Profile of David J. Anderson

You are at a picnic and a wasp is circling. You swat it away, but it buzzes back again and again, more persistent each time. The wasp seems angry.

Or is it? Can insects be "angry"?

David J. Anderson believes that what we perceive as insect anger may share a foundation with human frustration or aggression. His model is *Drosophila*, which "has been such a powerful system for studying so many aspects of behavior," he said, "that it's appropriate to ask whether flies have the building blocks of emotion."

"Insect emotion" is the latest focus of Anderson's group, which has previously worked on the developing nervous and circulatory systems. He enjoys the creative freedom of the pioneer, and his group is perpetually in transition to new fields.

Anderson, a professor at the California Institute of Technology in Pasadena, was named to the National Academy of Sciences in 2007.

"Aggressive behavior has a lot of negative consequences in society," Anderson said. "People are concerned about the extent to which the origins of impulsive violence are genetic or environmental."

His inaugural article, published in the April 15, 2008 issue of PNAS, considers how the environment acts on a fly's genes during its lifetime to affect its level of aggression (1). Fruit flies, like other insects, exhibit aggressive behavior, which is perhaps related to territoriality and competition for mates.

Fruit Fly Fights

Drosophila aggression has been documented for nearly a century, but research into this topic has recently been reinvigorated, thanks to new work by Edward Kravitz, who for many years studied aggression in lobsters and who also taught Anderson during a summer neurobiology course in 1979.

Across the animal kingdom, research has shown that males that mature in solitude tend to be more aggressive as adults than well-socialized individuals. Anderson's graduate student Liming Wang found that *Drosophila* males raised in solitary confinement are more likely to lunge at other flies in an experimental "fighting arena" containing a food patch. This fighting area could be reversed if the animals were switched to group housing and allowed to mature, the researchers found.

Based on these observations, Wang investigated how gene expression in the brain differed in flies raised alone and in group housing. In particular, he searched for the "pacifist genes" activated in group-housed flies.

David J. Anderson

Wang, Anderson, and their colleagues found 48 genes whose expression was higher in group-housed than in socially isolated flies. One of these genes, *Cyp6a20*, also belonged to a set of genes found by Ralph Greenspan that are down-regulated in flies bred for aggressiveness over 20 generations. *Drosophila* "rottweilers," Anderson calls them.

Cyp6a20 encodes an enzyme that is expressed in the antennae of the group-housed flies, the researchers found, suggesting that it may degrade an aggression-promoting pheromone. Their results show that it is possible for an animal's environment to cancel out or reinforce an inherited tendency to aggression.

In their aggressive *Drosophila*, the environment is able to modify the expression of one of the same genes that is apparently responsible for heritable differences in aggressiveness. In this way, "nature" and "nurture" have converged on a common molecular target, Anderson said.

The Natural

Anderson was born into an academic family and his fascination with science began early. His father, James, a theoretical physicist, brought the family along on his yearly summer visits to the Woods Hole Oceanographic Institution in Massachusetts. From the age of 7 until he was 16, David Anderson attended the Woods Hole Children's School of Science.

"The other students were the children of biologists, while my father was a physicist, and this created a certain aura of mystery about what was going on in these biology laboratories," he recalled. "And after I got too old for science school I got jobs washing test tubes in the laboratories, listening to postdocs and professors talk about research, and I just found it fascinating."

Anderson's father, a professor of physics at Stevens Institute of Technology (Hoboken, NJ), always supported him in his scientific career. His mother, Helene, a professor of Spanish and Portugese at NYU, did as well. As Anderson says, "Cross a physicist with a humanist and what do you get—a biologist."

"Having a physicist for a father has been a great educational opportunity to address the intellectual predispositions toward biology that are common in the physics community," Anderson said. "When I showed him my first paper, he joked, 'but David, where are all of the equations?'

"Growing up with my father was better preparation for being a biology professor at Caltech than anyone could possibly ask for. I had all of the arguments honed to a sharp edge. But I don't want to imply that he was dismissive of biology."

Anderson said his father has always been impressed with the way that biologists are able to find out things about seemingly impossibly complex biological mechanisms. And he has been vindicated by the increasing importance of mathematics in biological analysis. In fact, said Anderson, he has a surprise for his dad: his next paper will finally have equations!

A Spectacular Escape

Anderson attended Harvard University (Cambridge, MA) as an undergraduate. For his first research project, he studied the molecular signals at work when a scallop encounters its nemesis, the starfish.

"[The mollusk has] a spectacular escape response where it starts clapping its shell and jetting around and doing somersaults," he said.

This is a Biography of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on pages 5657–5663 in issue 15 of volume 105.

