INTERVIEW

Major Insights into Microbiology: An Interview with Luciano Marraffini

Kevin Davies and Luciano Marraffini

For more than a decade, Luciano Marraffini has played a major role in the CRISPR revolution. As a postdoc with Erik Sontheimer at Northwestern University, Marraffini demonstrated that CRISPR targets DNA, more or less like a restriction enzyme. As a young faculty member at The Rockefeller University, Marraffini forged an important collaboration with Feng Zhang in early 2012 that laid the foundation for the landmark demonstration of genome editing in mammalian cells. He continues to conduct important research on various aspects of CRISPR and microbiology, helping to preserve Rockefeller's steeped legacy in microbiology research.

Marraffini recently hosted Kevin Davies to discuss his career path, research highlights, and some future plans. (This interview has been lightly edited for length and clarity.)

Davies: Luciano, you had a fabulous 2019—appointment to the Howard Hughes Medical Institute, election into the National Academy of Sciences and tenure. Congratulations!

Marraffini: Thank you. Yes, it was an incredible year. I do not think there is going to be another one like this. But I'll take it.

Davies: You're originally from Argentina. What brought you to the United States?

Marraffini: I studied biochemistry as an undergrad. When I finished school, I decided to apply to American schools for my PhD and was fortunate to get accepted at the University of Chicago, which is where I ended up doing my PhD.

Davies: You hadn't heard of CRISPR at this point?

Marraffini: No! I started my PhD in 2002 in the lab of Olaf Schneewind, studying bacterial pathogenesis. We studied *Staphylococcus aureus* pathogenesis, as well as *Bacillus anthracis* pathogenesis. At that time, when the anthrax scare was taken very seriously, there was a lot of funding for research on anthrax.

During my PhD, there was a professor named Malcolm Casadaban. He passed away, unfortunately. But he introduced me to CRISPR. He used to talk to people in Olaf's lab. He was a person who got very excited about many things in science. One day, he came to my bench and started talking about bioinformatics studies on CRISPR—[Mojica], Koonin, and Bolotin—the impression I had was that this was similar to restriction systems.

Malcolm was a phage person. He developed a very cool technology in the 1980s called mu transposons, which enabled lots of genetics screens... He was very savvy on phage, bacteria interactions, and technology. He found CRISPR had the possibility of having something programmable at the time. It was very exciting, I assume, for a lot of people.

When I was finishing my PhD, I didn't know exactly what to do. I decided to give CRISPR a try—to see if this prediction that CRISPR was a defense system that would prevent phage or plasmid infection was true. This is one of these things that you have to be a little bit lucky.

I was trained in staphylococcal genetics. So, my natural instinct was to look at whether there is any staphylococcus that has a CRISPR system. I was confident that if there was one, I should be able to test it somehow.

I found one. It's called *Staphylococcus epidermidis*. We know it has a type III system, and one of the spacers matched a conjugative plasmid that is common among staphylococci. This was a bit of luck because at the same time, Rodolphe [Barrangou] was probably finishing CRISPR mutations against phages—the first experimental paper on CRISPR.¹

It was good for me that even without knowing who Rodolphe was (or what he was working on), I started working on plasmids and not with phages. Otherwise, I would have been scooped by Rodolphe. The study with staphylococcus plasmids transitioned into my post-doc position with Erik Sontheimer.

In my last year of grad school, I was able to demonstrate that this particular spacer in a CRISPR system prevents the transfer of conjugative plasmids into staphylococci. Erik was working on RNA interference, which is what all the bioinformatics papers were predicting—that this was like RNAi in bacteria. It seemed like a good prediction because it was mediated by small RNAs, and there is no RNAi in bacteria. Maybe this was the missing link.

Erik had very good publications on RNAi and *Drosophila* at Northwestern. I sent him my formal application. Then, we met and Erik looked at the data. He probably knew everything that could be learned at that time on CRISPR. It was not that much. He immediately got on board. I think he saw the same thing that some others and I who started very early saw in CRISPR—something really, really cool. He accepted me into his lab. I'm very grateful to Erik because it was a leap in the dark.

Davies: His was not a microbiology lab, so that was a bit of a leap for him, too.

Marraffini: Right, by accepting me as a postdoc, he had to open a new line of research in his lab, which can be difficult. He did not, of course, have funding to work on CRISPR. Nevertheless, he accepted me, and we got a small National Institutes of Health (NIH) grant. I also applied for a postdoctoral fellowship with Jane Coffin Childs. That was the initial funding we needed. It was in Erik's lab that we had really pursued the idea of DNA targeting versus RNA targeting.

