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A CRISPR VISION

Emmanuelle Charpentier spent years moving labs and relishing solitude. Then the co-discovery of CRISPR–Cas9 explosively changed her life.

BY ALISON ABBOTT

Emmanuelle Charpentier's office is bare, save for her computer. Her pictures, still encased in bubble wrap, are stacked in one corner, and unpacked cardboard boxes stuffed with books and papers are lined up in the adjacent room. But across the corridor, her laboratory is buzzing with activity. When Charpentier moved to Berlin six months ago, she had her science up and running within weeks, but decided that the rest could wait. "We were all determined to get the research going as fast as possible," she says, leaning forward from her still-pristine office chair.

Charpentier's workspace is a fitting reflection of her scientific life — one in which she always seems to be moving while keeping science on the go. Now 48, she has climbed her way up the academic ladder by way of 9 different institutes in 5 different countries over the past 20 years. "I always had to build up new labs from scratch, on my own," she says. Her eureka moments have occurred amid packing boxes and, after years on short-term grants, she was 45 before she was able to employ her own technician. "She's so resourceful, she could start a lab on a desert island," says Patrice Courvalin, her PhD supervisor at the Pasteur Institute in Paris.

The itinerant lifestyle doesn't seem to have hampered the microbiologist as she has carefully dissected the systems by which bacteria control their genomes. Charpentier is now acknowledged as one of the key inventors of the gene-editing technology known as CRISPR–Cas9, which is revolutionizing biomedical researchers' ability to manipulate and understand genes. This year, she has already won ten prestigious science prizes, and has officially taken up a cherished appointment as a director of the Max Planck Institute for Infection Biology in Berlin. The gene-therapy company that she co-founded in 2013, CRISPR Therapeutics, has become one of the world's most richly financed preclinical biotech companies, and she is in the middle of a high-profile patent dispute over the technology. Last September, Charpentier's phone kept on ringing. Journalists from around the world were trying to reach her, thinking — prematurely, as it turned out — that the imminent announcement of the 2015 Nobel prizes might well include her.

The academic limelight is not a comfortable place for Charpentier, which is why she remains the least well known member of the small international group tipped for the 'CRISPR Nobel', if it arrives. "Jean-Paul Sartre, the French philosopher, warned that winning prizes turned

Emmanuelle Charpentier: a key inventor of the gene-editing technology CRISPR–Cas9.

you into an institution — I am just trying to keep working and keep my feet on the ground," she says. She seems to be succeeding, this week publishing a paper[1] in *Nature* that reveals the mechanism of a CRISPR system that might prove even more efficient than CRISPR–Cas9.

Colleagues who know Charpentier well describe her as intense, modest and driven. "She's a tiny person, with a very strong will — and she can be pretty stubborn," says Rodger Novak, who was a postdoctoral researcher with her in the 1990s and is now chief executive of CRISPR Therapeutics. As Courvalin sees it, "She is like a dog with a bone — tenacious."

MEDICAL MISSION

Small and slight, with eyes so dark that they seem black, Charpentier looks as restless as she evidently is. Growing up in a small town near Paris, she had a clear idea from the start of what she wanted in life: to do something to advance medicine. A visit to an aunt, a missionary who was living in an old convent, set her dreaming of being able to do this "in a lovely setting, where you can be a bit alone with yourself".

Her socially engaged parents, she says, supported her ideas without guiding her in any direction. She pursued piano and ballet — but her leaning towards medicine eventually flowered into studies in life sciences. As an undergraduate at Pierre and Marie Curie University in Paris, she decided to do her PhD at the nearby Pasteur Institute, which was gaining a strong reputation in basic research and had a programme on antibiotic resistance that she wanted to join. Her PhD project involved analysing pieces of bacterial DNA that move around the genome and between cells, allowing drug resistance to be transferred.

> "SHE'S SO RESOURCEFUL, SHE COULD START A LAB ON A DESERT ISLAND."

