THE INNER WORKINGS OF THE BRAIN

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JUST LIKE SEQUENCING BEFORE IT, GENE EDITING TECHNOLOGY IS GETTING FASTER, EASIER, AND MORE ACCESSIBLE. IT'S OPENING UP SOME VERY EXCITING RESEARCH OPPORTUNITIES!

What are some of the hottest topics in genomics right now? Gene editing, and synthetic biology, are probably pretty high on that list. Where are the best places to work as a genomicist? The Broad Institute and the New York Genome Center are probably pretty high on that list. With all of that on his CV, that makes Neville Sanjana one of the most exciting researchers out there. The phrase 'cutting-edge' gets thrown around a lot, but Neville's work definitely deserves that label.

FLG: You went to Stanford University for your undergraduate degree in, Symbolic Systems and English Literature. It's been described to me as an interdisciplinary program concentration on 'The Science Of The Mind'. As intellectually attractive as it sounds, it seems like it's the kind of program you need to seek out quite specifically. The inner workings of the brain are still something you work on today. Where did that curiosity first come from?

NS: Growing up in San Diego, I had a school friend whose father was a cognitive scientist at the University of California, San Diego. In fact, his father was the founding chair of the first cognitive science department in the United States. So through being in that house, and hearing what kinds of problems a cognitive scientist thinks about, it stayed with me and gave me that early exposure.

Of course, many schools have a cognitive science major, but at Stanford they really created their own version of it with the Symbolic Systems Program. It included a lot of traditional cognitive science like cognitive psychology and neuroscience but also had this whole different side to it with logic, linguistics, and computational linguistics. It was very computationally focused and the underlying idea was that the mind is a computer. One of the major questions was how do we design experiments to understand the computations that the mind performs

FLG: Where did the English Literature side of things fit in?

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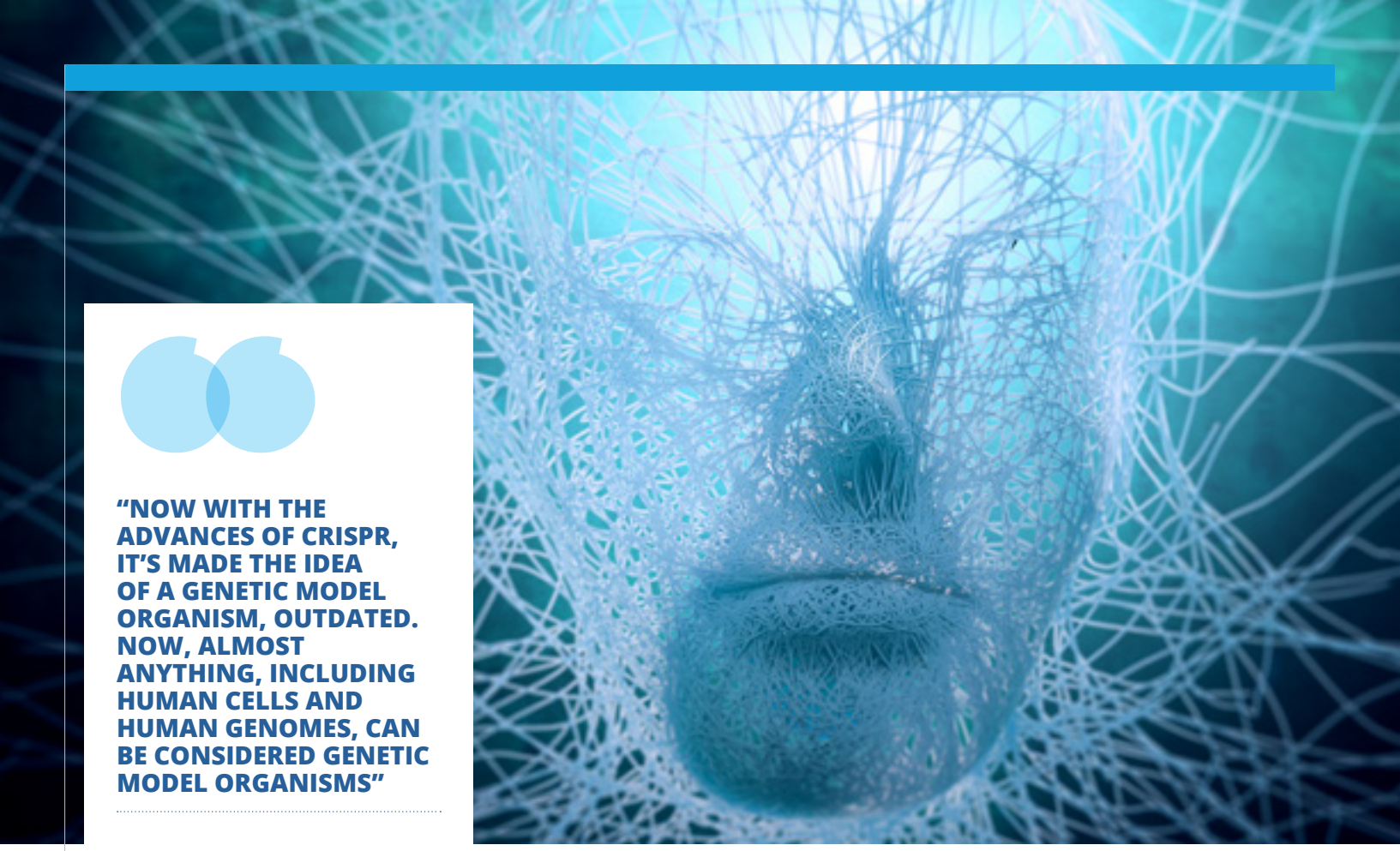
NS: I think in science it always helps to write. One day I stumbled into a class on Charles Dickens, and really just enjoyed the enthusiasm of the professor, Christine Alfano, who was an expert about Victorian era literature. It was a wonderful department with master writer-scholars like short story author Tobias Wolff and James Joyce scholar Brett Bourbon. An amazing faculty — in fact, I think they were ranked as the best PhD program in English Literature in the United States during that time. So, it's something I just stumbled into through traditional English literature, and it developed into a serious interest. It was fun for me but also very useful as science involves so much writing and critical reasoning.