Davies: That led to one of the milestone papers in the early history of CRISPR.² What stands out as you look back at that period?

Marraffini: The paper² is very clear at the end that if CRISPRs are programmable—I think we use the term “DNA destruction”—systems, they have many biotechnological applications. That turned out to be true, and it is not a minor biotechnological application. It is something

that is going to change the world, right? That is a very good feeling.

I was always a champion of DNA targeting by CRISPR systems. But then after many years of research, well, there are systems that target RNA, and I learned how science is never definitive. That's the beauty of it—even these type III systems use guide RNAs to recognize RNA, and then cleave the DNA. I have to credit to Erik when we wrote the paper. He put in a lot of cautionary sentences.

That paper also taught me you just need to present all the possibilities. The paper was submitted in the summer of 2008 and accepted in November.²

Davies: This was a year or so after the Barrangou et al.¹ paper?

Marraffini: Yes. Rodolphe's paper in 2007¹ had a lot of implications. For me personally, it was very encouraging. I was happy I was not scooped, and we still have work to present on plasmids. But more importantly, I saw that CRISPR was very interesting, not only to me, because the paper was published in *Science*. That probably helped Erik make the decision of “Yes, I'll take this guy.”

Then Jill Banfield had another *Science* publication in early 2008.³ These are very high-profile papers. It was very encouraging.

Davies: Did you feel that you had enough scientific success that you could now look for a junior faculty position?

Marraffini: I thought that there was enough to start my own lab. CRISPR as a field was just beginning, and there were so many things to do. CRISPR had lots of unique features that immediately attracted scientists who were interested in basic science because this adaptive immunity in prokaryotes, RNA-guided nucleases, those two things alone go a long way—at least when I interviewed for jobs because I think they were very attractive.

Very little was known. I had a system, I had all the tools, and it seemed like the system was working. I think it made me a successful candidate.

Davies: Obviously, you ended up here in New York at Rockefeller. What persuaded you to come here?

Marraffini: Yes, I interviewed at Harvard and MIT. I still have a very high regard for MIT because that was the other offer that was difficult to turn down. Yale, NIH, and others. New York is, of course, very international. We are immigrants, and my wife really liked the idea of living in New York. We started here in 2010.

Davies: How did the collaboration with Feng Zhang begin?

Marraffini: He emailed me, very early in 2012, during the holiday break. That is another example of how some people paid attention to the paper in 2008 with Erik. Feng was one of them.

He mentioned in his email, “I read your work on *Staphylococcus* and other CRISPR systems that target, destroy DNA, and I would like to use it for gene editing in human cells. Would you like to collaborate?” I immediately said yes.

I was very excited about the email. I had to Google who Feng was. He had all the credentials. He’d been working in genome editing. It was a very good collaboration. We were just two very young PIs: one with all the expertise on gene editing, whereas I had all the expertise on CRISPR. We teamed up and created something that I’m very proud of.

Davies: Prior to Feng’s outreach, were you considering gene editing as an application of CRISPR?

Marraffini: I started the lab with the type III system, continuing what I was doing in Erik’s lab. Quickly, we transitioned to type II. That was something that I wanted to do, being here at Rockefeller.

Rockefeller has many famous scientists, but one of them I admire is Oswald Avery, who in the 1930s and 1940s demonstrated that DNA is the molecule that carries genetic information by studying the transformation of pneumococci, *Streptococcus pneumoniae*. When I came here, I decided to check if CRISPR could prevent the DNA uptake that pneumococcus does.

I repeated all the Avery experiments, but I had to put a CRISPR system into *S. pneumoniae*. I don’t think there is any strain of *S. pneumoniae* that carries CRISPR. The closest I could find was *Streptococcus pyogenes*. That is how I started working on *S. pyogenes* Cas9.

I put CRISPR into pneumococci in 2011. We started doing all these Avery experiments. We even did the earlier Griffith experiment—the famous experiment of transformation in mice.

We published that paper in *Cell Host and Microbe*.⁴ But around 2011–2012, we had been working with Cas9 and were very familiar with how to program it because we had to program Cas9 to target the capsule genes. *S. pneumoniae* do not have a capsule gene, so they form very little colonies. When they acquire the capsule genes, they form these big and glossy colonies that are pathogenic.

The idea was to transform pneumococci with these capsule genes. Of course, the *S. pyogenes* system does not naturally target the capsule genes of pneumococci. So,

we programmed it. We learned during 2011–2012 how to program Cas9 against any DNA that we wanted to.

