Anthony De Tomaso: Finding the origins of adaptive immunity

As a student, Anthony De Tomaso was fascinated by many aspects of biology, from molecular biology to marine biology and development to immunology. Now as head of the same lab that he joined as a postdoctoral fellow, De Tomaso has marshaled his diverse scientific passions into a single project—tracing the evolution of the mammalian immune system by unraveling that of a sea squirt.

Anthony De Tomaso

The adaptive immune system in vertebrates seems to have come out of nowhere; B and T cells and mechanisms used to recognize and remember specific pathogens are missing in species that are only a step below vertebrates on the evolutionary ladder. Anthony De Tomaso is trying to figure out this puzzle, immunology's version of the big bang, using what he calls "the swing species between vertebrates and invertebrates"—the sea squirt.

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This model, also known as *Botryllus schlosseri*, is a translucent bag of biological tricks that De Tomaso wants to understand. *Botryllus* starts off looking like a tadpole, but matures into an adult that grows via asexual budding into a genetically homogenous colony. Adult stem cells within each separate bud then regenerate that bud on a weekly basis. Contact between two genetically similar colonies of buds causes the two to fuse into a chimeric colony in which stem cells from each animal compete to take over the germline of the other. Dissimilar colonies, by contrast, remain separate, owing to an inflammatory reaction that resembles the rejection of a transplanted organ.

As a postdoc, De Tomaso helped identify the gene that controls the choice between fusion and rejection, which came to be known as the fusion histocompatibility (*FuHC*) gene (1, 2). The same gene also seemed to protect the animal against parasitic stem cells from another sea squirt (3). Later, De Tomaso took over for his former mentor as head of the lab. His group then uncovered a potential receptor for FuHC (4). They are now trying to understand how the receptor's signals decide between a fusion or rejection reaction. In a recent interview, De Tomaso explained how his unique research model allows him to indulge his varied scientific interests.

THE ROAD TO SELF-AWARENESS

How did you get interested in scientific research?

During the summer of my junior year at Stanford, I took a class in subtidal zoology. But then I wanted to see marine biology in action. So I got an internship at Stanford's Hopkins Marine Station, where the summer classes are very intense; there are 12–14-hour days, with labs, lectures, independent research, etc. I had never been a good book learner, so it wasn't until this research experience that my interests in science solidified.

As an undergrad, how did you deal with the intensity of the internship?

Well, I had learned diving in high school. And this class had the added attraction that every morning you went diving in different areas around the Monterey Bay. So that helped.

What impressions did you take away from this experience?

I understood how diverse developmental mechanisms truly are. It also made me realize that so many marine species have some enhanced mechanism, function, or tissue that makes it easy for us to study them—like a squid's giant axon or a regenerating arm on a starfish.

Did you pursue marine biology for your graduate work?

No, I ended up doing something very different. I went to Washington University in St. Louis and became a student of regular old mammalian biology. My thesis project included a lot of cellular and molecular biology, which are my first loves.

How did immunology and* Botryllus *get on your radar?

After four years in the Midwest, I was ready to come back to California, which is where I'm from. I was thinking of working on hematopoeitic stem cells and went to talk to Irv Weissman about a postdoc position in his lab. I knew about Irv from my marine biology days. He had a lab at the marine station, where he had been studying the fusion/rejection reaction in sea squirts since 1980, because it resembles the way tissue transplants are accepted or rejected in mice and humans. He reeled me into the project.

What was his pitch?

Irv started talking about *Botryllus* and all the interesting biology behind it: self/nonself, or "allo," recognition, the constant asexual reproduction, and the relationship between these two features. He also threw down a gauntlet—to discover the genes underlying allorecognition. The challenge was that there was no bail-out point for that project. We would find the genes, or we would not.

Were you a hard sell?

No, I went for it, because as a postdoc, I wanted to be working on a project that was broad enough and interesting enough to form the basis of my own lab someday, and I realized right away that Irv was giving me a completely unique opportunity. I knew that I was gambling my career by doing something that was not mainstream, but if I was going to take a risk like that, at least I was in the right lab. We had money, expertise, and

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De Tomaso studies the mechanisms that allow two genetically identical colonies of *Botryllus* to fuse.

great collaborators. Irv had already invested so much into the basic infrastructure—bringing the animals into the lab, creating partially inbred strains, and so on. It was a great combination.

THE RULES OF FUSION

Did your gamble pay off?