FLG: Your next stop was your PhD in Cellular and Computational Neuroscience. How did that come about?

NS: I was very lucky that the Symbolic Systems lab in which I did my undergraduate thesis — Josh Tenenbaum's lab — also moved from Stanford to MIT that same year. It is no exaggeration to say that, in large part, I came to MIT by following Josh, who was working at the cutting edge of reverse-engineering the algorithms that the human mind uses to learn. With Josh, I was working on probabilistic models of how humans make decisions, and how human learn new concepts.

Once at MIT, I joined Sebastian Seung's lab, a non-traditional yet very innovative neuroscience group in the department of Brain and Cognitive Science. It was a perfect place for somebody like me who wanted to hedge their bets between studying cognitive science and neuroscience. Over time, I veered more into the neuroscience side of things and got really into molecular and cellular neuroscience.

Through being in his lab I became interested in developmental neuroscience, which actually carries forward to what I do in my own lab today actually. For my PhD thesis, I built a time-lapse microscope to image fluorescently-labeled axons from rodent brains over very long time period. I was able to do is track the trajectory of these growing axons as they go out and make synapses and find other neurons to connect to. And a lot of the work that I'd done in probabilistic modelling really came into play here because we ended up using the same kind of models to understand the trajectories of these nascent axons wiring up the brain. →



"NOW WITH THE ADVANCES OF CRISPR, IT'S MADE THE IDEA OF A GENETIC MODEL ORGANISM, OUTDATED. NOW, ALMOST ANYTHING, INCLUDING HUMAN CELLS AND HUMAN GENOMES, CAN BE CONSIDERED GENETIC MODEL ORGANISMS"

FLG: How did you find swapping California for the East Coast, with MIT and the Broad?

NS: I grew up in San Diego, so the East Coast took a few years to get used to. But, in the end, I really enjoyed my time in Cambridge. It's a fantastic environment with a real density of science. The Broad Institute, where I did my post doc, was a fantastic place to do genomics and genetics. It felt like being at the center of the genomics universe.

FLG: CRISPR is one of the sexiest buzzwords out there at the moment. How have you seen the field develop over the past six years?

NS: Well, I feel incredibly lucky to have been a postdoctoral fellow with Feng Zhang, my mentor at the Broad Institute. A lot of things are about timing. Gene editing has a long history with previous technologies like zinc fingers nucleases and TALENs. CRISPR itself has a long history within microbiology, dating back to the late 1980s.