Her years at the Pasteur Institute were formative. Her department in the historic institution was "young and fun", she says. She loved to study at the old St Geneviève library close to Notre-Dame Cathedral, happily isolated in the triangle of light from the green-topped desk lamps. "I realized I had found my environment," she says. Her ambition was to lead a lab at the Pasteur, and she decided that this would require a postdoc period abroad to gain expertise. "I was a typical French student of the 1990s — I imagined that after a short excursion I would work the rest of my life at home."

Charpentier sent out 50 or so exploratory letters to labs in the United States, and got a postbag full of offers in reply. She chose a position with microbiologist Elaine Tuomanen at the Rockefeller University in New York City to work on the pathogen *Streptococcus pneumoniae*. This microbe, which is a major cause of pneumonia, meningitis and septicaemia, has a particularly free-wheeling relationship with mobile genetic elements, shifting them about its genome while maintaining its vicious pathogenicity. Tuomanen's lab had priority access to it's recently sequenced genome, offering the tantalizing prospect of discovering where these elements were landing and what happened when they did.

Charpentier carried out a stream of painstaking experiments to work out how the pathogen monitors and controls such elements, and contributed to a study identifying how the pathogen acquires resistance to vancomycin, an antibiotic of last resort[2]. She had set out for New York with some trepidation but, absorbed in her work, was surprised to find that she wasn't homesick. When Tuomanen moved her lab to Memphis, Tennessee, Charpentier wanted to stay, so she found a home in the lab of skin-cell biologist Pamela Cowin at New York University School of Medicine, where she also had the opportunity to learn about mammalian genes through working on mice.

Cowin remembers Charpentier as her first postdoc who did not need looking after. "She just ran with the programme," she says. "She was driven, meticulous, precise and detail-oriented" — as well as a rather quiet, private person. Charpentier soon discovered that genetically modifying mice was a lot harder than manipulating bacteria. She spent two years on the project and emerged with a paper on the regulation of hair growth, a solid grounding in mammalian genetics and a strong desire to develop better tools for genetic engineering.

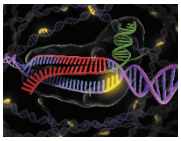
After another postdoc in New York, Charpentier knew that her next step needed to be complete independence — and a move back to Europe. Her time in the United States had taught her that she was European rather than solely French, and she chose Vienna. She arrived at the university there in 2002, and spent the next seven years running a small lab that was precariously dependent on short-term grants. "I had to survive on my own," she says. Nevertheless, "I had in mind to understand how every biochemical pathway in a bacterium was regulated." It was an exciting time scientifically, with the importance of small RNA molecules in regulating genes being revealed, and she embarked on many different projects on various bacteria — possibly too many, she admits, but she kept winning the grants. She discovered an RNA that controls the synthesis of a class of molecules that are important for virulence in the bacterium *Streptococcus pyogenes*[3].

It was in Vienna that Charpentier first found herself thinking about CRISPR. In the early 2000s, this was a niche area: only a handful of microbiologists were paying attention to the newly discovered, curiously patterned stretch of DNA called CRISPR in the genome of some bacteria, where it serves as part of a defence system against viruses. By copying part of an invading virus' DNA and inserting it into that stretch, bacteria are able to recognize the virus if it invades again, and attack it by cutting its DNA. Different CRISPR systems have different ways of organizing that attack; all of the systems known at the time involved an RNA molecule called CRISPR RNA.

Charpentier was interested in identifying sites in the genome of *S. pyogenes* that made regulatory RNAs — and found that bioinformatics took her only so far. So she forged a collaboration with molecular microbiologist Jörg Vogel, then a junior group leader at the Max Planck Institute for Infection Biology, who was developing methods for large-scale mapping of RNAs in a genome. He agreed to map *S. pyogenes* — and by 2008 he had sequences of all of the small RNAs generated by the bacterium.