My student, Wenyan Jiang, and postdoc David Bikard—now a professor at the Pasteur Institute in France—started playing with this. Immediately, they saw that we can make mutants in pneumococci. This is a funny story that has never been made public. I’m ashamed to admit that both of them pressed, “Let’s publish this method of making mutants with CRISPR and Cas9.” I said, “I don’t know. There are too many methods to make mutants in pneumococci. I’m not sure that this will be a big deal.”

That makes sense: being just a new lab, you try to do the most significant science. I only had three or four people in the lab. So, I had to decide which projects to prioritize. So we passed, which, of course, it wouldn’t have been gene editing of eukaryotic cells, but it could have been the first gene editing paper using Cas9, very early on. At the same time, things work out.

Davies: Was your choice influenced by the fact that it was the same organism that Charpentier had been using? Was it clear that this was a very tractable system?

Marraffini: There are two aspects. One is that in 2010, Emmanuelle published that tracrRNA paper.⁵ So, I felt confident that I understood what was required to transport the Cas9 system from one organism to another. Now, we see it as trivial. But it is not that trivial because maybe you need some genes other than Cas9.

But at the time, with the description of the tracer, it felt that this was probably the minimal system—which, by the way, is what I told Feng when he contacted me because he cited my previous work on staphylococci. I said, “No, we’ve been working with Cas9 and pyogenes. This is the one that we’re able to transport into a different organism.”

Of course, the jump from *S. pneumoniae* to *S. pyogenes* is not the same as jumping into eukaryotic cells. But at least it is something. And we knew how to program it, the PAM it required. Everything was working so well. I’ll let him comment on whether that was important—persuasive or not. That was, in the end, what we published. The paper with Feng is mostly based on pyogenes Cas9.⁶ Emmanuelle’s paper gave me the confidence that I understood the system.

The other part is the environment at Rockefeller, which has the largest collection of *S. pyogenes* strains in the world. There was a scientist at Rockefeller early on, before Avery, named Rebecca Lancefield. She is one of the mothers, so to speak, of serotyping. She collected both serum and bacteria from people with strep infections, and using the serotype, she was able to see which serum reacted to different isolates. She established a strain classification,

which was very important not only for strep but also for other diseases that follow the same kind of methodology.

We have a collection here from the late 1800s of *S. pyogenes*, which at some point we're going to sequence and see if there are some other Cas9s in there. We have 5,000 strains! Another famous Rockefeller lab is Vince Fischetti. He has inherited the *S. pyogenes* collection. Vince's lab is the number 1 lab in the world that studies *S. pyogenes*. I felt confident that I had an expert here that could help me with this experiment. Those were the reasons to move into pyogenes Cas9.

Davies: Your collaboration with Feng Zhang began in early 2012 and peaked 12 months later?

Marraffini: Right. As you can imagine, transporting the system from bacteria into human cells, you're a little bit in the dark. You need to add nuclear localization signals to target different targets. Everything seems very simple now. But at the time, we did not know. We performed routine checks—for example, does Cas9 with a nuclear localization signal work in bacteria? If it works in bacteria, then you have the green light to move it. You hope that it still works in human cells. If, immediately after putting this sequence in Cas9, it stops working in bacteria, then you know that it's not going to work. We did those kinds of checks.

We also checked different targets, which again seems very trivial—Cas9 cleaves anything. But we wanted to cleave a particular human locus—in HEK293 cells, we wanted to make sure that Cas9 was actually able to target and cleave that DNA. If that happens in bacteria, then, again, we feel confident.

Davies: How confident were you that this was going to work in human cells? Were you encouraged by the fact that ZFNs and TALENs are also able to work in that context?

Marraffini: I don't think we thought too much about it. We were not afraid of it not working. I don't think we would have stopped doing the experiments just because it seemed unlikely that it was going to work. At the same time, human chromatin is very complex. What if Cas9 is kicked off the human DNA by all these structures that are not present in phages or bacterial chromosomes? It might have not worked. But I don't think we had that worry in mind. We were going to try. Fortunately, we did not give up because it didn't work immediately! We started the collaboration in 2012, and it took several months to start working.

Davies: Do you recall when the idea for the single-guide RNA became a practical part of the system?

Marraffini: The Doudna–Charpentier paper is when we all learned about the single-guide RNA.⁷ That was online in June 2012. Of course, it was an improvement of the system. Instead of having to deal with three elements of the guide RNA, the tracer RNA, and Cas9...

At the time their paper came out, I think we had already started working with the three-component system. Everything was cloned as a three-component system. I don't think we switched immediately. In the end, after seeing the paper, we recognized the value of the single-guide RNA. There is a figure in the paper where we used a single guide. But the first experiments were all using three components.