When I started working in Feng's lab, we were using TALE proteins, which are programmable nucleases like CRISPR. In the case of TALEs, you had to clone a new protein every time you wanted to target a new sequence. Similar to zinc fingers, TALEs are not terribly easy to use because they are very repetitive protein domains that need to be arranged in a specific order. In contrast, CRISPR is both a more efficient and also much easier to program by virtue of its RNA-based targeting. It's a quantum leap forward.

FLG: The development of relatively fast and accessible sequencing tools has been pretty exciting. But there are plenty of people out there who see genome editing, as the thing that's really going to be flicking on the power for genomics. We're going from sequencing everything, to going into the genome and testing what all of this stuff actually does. What's your take?

NS: I agree — there is a huge potential for these technologies and much of it has not been explored yet. If you make the analogy with computers, it seems obvious that writing data is equally important as reading data. Really, a lot of the power of personal computers and other devices in our life is that they can both read and write data very, very easily and that's something that we don't currently have in genomics. It's very limited. Previously you had the ability to manipulate the DNA of traditional genetic model organisms like yeast, fruit flies, or mice, to some degree. Now with the advances of CRISPR, the idea of a genetic model organism is itself outdated. Now, almost anything, including human cells and human genomes, can be considered genetic model organisms.

FLG: There's another story that you're involved in that made the headlines in a big way a couple of months back. HGP-Write. How did that all come about?

NS: Well, my involvement in that is probably smaller than many of the other authors, but for me that came about through a meeting organised by Jef Boeke, George Church, Nancy Kelley and a few others. And it just was a really interesting, thought-provoking meeting. Significant progress has been made in synthesising entire chromosomes for other organisms like yeast, so we're thinking about what would be the potential applications and what technology is needed to synthesize an entire human chromosome? You could think of this as a parallel technology to genome editing. Genome editing takes an existing chromosome, or genome, and puts in particular changes. But it's a different capability to really be able to just create an entire chromosome, or string of DNA. They're

both technologies that are going to enable new kinds of science. As a scientist, you need to be able to dream about the future if you want to eventually get there.

FLG: What do you think the project's lasting legacy might be? Is it more about what the problems and opportunities you guys end up identifying along the way?

NS: When the human genome project was completed, a lot of people thought a lot of answers would be evident. Like what causes cancer or the mechanisms of neurodevelopmental disorders. We only started to get that about ten years later. So a lot of people have been questioning what we actually learned. But for geneticists and genome scientists, the discoveries have been continuous and are still coming. Sequencing the human genome is like landing a man on the moon: it's a tremendous shining achievement for humanity but it is in many ways a first step towards greater explorations. So while sequencing one genome didn't give us all the answers, the technologies that came from sequencing that one genome have enabled a lot of science in the following years.

If we compare HGP-Write to the timeline of the Read project, I think we're at that planning stage now, like in the mid to late 80's when people were making gene sequencing a priority. At that time, sequencing an entire human genome still looked like a ridiculous task. It's a multi-generational effort where this work might benefit people 20, 30, 40, or even 50 years from now. As we look ahead into the distance and start dreaming big, it's these first steps that are pivotal in encouraging you to reach those aspirational scientific goals.

FLG: Through all of this, we haven't even talked about your move to New York yet. You're now at The New York Genome Center and an assistant Professor of Biology at NYU. What attracted you to New York?

NS: I came from a very department-oriented background, through my PhD, as most people I interacted with were within the department. Towards the end of my time as a PhD student, I was able to work with two very talented postdoctoral fellows — Erez Levanon and Billy Li — in George Church's Lab at Harvard Medical School. It was through a joint project with them that I really got exposed to this brave new world of high-throughput sequencing and the larger genomics community. I realised that just across the street from my neuroscience department, there was the Broad Institute which was making amazing discoveries using these new sequencing technologies. Through my friends in the Church Lab and in the neuroscience community, I was introduced to Feng Zhang and given the incredible opportunity to join the Zhang lab at the Broad. I thought it was fantastic. I loved this idea of something that takes advantage of all the institutions around it and brings scientists together. It was a hotbed of new ideas and technologies and much of it was due to the tremendous cross-pollination. There are people from MIT, Harvard, Harvard Medical School, Massachusetts General Hospital, and many other Boston research centers. It's just a very mixed environment that I thought was very attuned to the future of science, and the nature of what science should be, which is to say collaborative.

