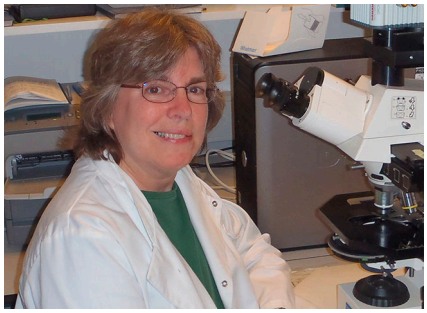
Margaret Robinson: Vesicles wear fancy coats

Robinson studies the function and evolution of coated vesicle adaptor proteins.

The vesicular coat protein clathrin was discovered in 1975. Since then, remarkable progress has been made in understanding vesicular biology. Yet researchers today are still working to understand how clathrin and other vesicular coat proteins give vesicles their characteristic structure and manage vesicular cargoes.

Margaret "Scottie" Robinson first heard about clathrin-coated vesicles soon after the coat protein's discovery and was immediately determined to work on them. Robinson is well known for her work on adaptor proteins (adaptins; AP), a class of proteins that make up part of the vesicle coat (1–5). Some adaptins mediate the interaction of clathrin with membranes and with cargo proteins (2), whereas others dispense with clathrin completely (4, 5). Altogether, adaptins comprise an ancient gene family with deep connections to human disease, Robinson explained when we called her at her lab at the Cambridge Institute for Medical Research.



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Margaret "Scottie" Robinson

LESSONS LEARNED

Did you have any role models growing up?

I remember an assignment from grade school where we were supposed to write about what we wanted to be when we grew up. I wrote about some people I admired. One of them was Margaret Hamilton, who played the witch in *The Wizard of Oz*. I thought it would be cool to be an actress, but I never wanted to play the princess. I wanted to be the villain. [*Laughs*] Another person I admired was Marie Curie. I used to get books out of the school library about chemistry. I suppose that was when I first became interested in science. I wanted a chemistry set and everything.

But I sometimes wonder: if there hadn't been a science requirement in college, would I have necessarily taken biology? I was actually planning to major in English or theater until I took an introductory biology course to satisfy my science requirements. In that class, Jeanne Powell gave a lecture on cells and showed us electron micrographs. That's when I really got interested in cell biology. I just couldn't believe the kinds of things that were in our cells. But I didn't go straight to graduate school after college because I wasn't sure what I wanted to do.

But you ended up in graduate school at Harvard…

Yes, and then I took a summer course at Woods Hole where I learned about coated vesicles. Brown and Goldstein had just discovered that they could sort LDL receptors, and Barbara Pearse had just discovered that they were made mainly out of a protein she identified called clathrin. I thought coated vesicles were fascinating. The problem was there was really nobody at Harvard Medical School who was working on them.

In retrospect, what I should have done was to say, "Okay, I can work on this as a postdoc." But somehow I thought that, if I didn't start addressing the question of how coated vesicles can sort their cargo right away, somebody else would answer it—which, of course, was silly because that took many, many years.

> "I thought coated vesicles were fascinating."

I joined David Albertini's lab because he was a brand-new faculty member and he was willing to let me work on anything I liked. But that was a mistake because I didn't have the background I needed to succeed. My work didn't go very well, and I was nearly kicked out of graduate school because I was too stubborn to change my tack. Finally I had to stop working on coated vesicles and work on something closer to what David's lab was doing.

These days I advise students to use their time in graduate school to learn to think like a scientist. Of course, one has to follow one's passion, but one can do that as a postdoc.

A WISH FULFILLED

You did eventually get to study coated vesicles…

I managed to convince Barbara Pearse to let me do a postdoc with her and also convinced the NIH to fund me in her lab. I wanted to find out what it was in clathrin-coated vesicles that binds to cargo. Eventually I succeeded in purifying components of the coat that were not clathrin and that we now know act as adaptor proteins. They sit between clathrin, which forms the vesicle's outer shell, and the vesicle membrane. Probably the most exciting result from my postdoctoral work was when I realized there were two different populations of clathrin-coated vesicles, one that used AP-2 at the plasma membrane and one that used AP-1 and was associated with intracellular membranes.

The AP proteins are multimeric complexes…

Right. AP-1 and AP-2 are both heterotetramers with related sets of subunits. They have two large subunits, and the beta subunit is fairly closely related in AP-1 and AP-2. Our evolutionary ancestors had just a single beta subunit, but in vertebrates AP-1 uses beta 1 and AP-2 uses beta 2. The other large subunit—called alpha at the plasma membrane and gamma at the intracellular membrane—is quite divergent. Then there's a medium-sized mu subunit and a small sigma subunit.

