shown that upon infecting a cell, *Listeria* facilitates the translocation of a host deacetylase protein to the nucleus, where it interacts with host cell chromatin and induces histone modifications that affect the host cell's transcription programs—to the advantage of *Listeria*.

Cossart Contemplates

Big questions. Cossart says she has been successful because she has thought through projects before taking the plunge in the laboratory and because she wants to address important questions. "Important questions give important results and identify major mechanisms. When I switched from protein-DNA interactions to studying *Listeria,* together with my colleague Brigitte Gicquel, it took several months to think of what would be interesting aspects to study and what should be our future directions. My major concern was how this pathogen interacts with its host. It was known that the bacteria are infecting us through food, but not much else was known."

Factors for success. "There is no secret to success, really. It's hard work, long hours in the lab, and good health. You also need to be optimistic, because there are up and down periods. One needs to sometimes abandon projects and to start new ones. It is important also to go your own way and stick to one's ideas. I also think it is important to have a substantial amount of money in order to take on risky projects!"

A balanced life. "I have three daughters and I am therefore completely aware that life can be complicated; that it is hard to do science and at the same time raise kids. I think I understand for the students that work with me, both men and women, that life outside of the lab, that private life can affect their science. We all have to combine our personal lives with our scientific lives, to organize and create our own balance."

Greatest Hits

- Identified the two main bacterial proteins, Internalin A and InlB, used by *Listeria monocytogenes* to interact with human cell-membrane receptors, allowing for membrane remodeling, cytoskeletal rearrangements, and entry into the cells.
- Identified E-cadherin as the receptor for Internalin A.
- Identified the protein ActA, required for mobility of *L. monocytogenes* within the mammalian host cell.
- Created a humanized mouse model to study the natural route of an *L. monocytogenes* infection—through infection of gut epithelial cells.
- Utilized comparative genomics to identify a series of *L. monocytogenes* virulence factors, necessary for intracellular survival and infection.
- Discovered that an RNA thermosensor was responsible for activation of virulence factors at host body temperature.
- Discovered several new types of regulatory noncoding RNAs important for virulence.
- Revealed that disruption of host mitochondrial function by a bacterial toxin promotes efficiency of early *L. monocytogenes* infection.
- Identified the mechanism by which *Listeria* impairs SUMOylation of host proteins to promote efficient infection.

Tags

noncoding RNA, Listeria monocytogenes, infectious disease, disease/medicine and cell & molecular biology

Add a Comment

You

Related Articles

Chagas Watchdogs

By Tracy Vence

Can screening dogs for *Trypanosoma cruzi* antibodies inform public health officials about the risk of Chagas disease to people?

Entry Requirements

By Nicholette Zeliadt

Recent developments in cell transfection and molecular delivery technologies

Head Scratchers

By Mary Beth Aberlin

Many natural phenomena elude our understanding.