I really enjoyed my time at the Broad. It's a place that made me successful, and a place where I learned a lot from colleagues around me. Out of all the places I went on to interview at, the New York Genome Center just stood out as being a place that had the same vision and the same kind of ideas that I first encountered at the Broad. There are no departments, really, here at the Genome Center, we have core faculty and core labs, but none of those traditional boundaries. Everybody works with everybody and that is fantastic.

So there are two things that really brought me here: it felt like the best of what I'd seen in Cambridge and at the Broad, and also the junior faculty here were clearly the dream team of human genetics. Each of them also have an appointment somewhere else in New York, which makes the Genome Center really a cross-roads of great genomics for the city. My joint appointment is with NYU's Department of Biology, which was, of all the departments that I interviewed in across the country, the most friendly and collaborative department that I experienced. NYU Biology also has tremendous diversity. Coming from a neuroscience department, it's refreshing to interact with everyone from plant biologists to computational scientists solving protein structures.

FLG: Your team is developing technologies to understand how human genetic variants cause disease of the nervous system and cancer. Can you tell us a bit more about the work you guys are doing and the people in your lab?

NS: I just started in April, but the lab is essentially focused in two directions. The vision for the first direction is new genome engineering technology development to understand the noncoding genome. One of the tools we rely upon are high-throughput CRISPR screens, which take advantage of the easy programmability of the CRISPR system to look at many genetic variants in parallel.

In terms of the disease domains, we focus quite a lot on cancer. We've worked on drug resistance in melanoma and in vivo models of metastasis with lung cancer. We're synthesising large libraries of single guide RNAs (sgRNA) that are used to guide the CRISPR enzymes like Cas9 to different locations in the genome. We're doing this to ask genome-wide questions. For example, what are all the possible loss of function mutations that will trigger metastasis from a primary tumor into the lung? Or what are all possible mutations that can trigger resistance to a BRAF inhibitor? BRAF mutations are one of the most common mutations you find in melanoma, so we want to find out, before you get to a clinical trial, if you can predict what it is about a mutation that creates drug resistance and how to pair patients with more effective drug combinations. A good example is the cocktail of drugs used in HIV as a good example: One drug by itself promotes resistance, but if you mix these three drugs together, it keeps the disease very well controlled.

The other half of my lab kind of continues in the neuroscience direction along some of the interests I developed during my PhD. This side of the lab also uses genome engineering, but uses it in a very different way. Rather than testing hundreds and thousands of mutations in parallel, here we take some nominated mutations based on patient sequencing. Right now, we have invested significant efforts into understand the mechanisms underlying mutations found in autism. We are looking at de novo mutations, which means mutations that have arisen in the germ line cells of the parents. We look at them by putting them into human stem cells. We worked for many years on making very efficient ways to rapidly differentiate those stem cells into specific kinds of neurons. Neurons are something that we obviously can't get directly from human patients. Thus, gene editing in stem cells and differentiating the stem cells into cortical neurons gives us a powerful platform for understanding what is going wrong in neuron with autism-associated mutations. It means we can try to understand the mechanisms that lead to autism. We hope that in the future we might be able to develop this into a platform that could be used for something like a drug screen, or a high throughput CRISPR screen, to help develop therapeutics.

Right now, we're at the stage where we might be able to diagnose some of these kids based on genome sequencing, as the cost of sequencing becomes cheaper and establishes itself in the clinic. But what we want to do is the next step: To understand the mechanism, so we can actually think about treatments. →

FLG: How have you found the jump from being part of someone else's lab to having your own lab and calling all the shots?

NS: You know, I feel very lucky. Anybody who's doing a PhD or a post doc knows, it's an incredibly competitive job. I'm very grateful for the opportunities that I've been given. I'm very positive that this is an opportunity where we can do a lot and hopefully have a big impact on diseases that affect so many people. The really great thing about the New York Genome Center is that everybody here is new and that itself provides a lot of raw energy. Even the veterans have only been here 2 or 3 years. There's something really powerful about being in a new place and building an organisation together.

FLG: Twist Bioscience very kindly introduced us to you, so it'd be great to hear about what your relationship is with them. What do you look for in partnerships with suppliers?