The first thing that the researchers noticed was a super-abundance of a novel small RNA that they called trans-activating CRISPR RNA (tracrRNA). From its sequence and position on the genome — it was at a location that Charpentier's bioinformatics had predicted as being close to the CRISPR site — they realized that it was highly likely to be involved in a CRISPR system that had not previously been described. Charpentier and her colleagues began a long series of experiments to explore this system, identifying that it had just three components — tracrRNA, CRISPR RNA and the Cas9 protein. This was a surprise: "Other CRISPR systems involved just one RNA and many proteins, and no one had really thought that two RNAs might be involved," says Charpentier. The system was so exceptionally simple that she realized that it might one day be harnessed as a powerful genetic engineering tool. If the components could be controlled, it might provide the long-sought ability to find, cut and potentially alter DNA at a chosen, precise site in a genome.

But how exactly was this CRISPR system working? Charpentier suspected that the two RNAs might actually interact with each other to guide Cas9 to a particular DNA sequence in the virus. The concept was radical; that type of teamwork is routine for proteins, but not for RNAs. But Charpentier "always looked for the unexpected rather than the expected in a genome", says Tuomanen. "She is a very counter-culture person." Charpentier remembers that it was hard to persuade any of her young students to follow up her intuition and perform the key experiment to test whether the two RNAs might interact, but eventually a masters student at the University of Vienna, Elitza Deltcheva, volunteered.



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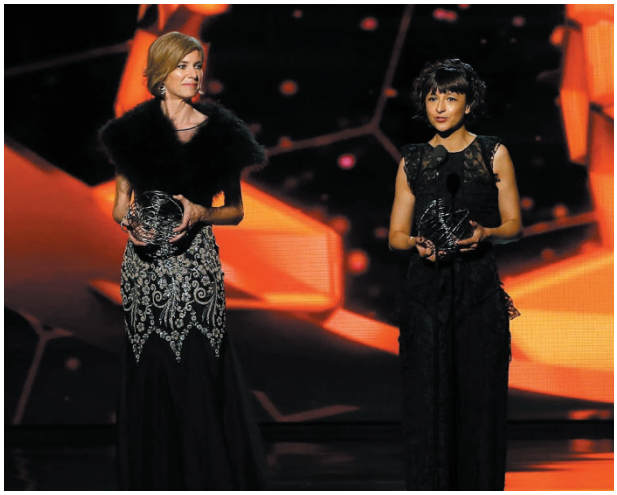
By then, it was June 2009, and Charpentier was again on the move. She had never felt completely at home in Vienna, where she says the grandiose architecture oppressed her. And she knew that she had to find more security and support. "At this time in my career, I needed the luxury of being able to focus on finalizing a big, cool story," she says. She took a position at the newly created, well provisioned Umeå Centre for Microbial Research in northern Sweden. The pretty, human-scale architecture of the old town made her feel comfortable, and she even learned to like the long, dark winters, which made her lose the feeling of time, allowing an even greater focus on work.

In summer 2009, she was still commuting between Austria and Sweden when Deltcheva called her in Umeå at 8 p.m. to tell her that the experiments had worked. "I was very, very happy," Charpentier says. But she told no one. Vogel says that it was "a very intense time". He recalls getting a call from Charpentier one night that August when he was driving on a country road outside Berlin. "I stood on the kerbside for ages while we discussed when would be the right time to publish, because by then we had actually got the story."

> "THE SCIENTIST THAT I AM GOT ME HERE, AND THAT IS THE SCIENTIST THAT I WANT TO REMAIN."



STEPHEN LAM/REUTERS

Jennifer Doudna (left) and Emmanuelle Charpentier receive the Breakthrough Prize in Life Sciences in November 2014.

They both knew that this discovery was going to be a game-changer, but both were afraid of being scooped if word of the system they had stumbled on got out. To make sure that publication would not be drawn out by referees' queries, they worked doggedly and silently for more than a year to cover as many bases as they could think of before submitting to *Nature*[4].