"[But] I got completely waylaid and diverted into basic cell biology and the study of biological membrane structure and function. The link to that was my interest in receptors for chemical signals that communicate information between animals. Now, 30 years later, I've come full circle and I'm studying chemical communication between animals and how it affects their behavior."

Anderson completed his undergraduate thesis on the structure and function of biological membranes in the lab of Daniel Branton, who is still a professor at Harvard.

Interested in how lipid and protein domains are segregated in membranes, Anderson decided on Rockefeller University in New York for his Ph.D. studies. He embarked on a study of "gap junctions," islands of proteins in the cell membrane that hold cells together and through which chemical signals pass. Fortunately, it took him only 6 months to realize that the problems he faced were too difficult.

"There were no biochemical assays for gap junction proteins," he said, "and it was really premature to be trying to study them in that way."

So he switched to the lab of cell biologist Günter Blobel to address how the synaptic receptor for acetylcholine, a neurotransmitter, was synthesized and assembled.

"It's a very complicated multisubunit membrane protein," Anderson said. "This was one of the first efforts to start to apply molecular biological techniques to the study of membrane-excitability proteins in the nervous system."

To obtain raw material, Anderson ground up the electric organs of electric rays, which produce acetylcholine receptors in great numbers. He then isolated messenger RNAs for the receptor subunits, translated them in a cell-free system, and determined how they were inserted into the endoplasmic reticulum.

"That was an important step forward," he said, "because it showed that these types of proteins were amenable to this kind of approach."

Switches of Fate

Woods Hole remained a important base for Anderson. He remembered attending a meeting there in 1980.

"It was one of the first on the topic of molecular genetic neuroscience, and there were several very influential people, like Francis Crick and others. For me as a graduate student, it was really a privilege to attend. It had a big influence on my thinking and direction," he said.

The Blobel lab had focused on the biology of individual cells, but Anderson wanted to study the development of the multicellular nervous system. For that, he realized he needed to become an expert in "molecular cloning." With this approach, he would be able to isolate and amplify DNA that coded for the signals between cells and the switches that control fate.

"It seemed like there was a great opportunity to begin to solve some of the riddles of neural development," he said.

For his postdoctoral work, Anderson chose Richard Axel's group at Columbia (New York).

Blobel, Axel, Martin Raff, and the late Seymour Benzer have been the most influential figures in Anderson's career. Axel and Benzer supported his pioneering drive, encouraging him "not to be afraid to change fields even when the field was popular and things were going well," he said.

"It's humbling to realize that one of the most important things you've discovered was by mistake."

Benzer, who died in November 2007, was a physicist turned biologist at the California Institute of Technology, who pioneered the use of genetics to study behavior in fruit flies. In 1985, he chaired the departmental search committee and "made an extended personal effort" to recruit Anderson to Caltech.

"What I like about Caltech," Anderson said, "is that unlike in a medical school where I would have been in a neurobiology or biochemistry department, here I am in a biology division that spans all different areas of biological science, all of the way from people studying DNA replication in yeast to people studying neurons in the monkey cortex."

"What I did was initiate a two-pronged approach to the problem of fate determination that I continued in my lab [at Caltech]," Anderson recalled.

He developed methods for isolating neural progenitor cells from developing tissue by using specific cell surface antibodies; at the same time, he cloned genes that were molecular markers of cell types he had isolated.

"The idea was that I could work backwards from one of those genes to identifying other genes that were regulators of those genes, and ultimately try to link the two levels of analysis, cellular, and molecular," he said.

Embryonic Alchemy

Anderson had to identify the markers himself. "I started by cutting sections through developing embryos in the region of the nervous system that I was interested in," he said, "and then staining those sections with a battery of many different antibodies, to try to find antibodies that labeled cells where I knew progenitors were forming."

Once he had the antibodies, he could feed a cell suspension through a fluorescence-activated cell sorter to extract the subpopulation he was interested in.

"Because I knew roughly from where in the embryo those cells were coming, I could then formulate hypotheses about the derivatives they would produce, and try to coax the cells to develop along these pathways," he said. "There's a lot of alchemy involved."

In 2001, concerned about how the American public expected stem cell research to produce medical cures immediately, Anderson wrote an article in the New York Times (2).

"I was trying to explain to the general public how much of this kind of research had a hit or miss quality to it, and that that's why progress was slow," he said.

Fortunate Phenotypes

The diversity of his colleagues' interests slowly percolated into Anderson's lab. Until ≈1997, his group focused mainly on extending the work he had done at Columbia.

In 1992, his laboratory had isolated neural stem cells in culture (3); in 1999, they found the stem cells in neural crest tissue (4). In parallel, they identified several factors that drive the stem cells toward different fates (5).