When I started my own lab I wanted to pursue the AP proteins in more depth. Barbara was great because she let me publish single-author papers when I was a postdoc and she was very happy to let me take some of the work with me.

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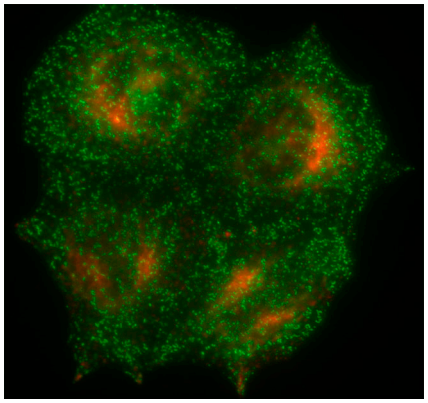

IMAGE COURTESY OF SCOTTIE ROBINSON

Vesicles bearing the adaptor complexes AP-1 (red) and AP-2 (green) localize to different parts of the cell.

By this time I decided to bite the bullet and work with DNA [*laughs*] because I realized that in order to characterize these complexes thoroughly I would really need to clone the subunits. In the end, Tommy Kirchhausen cloned the medium and small subunits, and I cloned the large ones.

A DEEP CONNECTION

There turned out to be several other AP complexes…

Both my lab and Juan Bonifacino's lab at NIH had the same idea at around the same time: to look at these interesting proteins that were about 30% identical to subunits of AP-1 and AP-2. I was interested in these proteins because I wanted to look for adaptors at other cellular locations. We both managed to find another AP complex, AP-3, which interacts very well with lysosomal membrane proteins such as LAMP1. It also interacts with tyrosinase, which is the key enzyme in melanin biosynthesis, so AP-3 is important for tyrosinase trafficking to premelanosomes. That's why mutants in AP-3 subunits are associated with coat-color mutations in mice and, as Juan showed, a form of albinism in humans called Hermansky-Pudlak syndrome.

Both the pearl mouse and Hermansky-Pudlak syndrome humans have mutations in the AP-3 beta subunit. On the other hand, mocha mice have a mutation in the other large AP-3 subunit, which is called delta. What's interesting is that in vertebrates there are two AP-3 beta isoforms—beta 3a, which is expressed ubiquitously, and beta 3b, which is only expressed in neurons and neuroendocrine cells—but there's only a single delta gene. Beta-mutated pearl mice seem fairly normal except for the coat color and a blood clotting defect. In contrast, delta-mutated mocha mice not only have the abnormal coat color; they're also very hyperactive, have a poor sense of balance, and become deaf. Pearl mice are similar to humans with Hermansky-Pudlak syndrome type 2. So far no delta mutation has been identified in humans, but it's possible they simply haven't been described yet.

> "We're very keen on what AP-4 and AP-5 might be trafficking."

What about the other related complexes, AP-4 and AP-5?

AP-3 can bind to clathrin in certain organisms, but AP-4 absolutely doesn't use clathrin. AP-4 is a real mystery because we don't know what it could be using as a scaffold. There's very little of it in cells, and the knockout mice have a fairly mild phenotype. But in humans, AP-4 mutations cause severe intellectual disability, spastic paraplegia, and other things. So although it's expressed ubiquitously, AP-4 mutations seem to primarily affect neurons, presumably because important neuronal cargoes are being missorted. We're currently trying to identify AP-4 cargos in an effort to explain this phenotype.

AP-5, on the other hand, was a big surprise to us. There was something in the human genome database that looked like a mu subunit, but we thought it was the result of a recombination event and probably part of a different protein subfamily. In fact, we subsequently realized that this protein is part of an evolutionarily ancient AP complex and that mutations in the large zeta subunit are involved in hereditary spastic paraplegia. There's also another complex that is even more ancient than AP-5. We call it TSET, and it seems to be a missing link between AP-5 and another type of coat, COPI.

Are you following up on these proteins?

We're very keen on what AP-4 and AP-5 might be trafficking because both of them cause hereditary spastic paraplegia but they're otherwise not very similar. They don't colocalize, and we're not even convinced that AP-5 makes vesicles. We're planning to use a proteomics approach to study this question, either using knockdowns or what we call a "knocksideways" approach, to try to understand more about the function of AP-4 and AP-5. With knocksideways we can very quickly trap a protein of interest on mitochondrial membranes, preventing it from reaching its target membrane. The cell has very little time to adapt to this change, so it generates very interesting effects. We'd like to make a knocksideways mouse. That's proven to be quite challenging, but we're still trying!

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Delicious biology: Members of the Robinson lab collaborated on an edible 3D replica of a cell, complete with chocolate frosting, to celebrate the work of a departing colleague.