NS: The key thing for us with pooled CRISPR screens is having a way to quickly use the sgRNA libraries that we design. Most labs don't have the capability to do very high throughput oligonucleotide synthesis in house and oligo libraries have historically been hard to purchase anywhere. Twist Bioscience seemed genuinely very interested in developing libraries for pooled CRISPR screens. They saw it as something that a lot of people might find useful. They've been ideal partners, in that they've wanted to understand our needs, and have helped us test things out. I think Twist Bioscience has done a nice job in recognising this market for pooled screening reagents, and thinking about how to make something that is both accessible and useful for anyone wanting to do a CRISPR pooled screen.

Many other companies in this space have just been either confused or extremely protective in a way that doesn't encourage innovation. The people that I've worked with at Twist, Maria Ramirez and Scott McCuine, have been great partners with a real interest in enabling others to do great science. In our own work, we're always thinking about how we can get these ideas and technologies out to as many people as possible. Since our first genome-wide CRISPR screen maybe a little over two years ago, I see one or two new papers being published using the technology every week. Overall, it's been wonderful to see the technology take off and even better to see industry collaborators interested in fostering that.

FLG: You're still early on in your career. Are there specific things you're hoping to achieve?

NS: I feel incredibly lucky to be here at a time where genome engineering is really becoming a reality. Every time we have new tools, you can just see much, much further and that naturally leads to better experiments and new results. So, I'm very grateful to have been a part of this genome engineering community and I feel like I want to continue developing these tools in order to understand the basic mechanisms of human disease. At a future stage, I hope to develop gene therapies that can help people where no drugs exist. And of course, there are many more things that don't have to do with human health or disease. There is tremendous potential to apply these tools in plant and crop biotechnology, looking at DNA as a storage medium, and other things that will be enabled by making writing DNA a routine procedure.

Right now, we can plan out experiments easily that just four years ago we could not do. We could not really easily modify genomes of human cells. That's the bottom line; that's something that enabled so much that science can do today.

"BUT PERSONALLY, WHAT I FOCUS ON EVERYDAY IN THE LAB IS REALLY BEING ABLE TO UNDERSTAND THE MECHANISTIC PHASES OF THE DISEASES. AND EVERY DAY WOULD YOU THINK THAT JUST FOUR YEARS AGO WE COULD NOT DO. WE COULD NOT REALLY EASILY MODIFY GENOMES OF HUMAN CELLS. THAT'S THE BOTTOM LINE; THAT'S SOMETHING THAT ENABLED SO MUCH THAT SCIENCE CAN DO TODAY"

FLG: What advice would you give to people just starting out now, struggling through their PhD's?

NS: I feel that being fearless is the hardest thing to do and also the most important. In new fields like genome engineering, you don't need a whole lot of experience but you do need a willingness to try many kinds of experiments, fail, and quickly move on. It's surprising how quickly expertise can develop. I knew almost nothing about molecular biology when I started my post doc. With the fantastic training environment where I was surrounded by people like Feng — people who are risk takers and innovators, eager to just try new experiments and put together new tools — I saw that you can actually cover a lot of ground pretty quickly. Even as a PhD student or technician in the lab, you have the ability to try out new things. If you thrown a lot of darts before you pick one direction, you will have many opportunities to hit the bullseye. ■

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Neville Sanjana, PhD, is a Core Member and Assistant Investigator at the New York Genome Center. He holds a joint appointment as Assistant Professor in the Department of Biology and the Center for Genomics and Systems Biology at NYU. As a bioengineer, Dr. Sanjana is focused on creating new tools to understand the impact of genetic changes on the nervous system and cancer evolution. His research interests include developing pooled screening approaches for functional genomics and using gene editing to create pluripotent stem cell and human neuron models of neurodevelopmental disorders.

Dr. Sanjana is a recipient of the NIH Pathway to Independence Award and is a Next Generation Leader for the Paul Allen Institute for Brain Science. Prior to joining NYGC and NYU, he was a Simons Postdoctoral Fellow at the Broad Institute of Harvard and MIT. Dr. Sanjana holds a PhD in Brain and Cognitive Sciences from MIT, a BS in Symbolic Systems and a BA in English from Stanford University.

“RATHER THAN TESTING HUNDREDS AND THOUSANDS OF MUTATIONS IN PARALLEL, HERE WE TAKE SOME NOMINATED MUTATIONS BASED ON PATIENT SEQUENCING. WE LOOK FOR DE NOVO MUTATIONS THAT HAVE ARISEN IN THE GERM LINE CELLS OF THE PARENTS. WE LOOK AT THEM BY PUTTING THEM INTO HUMAN CORTICAL STEM CELLS”