Yes, it did. And it helped that what was involved was simple, single-locus genetics. If two individuals share one or both alleles at the locus, they fuse; if they share neither, they reject each other—a very simple thing to track. We took a forward-genetics approach and identified the *FuHC* gene by positional cloning. This gene encodes a highly polymorphic, type I transmembrane immunoglobulin. We think it is the allorecognition ligand. It's the first connection that we've seen between the vertebrate and invertebrate immune systems.

We've recently identified another locus, called *fester*, that seems to fit the bill for an allorecognition receptor. *Fester* is very polymorphic and is also polygenic, meaning there could be several different receptors on the same animal.

How does this ligand–receptor system control fusion?

Functionally, this system is similar to how natural killer (NK) cells work in the vertebrate immune system. The NK cells' rejection reaction is inhibited when there is a self-MHC allele on their targets. We think that *fester* alleles similarly undergo an education process in which they learn about the FuHC ligands that they were coinherited with. If the receptors recognize a self-like ligand on another animal, a signal is sent that blocks a default rejection reaction.

My wild speculation is that tolerance is the key invention that everything else in our immune system is based on. Before we evolved a sense of what's foreign, we had to develop a sense of self. We've found the first clue as to how tolerance works in these animals. If we can teach cells to become tolerant, that would be a breakthrough in treating any disease where self-tolerance has broken down.

Besides allorecognition, what else are you using* Botryllus *to study?

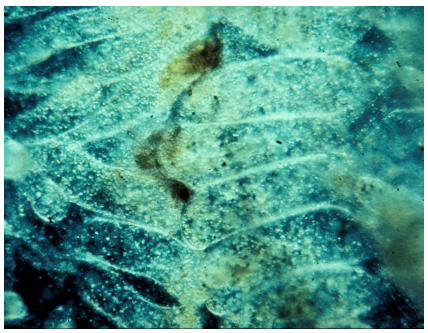
The adult *Botryllus* regenerates every organ inside a new bud every week. The old bud is destroyed by apoptosis, and the new bud, which has every major organ such as the heart, gut, etc., takes over. It's an asexual reproductive mechanism that's not seen in any other organism. We have found several candidate genes that are required for this unusual development and are working out where they fit in.

Botryllus is also a great model to understand stem cell biology. This animal has adult stem cells that try to take over the regeneration process when two colonies fuse. Because this parasitism is an inheritable trait, we've developed several winner and loser inbred strains of *Botryllus*. We are using them to understand how one stem cell outcompetes another and how the winning cells contribute to development.

OVERCOMING CHALLENGES

Genetically diverse colonies are the key to most of your studies. How do you find them?

All we have to do is to go by our local marina and scrape the bottoms of boats. About 90% of the colonies that we bring in at random will reject each other, because the locus is incredibly polymorphic, probably more so than our own MHC.



Scars (brown) mark the rejection reaction between two genetically dissimilar colonies.

Scraping animals off boat hulls? You haven't gotten into trouble for that?

Nobody seems to care about a sea squirt. And even if animal rights people did get interested, they'd love our facility. The animals are much happier in our clean water than in the marina with the oil and the gunk.

You recently made the transition from postdoc to PI right in the same lab. How did that go?

I've been running the lab for a year now. Things weren't as hard for me as they might be for others because I inherited the physical structure of Irv's lab, which gave me momentum. But there are the usual challenges—getting and renewing grants, expanding into new projects, getting new people.

> "It's the first connection that we've seen between the vertebrate and invertebrate immune systems."

Your model is very different from the mainstream animal models that most labs use today. Do you face skepticism for that from your peers?

Irv and I used to say that we work on an orphan model. But the people who understand why I am asking these questions are the same ones that have asked seminal questions in mainstream models like mice or *C. elegans*. They know that inbred mice are powerful tools, but they also know that by breeding out the variability, they are going to lose some of the biology. We shouldn't underestimate how important diversity is. My answer to skeptics is that we should be working on any organism that we can. And in each model, we should be exploiting the power of that system and asking the most relevant questions.

1. De Tomaso, A.W., and I.L. Weissman. 2004. *Science.* 303:977.
2. De Tomaso, A.W., et al. 2005. *Nature.* 438:454–459.
3. Laird, D.J., et al. 2005. *Cell.* 123:1351–1360.
4. Nyholm, S.V., et al. 2006. *Immunity.* 25:163–173.