Davies: Your *Science* paper—your student Wenyan Jiang was also a co-author—came out in January 2013.⁶ What was the reaction of the community? George Church had similar findings in the same issue, and many other groups besides.⁸

Marraffini: I remember speaking at a Rockefeller faculty meeting and people got really, really excited. In the end, this idea that David [Bikard] and Wenyan, my postdoc and student, had of using CRISPR for gene editing in bacteria, we did end up writing a paper. That was also published in early 2013 in *Nature Biotechnology*.⁹ I think that is the first bacterial use of gene editing using Cas9. I mostly talked about bacterial gene editing, and at the end, I said, "This also works in eukaryotes," and people went, "Wow!"

We think that when we publish papers, everybody reads them. But that's not true, right? I do not think that people immediately got the idea how revolutionary this was going to be. There is nobody to blame. At the beginning, it was just one more cool technique, and we'll see how useful it is.

I remember very quickly, maybe March–April 2013, I started receiving emails from my colleagues at Rockefeller saying, "We're going to try this. We need advice." Shortly after, the emails were, "This is amazing. It works incredibly well!" Sometimes, I review undergrads who apply for the PhD program at Rockefeller. Many of the letters of recommendation say, "We gave this undergrad the task of trying CRISPR, and he or she did fantastic work and set it up for my lab." That to me is a very good measurement of how revolutionary this technique is.

The *Science* papers, George and Feng's, at the beginning, probably they were seen as, "Okay, this is an interesting technique." But not everybody will jump because somebody who is doing gene editing of cells will completely shift the research program from TALENs, let us say, to CRISPR at that time, just because there is a paper. What they want is to try it and see how it works.

With CRISPR Cas9, at least the evidence that I have from these PhD applications, even the undergrads who had very little research experience, they were able to make it work, which is a reflection of how powerful and easy the technology is.

Davies: Several companies have emerged using the CRISPR technology that you helped launch. How did you get involved with Intellia?

Marraffini: Intellia was mainly the idea of a venture capitalist, Nessim Berningham. Early on, he wanted to speak with me. We had lunch, and he expressed his views for a company dedicated to human gene therapy. He believed that this was the right moment that CRISPR was going to be that area of research.

After talking to scientists, he became even more convinced, and looked for capital to start the company. When that happened, he invited me, Rodolphe [Barrangou], Erik [Sontheimer], and Jennifer [Doudna] to join the company.

That it is the dream of almost every scientist. If we can give back to society in any way, I think it's very important. If the CRISPR technologies, either the basic research that I did or the company that I co-founded, help develop cures for even a small number of genetic diseases, it would really be an amazing accomplishment.

Davies: What will be the secret of Intellia's success? Is it the people or the strategy or the delivery platform or the choice of therapeutic targets?

Marraffini: I've always been very impressed with the people who are in charge. Every time I go there, I come back very, very impressed—I have confidence in the company because I see the people who are working on this. Of course, they've made very good advances, which is a reflection of having good people. We will see.

Davies: Let's talk about the current scope of your research programs. I suppose you have moved beyond Cas9?

Marraffini: The goal of the lab is to understand the mechanistic level of all the different CRISPR systems. We've talked about type II, type III. But there are six different types. We are very lucky to have people such as Eugene Koonin (NCBI) who do an amazing job at discovering these new variants of CRISPR.

We try to characterize them in their native hosts or how they work in bacteria. We did that recently with

type VI, one of the exclusively RNA targeting systems. They do not protect by killing the invader. They mostly protect by killing the cell that is infected. It is more a eukaryotic-like type of defense than some sort of apoptosis, where the cell that is infected goes down, and the phage goes down as well. I think it is a new strategy for CRISPR systems.

I'm also interested in the other side of the coin. CRISPR basically is an anti-phage (and anti-plasmid) defense system against viral infections. Viruses of bacteria and archaea are the most diverse "organisms" on earth. It would be good to start focusing on how different phages—instead of how the same phages are defended by different CRISPR systems—maybe take one CRISPR system and see whether it can and how it defends against many different phages that have very different life-styles and lytic cycles. Probably there are going to be some surprises there...

CRISPR systems are the same in archaea and bacteria for the most part, but the DNA repair systems are very different. The phages are also very different. I have some personal curiosity about archaea that I'm interested in pursuing.

Davies: You mentioned we now have six CRISPR systems. Is that going to be the last word?

Marraffini: You'll have to ask Eugene! But probably not, right?

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