

Alejandro Sánchez Alvarado: Bootstrapping flatworms into the molecular age

Sánchez Alvarado studies tissue regeneration in planarians.

The flatworm *Schmidtea mediterranea* (a freshwater planarian) can regenerate its entire body—including complex structures such as reproductive organs and its head—from a fragment as small as 1/279th of the animal. What grants planarians this extraordinary regenerative capacity, and why don't humans share this power?

It takes a person as resilient as a planarian to answer these kinds of questions. Alejandro Sánchez Alvarado is just that kind of person, which is fortunate, because it was no small task to optimize the tools (1, 2) needed to develop planarians as a model organism suitable for the age of molecular biology and genetics (3–5). We called him at his new lab at the Stowers Institute for Medical Research in Kansas City, Missouri, to talk about his life, his career, and the aspirations he holds for his favorite flatworms.

LANGUAGE BARRIER

Where did you grow up?

I was born in Caracas. Venezuela is a relatively small country in Latin America, which, although well known for its natural wonders, is not particularly well known for its pre-Columbian history—nothing like Peru or Bolivia or Mexico. It was sort of a backwater of the colonies from the sixteenth to the eighteenth centuries. So I became very curious to learn about the rest of the world.

I began to feed this interest by collecting stamps, believe it or not. I would get stamps from people who were receiving correspondence from various corners of the world, and I would go to the library to learn more about the countries they came from. That was how I discovered that not everybody speaks Spanish [laughs].

It was a great hobby, which is probably now no longer a sustainable pastime, given how email has superseded regular mail.

How much English did you speak when you first came to the United States?

None at all! I went to college in the United States ultimately because that was where I could study molecular biology. And I wanted to study that because, during high school in Venezuela, I had a simply phenomenal biology teacher, Mr. Maldonado. For every new topic we studied, he would pose thought experiments to us. It was very engaging, and, by the time we got to molecular biology, I was hooked.

After high school, I was headed to medical school because that's what biologists did back then. If you wanted to do research, you had to have a "real" career, like being a physician. But I was there for probably three months, and I hated it because no one wanted to understand how things worked. You just had to memorize things. I talked to my father and told him what I really wanted to study was molecular biology. "It's the wave

of the future," I told him. Fortunately, my family was relatively wealthy at the time, so they could afford to send me abroad to study. I interviewed with three schools in the States that listed molecular biology as a major, and I chose Vanderbilt especially because it didn't have a Hispanic population to speak of. I knew that, if I went to a place with a large Hispanic community, I would hang out with them and never learn English.

A MATTER OF DEGREE

What made you decide to go to graduate school?

After college, I returned to Venezuela, only to discover that there were no research jobs for someone with a bachelor's degree. I had to do graduate school, but I didn't want to start right away. To tell you the truth, I was a little envious of some of my classmates from Vanderbilt, who were real world travelers, so I decided I would take a three-month

"We needed to develop tools that would allow us to perturb genetic activity at will."



PHOTO COURTESY OF TAJANA PIOTROWSKI

Alejandro Sánchez Alvarado

tour of Latin America first. It ended up taking me almost a year. I didn't anticipate the weather conditions, roads being underwater, lack of transportation, and the fact that Brazil is a humongous country [laughs]. Then I was pickpocketed in Bolivia and lost all my money, my camera, everything. So I had to basically hitchhike, penniless, the rest of the way. By the time I got back my parents had given me up for dead, because I'd had no money and couldn't write, so they hadn't heard from me in months.

Eventually, I came back to the States for my PhD. I went to Cincinnati. Tom Doetschman was there, who had just trained with Oliver Smithies to learn this method called homologous recombination. He convinced Jeffrey Robbins to use this method to study heart development. I really wanted to learn how to do recombination in mammals, but I eventually became fascinated not by the method itself but with the biology of the mouse embryonic stem cells used in our experiments. I was smitten with the plasticity of the genome—how cells channel genetic information to produce specific outcomes. And that's what eventually led me to my postdoc at the Carnegie Institute with Don Brown. There, I wanted to study this remarkable transformation that some tadpoles undergo when their tails are amputated in the presence of retinoic acid: they grow hind limbs, instead of a new tail.

IMAGE COURTESY OF JOCHEN RINK



The remarkable plasticity of regeneration shown in a planarian with two heads.

What about that interested you?

