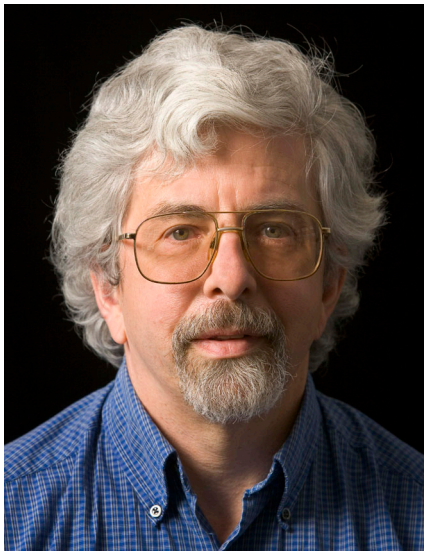
Vann Bennett: How ankyrin holds it all together

Since discovering ankyrin, Bennett has worked to understand its many functions at cell membranes.



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Vann Bennett

Pick any animal cell, and chances are you'll find a protein called ankyrin somewhere under the plasma membrane. Vann Bennett first discovered ankyrin (1, 2) as the anchor that connects the anion exchanger to the spectrin-based membrane skeleton in erythrocytes. Ankyrin is a member of a family of adaptors that perform similar roles for multiple membrane transporters as well as cell adhesion molecules such as E-cadherin, L1 CAMs, and dystroglycan—which helps explain its presence in multiple tissues (3).

As a postdoctoral researcher, Bennett spent a year developing the assay he would later use to purify ankyrin from red blood cells (4). When his former graduate research advisor recruited him to work at Burroughs Wellcome, Bennett told him it should take about 18 months to finish purifying and characterizing the protein, and then he wanted to move on to other projects. Instead, the study of ankyrin family members has taken up his entire career (1–6) and provided important insights into how plasma membrane domains are organized (3). We caught up with Bennett at his lab at Duke University to talk about ankyrin, as well as some of his other lifelong obsessions.

HISTORICAL INTEREST

When did you first consider pursuing a career in science?

It's hard to say. I really loved history when I was in high school, so I thought I would major in history in college and then go to medical school to become a doctor like my father. I started out as a history major, but I found that math and chemistry were easy, whereas I had to work really hard at writing my history essays. [Laughs] They probably weren't very good! Then I had a particularly inspiring chemistry professor at Stanford named John Brauman, who actually let me work in his lab for a couple of years, and after that I switched majors to chemistry and biology. I made up my own major by combining the biology and chemistry programs and trimming some of the courses I didn't want to take.

Did you go on to medical school as you had planned?

I did. When I arrived at Johns Hopkins for medical school, I was still thinking about going into medical practice, because that's what I thought people did with a medical degree. But my first summer, I was trying to think of some research that interested me. My advisor, Dan Nathans, suggested I might want to work with this young faculty member, Pedro Cuatrecasas. And when I heard about Pedro's work on the insulin receptor, I thought, "This is what I want to work on." After working in his lab a while I realized that I wanted to get a PhD, so I interrupted my medical studies right after the basic science courses to do that. Then I went back to finish my MD.

I think I decided to pursue a research career after I spent a summer on an experimental oncology floor and all the patients I was caring for had died by the end of the summer. I realized that every day we were working very hard and doing the best we could, but we did not understand the biology underlying these malignancies. This was something that, as a researcher, I could perhaps contribute to, whereas there were many excellent doctors in my medical school class, and if you subtracted me from the doctor population, it would not make much difference. [Laughs]

> "I was frequently asked after identifying ankyrin, "Is this just a red blood cell thing?"

RETAINING INTEREST

Why did you decide to do your postdoc in Dan Branton's lab?

At the time I thought, extremely naively, that the molecular biology revolution had passed and that the major discoveries in this area had already been made. Little did I know what was to come! But I was very excited by membranes. At that time this was an emerging area, and I felt it was attackable by someone with training in chemistry, as opposed to a genetics background.

Dan's lab worked on membrane proteins in red blood cells, so it was a good fit for me. One of the proteins they were working on was spectrin, which was known to be associated with red cell membranes, but it wasn't clear how. There was a belief at the time that perhaps spectrin didn't have a protein receptor and that instead it just bound directly to lipids. As it turns out, spectrin definitely binds to lipids, but there's also a critical protein component to the membrane linkage. I proposed to identify that protein, and, surprisingly, that's actually what I eventually did. [Laughs]

That protein turned out to be ankyrin. It connects membrane-spanning proteins floating in a fluid phospholipid bilayer with the spectrin skeleton on the inner surface of the plasma membrane, just like an anchor immobilizes boats floating in water. In our initial search for the spectrin-binding attachment site we expected the protein to itself be a membrane-spanning protein. Instead, spectrin bound indirectly to the membrane through ankryin.