"Those factors act by instruction rather than selection," he said. "This was a source of controversy in many fields. When you throw a growth factor on a plate of cells and you come back the next day and you see that all you have is neurons and no glia, is that because the growth factor directs the stem cell to become neurons at the expense of glia, an 'instructive' effect, or is it because the stem cell randomly generates both neurons and glia but in the presence of the growth factor only the neurons survive and the glia all die (a selective effect)? We were able to show that it's an instructive effect."

However, an accidental finding was soon to provoke a major detour for part of the lab. Anderson and his students found a receptor that guided the migration of neural crest stem cells in culture,

and they wanted to see what would happen when they knocked out the receptor's gene in transgenic mice.

"We found to our dismay that there was no effect on migration," he said. "But we also found that the embryos died at a fairly early age. So we decided to try to figure out why the embryos were dying."

They tagged the knockout cells with a marker that turned the cells blue. "I remember very clearly the day that my student who was working on this came in and said, 'well, we're lucky. We didn't have a cell migration phenotype, but we have an axon guidance phenotype.' And he showed me blue cells in a normal embryo and then blue cells in a mutant embryo, and there were these branched structures that he thought were nerve fibers. They were normal in the normal embryo and they were screwed up in the mutant embryo."

"I looked at it a little bit more and I said, 'wait a second, those are not nerve fibers, those are blood vessels!'"

Anderson's student Hai Wang had discovered a gene that marked arteries and distinguished them from veins. Subsequently, he found a counterpart in veins that interacted with the first gene (6).

"That was very important," Anderson said, "because it showed that arteries and veins were genetically specified and that their identity was not simply caused by physiological factors like blood flow and oxygen. I wrestled a lot with whether we should pursue this, and I have to credit my father the physicist with persuading me that this sounded like an important discovery."

He also cold-called the late Judah Folkman, who was well known for his work on tumor angiogenesis.

"He didn't know me from a hole in the wall," Anderson said. "But he was very excited and said, 'this is extremely important, you can't let this go.'"

Anderson continued to study the interactions between arteries and veins, and in later work, he found that that arteries, but not veins, are aligned with nerve fibers, which provide a template that dictates how arteries branch in developing skin (7).

"I often tell people that it's humbling to realize that one of the most important things that you've discovered was something that you discovered by mistake," he said.

Regulating Cell Fate

In his efforts to probe how stem cell fate is determined, Anderson said that two results stand out in particular.

A set of genes called the "achaete-scute complex" in *Drosophila* control transcription factors that determine neuronal stem cell fate. Anderson found that two genes transiently expressed in rat neurons were close homologs of the *Drosophila* genes (8).

"Across 400 million or 500 million years of evolution," he said, "there's been a parallel conservation of the amino acid sequence of these genes and the cell type whose development they control, which was a very important discovery."

"The complement to that discovery that seems to be getting more important, even though we're not working on it anymore, was the discovery of a repressor, which we call the neuron-restrictive silencer factor, or NRSF" (9).

Independently discovered by Gail Mandel, who named it "REST," the genetic silencing factor "shuts off neuronal genes in the liver and the kidney, and lung and fibroblasts and all those other non-neuronal tissues, and allows those genes to be expressed in the brain," Anderson said. "It's a fundamentally different mode of controlling tissue-specific gene expression."

The Flies Have It

Over the course of nearly 6 or 7 years, Anderson made a research transition away from the growth and transcription factors that determine neuronal fate.

"Stepping sounds too crisp," he said. "I did make a deliberate effort to change direction, but it was anything but a step."

According to him, the field of stem cell biology had advanced and expanded to the point that researchers with narrow focus and specialized technique were better positioned to contribute than he was.

In search of new territory, he decided to use his molecular techniques to identify and study the neural circuits that govern innate behavior in animals, such as the automatic avoidance triggered by certain olfactory receptors in *Drosophila* (10).

"Emotional behavior with quotes around it," he said. "In parallel with fruit flies, we're studying fear and aggression in mice."

His lab was one of the first to identify molecular markers for subtypes of neurons in the amygdala, a brain structure implicated in fear (11). Anderson's group is now using molecular genetic techniques they have developed together with Caltech colleague Henry Lester to determine the role of these neurons in fear behavior, by activating or silencing them (12). Their hope is that this work may lead to improved treatments for psychiatric disorders, such as depression or posttraumatic stress disorder.

While he remains fascinated by fly behavior, Anderson continues to face some skepticism from colleagues. "If you tell people you're studying emotional behavior in flies, they'll initially say, 'you're crazy!'" he said. "But then if you remind them about the angry wasp at the picnic table, they'll stop and say, 'I guess so. If a wasp can be angry, why can't a fly be angry, too?'"

Kaspar D. Mossman, *Science Writer*

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