Something that continues to be a source of fascination for me is to understand how cells make decisions about developmental fate, and how they all come together to generate form and function. How do they do this so well, and so reproducibly, generation after generation? That's especially interesting in the adult context, where there are tissues that are being regenerated that are not part of the immortal germ line.

There is tissue regeneration in humans: your nail beds and your skin are constantly regrowing throughout your lifetime. In a year, humans turn over a mass of cells equivalent to our entire body weight—that's maybe 50–70 billion cells per day—and yet we recognize ourselves in the mirror each morning, so these processes are under remarkable regulation. Cells need to know what to do and how to do it, and how to integrate newly formed tissues into preexisting tissues without losing the functional integrity of the organism in which these cells reside.

EXTRAORDINARY ABILITY

Some are better at regenerating than others; humans can't regenerate their heads like planarians can...

That's absolutely correct. And I'd like to know why. The bottom line is that if you look at the genomes of these various organisms, the genes that are present in different organisms' genomes are mostly the same. It's not like every species invented their own genome; we all share what appears to be a common set of molecules that are very ancient. So the question is, if we share all of these components and we share all of these common attributes, why is it that some

animals are so much more capable at regeneration than others?

But, to answer this question, we needed a good model in which to study the process of regeneration. Model organisms like *C. elegans* and *Drosophila* weren't selected because they're pretty; they were chosen because they exaggerate a particular attribute that we want to understand. I selected planarians because they have an amazing regenerative capacity; you can slice them and dice them in any imaginable way, and they'll just laugh at you and grow everything right back.

How do you go about establishing a new model system?

The reason why planarians probably didn't make it as a model system in modern biology is because at first it was not possible to move away from phenomenology and do functional mechanistic studies. It was apparent to me that, if we wanted a useable system, we needed to develop tools that would allow us to perturb genetic activity at will, as can be done in other model organisms like yeast, flies, and mice.

Our first major advance came while I was still at the Carnegie Institution. Andy Fire, who pioneered the use of RNAi in *C. elegans*, was also there. He convinced me to try injecting double-stranded RNA into planarians. At first I was afraid it wouldn't work because it was supposed to be very hard to inject planarians, but I tried it and it worked on the first try! That was a good lesson for me: don't overthink it, just do the experiment and go from there.

Is there a planarian genome available?

Yes, there is. It's still being refined, but what's been found so far is that there are around 20,000 genes in these guys, about the same number that you and I have. If you look at the families of genes that are represented in this genome, there is nothing really extraordinary that jumps to the eye. So now we're operating from the hypothesis that either there are some very special genes that, when duplicated, carry out the animal's extraordinary regenerative

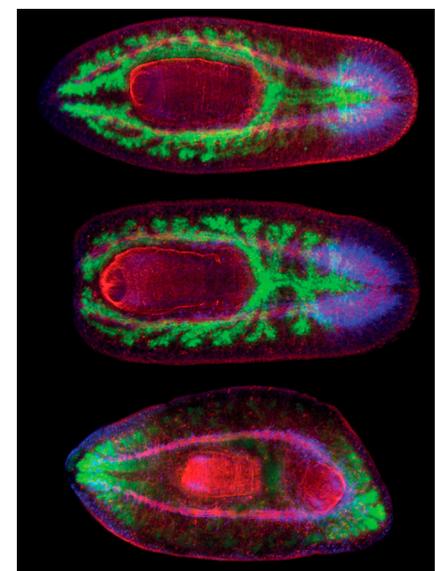
capacities, or else that the difference between us and them lies in the way in which these genes are deployed in time and space. I like the second idea better.

Now we can do more targeted tests with RNAi, because we know what's there to be interfered with. The other thing that having the genome mapped out gives us is the ability to start thinking about going beyond just knocking a gene down with RNAi. We need to begin doing traditional genetics with these guys—generating and testing alleles, for example—because that's the way evolution works, by testing out new alleles. My dream would be to be able to

modify every nucleotide in the genome of these animals at will, pretty much like what people do in mice. That would open up a lot of new horizons.

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“Don't overthink it, just do the experiment and go from there.”



Knocking down Hedgehog (bottom two images) prevents normal regeneration patterns after head and tail amputation in flatworms labeled for neurons (blue), gastrovascular system (green), and central nervous system (red).

IMAGE COURTESY OF JOCHEN RINK, KYIE GURLEY, SARAH ELLIOTT, AND ALEJANDRO SÁNCHEZ ALVARADO