Text and Interview by Caitlin Sedwick
csedwick@gmail.com

I was frequently asked after identifying ankyrin, "Is this just a red blood cell thing?" So I went looking for it in other tissues, and, amazingly, I found ankyrin immunoreactivity in every tissue I tested. This protein turned out to be an opening into basic organization processes in many cells and tissues.

It turns out that ankyrin is present in the cells of most multicellular animals. In terms of evolution, we now have some idea of how this might have happened: we've found that membrane-spanning proteins interact with ankyrin through short, intrinsically unstructured peptide segments. These unstructured proteins are the most rapidly evolving part of our genome, and we think that intrinsically unstructured peptide segments have fueled much of the rapid acquisition of vertebrate adaptations such as myelination. We can actually track the ability to bind ankyrins in proteins such as voltage-gated sodium channels: *Drosophila* sodium channels completely lack an ankyrin-binding motif and so do those of echinoderms, but an ankyrin-binding site is present in its modern form in voltage-gated sodium channels of zebrafish.

Are ankyrins important because they help lend physical resilience to cell membranes?
That's likely one component. It's a thought that arose from the work on red blood cells, where defects in these proteins lead to fragile membranes. In fact, in single molecule studies with our collaborator Piotr Marszalek, we've actually found that ankyrins behave as springs. Although cells and tissues must deal with mechanical stresses, they also need mechanisms for the transport and retention of proteins in specialized regions of the membrane. That, I think, is ankyrins' primary importance.

Spectrin and ankyrin are found at well-organized areas—functional domains—of the plasma membrane. A good example of that would be excitable membranes; probably the best-known system where ankyrins and spectrin operate is in the axon initial segments in the central nervous system. These are ankyrin-rich domains, and, if you knock out ankyrin in mice, as we and others have done, then the entire initial segment loses its properties.

"Spectrin and ankyrin are found at well-organized areas—functional domains—of the plasma membrane."

INTERESTING PLACES

Where else do ankyrins function?
One big surprise came from our work on ankyrin-B knockout mice in the '90s. These animals have very severely disturbed nervous system formation, which we expected. But they also have abnormalities in their skeletal muscle and abnormal function of their neonatal cardiomyocytes. Later on, a postdoc of mine, Peter Mohler, helped to show that mutation in the ankyrin-B gene can cause a human cardiac arrhythmia called long QT syndrome. This mutation causes ankyrin to lose the ability to coordinate normal calcium dynamics.

We've also found that ankyrin-B mutations are likely to have a role in the function of pancreatic β cells and appropriate regulation of insulin secretion. But one of the biggest surprises has been that ankyrin mutant mice have symptoms of accelerated aging in multiple organ systems. Now, we're not talking about extreme accelerations such as progeria, but, instead, the appearance of age-related disorders like cardiac sinus bradycardia or diabetes in people in their 50s and 60s instead of their 70s and 80s. This is something we're very excited about, and we're trying to understand exactly how it's working.

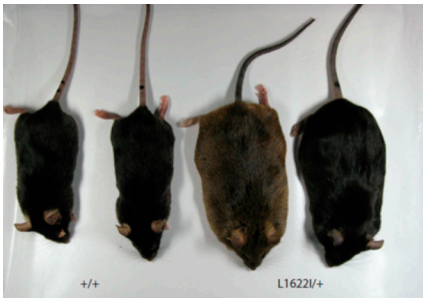

PHOTO COURTESY OF JANE HEALY; PHOTOGRAPHY BY KENT NILSSON

Mice bearing a mutation in ankyrin B (right two) exhibit cardiac arrhythmia and defective pancreatic function.


PHOTO COURTESY OF BOB FRENCH

Bennett (in yellow) and a guide shoot some scary Seal River rapids.

It sounds as if you have your hands full in the lab. What about outside of it?
Well, my wife is French. She has many French relatives and a French addiction, so we visit France whenever we can. I also have an Arctic addiction that she doesn't share: for some reason, I'm fascinated with being above the Arctic Circle, especially in the summer. My friend Peter Agre and I have gone on a number of canoe and camping excursions up there; we navigated the Seal River in Canada in 2004, which is not above the Arctic circle but is far enough north to have polar bears.

Do you have any upcoming excursions?
There's a route called the Porcupine River that starts in the Yukon and goes 600 miles until it hits the Yukon River in the middle of Alaska. If I can get a group of maybe seven or eight people, plus guides, we could do this as a canoeing trip that would take about three weeks. Of course, it's hard to get together that many people who can commit to a trip that long. I told my wife I'm willing to go alone, but apparently I'm not allowed.

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