Charpentier was unknown in the then-small CRISPR world. She presented the work for the first time in October 2010 at a CRISPR meeting in Wageningen, the Netherlands, a few weeks after submitting it for publication. "It was a highlight of the meeting — a beautiful story that was extremely unexpected and came right out of the blue," says microbiologist John van der Oost of Wageningen University, who organized the meeting. Charpentier didn't mind being an outsider. "I have never really wanted to be part of a cosy scientific community," she says. And she was already thinking ahead to the next step — how this neat dual-guide RNA system actually led to cleavage of DNA.

At a 2011 American Society for Microbiology conference in San Juan, Puerto Rico, she met structural biologist Jennifer Doudna of the University of California, Berkeley. Doudna was immediately charmed. "I loved her intensity, which was apparent from the moment I met her," she says. They began a collaboration that swiftly led to the second key discovery showing how Cas9 cleaved DNA[5]. With the mechanism elucidated, researchers went on to show that the system could indeed be adapted to make targeted cuts in a genome and to modify a sequence. The technique has since been embraced by labs around the world.

Charpentier, meanwhile, made two decisions. The first was in deference to her original ambition to do something to advance medicine. She contacted Novak, who was by then working at the pharmaceutical firm Sanofi in Paris, with the intention of co-founding a company to exploit the methodology for human gene therapy. CRISPR Therapeutics, based in Cambridge, Massachusetts, and Basel, Switzerland, was born in November 2013 with a third co-founder, Shaun Foy, and Charpentier remains chair of its scientific advisory board.

The second decision was in deference to her ambition to fully dedicate her time to basic research in gene regulation. For this she wanted a permanent post, with more institutional support.

In 2013, she moved to Germany to become a professor at the Hanover Medical School and a department chief at the Helmholtz Centre for Infection Research in nearby Braunschweig, where she finally got her own technicians and built up a lab of 16 PhD students and postdocs. Just over two years later, she was recruited by the Max Planck Institute in Berlin. Now she has generous technical and institutional support, and her labs are in the elegant, nineteenth-century campus of the Charité teaching hospital, an environment she can relax in. Maybe in a few years, she says, she'll even find a few moments for reading philosophy.

But right now, fame and prize-winning leave little time for that. She values the recognition, engaging fully with the publicity activities that each prize requires — but notes anxiously that on average, each takes two full days from work. She declines to discuss the high-profile and rather complicated patent dispute between herself — alongside Doudna and Berkeley — and the Broad Institute of MIT and Harvard in Cambridge, Massachusetts. She leaves that to the patent lawyers, who are currently arguing it out.

Her focus is still on research, and her latest paper[1] — an elaboration of a CRISPR system that is even simpler than CRISPR–Cas9 — was once again finalized in the middle of a lab move. The work shows that a protein called Cpf1 can do the jobs of both tracrRNA and the Cas9 protein — "a very important contribution", says van der Oost, and part of a flurry of recent studies on this system[6,7]. But Charpentier is keen not to be defined by CRISPR, which is just one of five themes in her lab; others include the mechanisms by which pathogens interact with host immune cells and the molecular complexes that regulate the behaviour of bacterial chromosomes.

Reflecting back, she feels that her life has been tougher than it need have been. She notes that now there are more sources of major grants to help young investigators to start their own independent labs. And although her goals to further medicine and improve genetic-engineering tools have been met, her ambitions have not waned. "I haven't changed, and I won't change," she says. "The scientist that I am got me here, and that is the scientist that I want to remain."

But some things have changed. Charpentier is not an outsider any more: she is an established member of the rapidly expanding CRISPR community and is inundated with invitations to give talks. Her mischievous ambition, however, is to show up at a CRISPR meeting and report the discovery of something entirely different, but equally important. She has a few things up her sleeve, she says. ■

Alison Abbott *is* Nature*'s senior European correspondent.*

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