Nihal Altan-Bonnet: Tracking viruses that hijack membranes

Altan-Bonnet explores where membrane traffic meets health.

In some ways, Nihal Altan-Bonnet's career shares similarities with a national security agent, pursuing invaders. As a graduate student in Sandy Simon's laboratory at The Rockefeller University, she showed that drug-resistant tumor cells were sequestering chemotherapy drugs away in acidified membrane compartments for deportation from the cell (1). That began her studies of phenomena in which the normal functioning of the secretory pathway was usurped for more nefarious purposes.

As a postdoc with Jennifer Lippincott-Schwartz at the NIH in the early 2000s, Altan-Bonnet discovered that the small GTPase Arf1 regulates the Golgi's function as a docking platform for proteins needed during mitosis (2). As she was moving to Rutgers University in 2006 to set up her own laboratory, a golden collaboration landed in her lap. That led her group to uncover Arf1's role in the hijacking of the secretory pathway by positive-stranded RNA viruses, including those that cause polio, the common cold, and hepatitis C (3, 4).

In 2013, she returned to the NIH in Bethesda, Maryland, where her group revealed a new mode of viral transmission: hundreds of stowaway virus particles are packed into phosphatidylserine-rich vesicles, expunged and picked up en masse by the next cell (5). Altan-Bonnet spoke with *JCB* about the sneaky moves viruses make and her solution to the two-body problem.

> "[Viruses] hijack molecules from the cell that can give them access to many more molecules"

MEMBRANES AS DOCKS

Your postdoctoral work revealed a new role for the Golgi as a docking platform for mitotic proteins. Was that controversial?

At the time, much of the controversy in the field surrounded what determines organelle identity. When Jennifer started using GFP-tagged organelle-resident proteins in conjunction with live imaging to watch the dynamics of these organelles, what she observed was that the Golgi was essentially an outpost of the ER and needed continual membrane input from the ER to be maintained.

In mitosis, we observed that the flux from the ER would stop, and consequently, the Golgi rapidly resorbed back into the ER compartment. I was very much interested in finding a functional relevance to why disassembling the Golgi would be important for the cell. We found that there are molecules on the surface of the Golgi, which regulate chromosomal division and the actin furrow. We proposed that the Golgi works as a "dynamic platform" where proteins dock during interphase and then in mitosis, they are released and can bind their mitotic targets.

What was controlling Golgi protein docking?

The small GTPase Arf1 needed to be inactivated for the Golgi membranes to be resorbed during mitosis. That's critical to this idea of a dynamic platform, too, because Arf1 is a recruiter of effectors to membranes. When Arf1 becomes inactivated at the onset of mitosis, the membranes are resorbed and these effector proteins are no longer sequestered on that membrane platform.

This idea that Arf1 activity is important for recruiting a large network of cellular proteins was one of the things that I carried over to my independent work.

Why did you switch to probing this system in virally infected cells?

Six months before the end of my postdoc, I was contacted by Ellie Ehrenfeld at NIH, who wanted to understand why poliovirus replication was sensitive to the drug brefeldin A, an Arf1 inhibitor. She asked if I wanted to look at Arf1 during poliovirus infection. Even though I was about five-months pregnant with our second child,

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Nihal Altan-Bonnet

I had no qualms about working with live viruses. I was *that* excited.

It was a completely whole new world for me, and I had a very rudimentary knowledge of virology. I just jumped right in and started spending long hours at the microscope to image the dynamics of poliovirus infections.

Once we could follow infections on the microscope, we expressed Arf1-GFP in the cells, and watched what happened.

And what did you see?

It was just incredible. Arf1 was being recruited by poliovirus to all these membrane platforms where the virus was replicating. As soon as we inactivated Arf1, it came off these platforms and the virus stopped replicating.

I had the idea that maybe Arf1 was being used by the virus to bring in some other host components needed for replication. I had planned to continue at Rutgers investigating the molecules on the Golgi regulating mitosis, but I was so excited and intrigued with what I saw happening in the cell during poliovirus infection that I completely switched gears and applied myself 100% to that.

What did you find?

We did an imaging-based screen to look for Arf1 effectors on those replication membranes. Much to our surprise we found only a small subset of effectors, including phosphatidylinositol 4-kinase III β (PI4KIIIβ). So over time, the virus was remodeling the entire host secretory pathway and forming these new mini organelles that were highly enriched in

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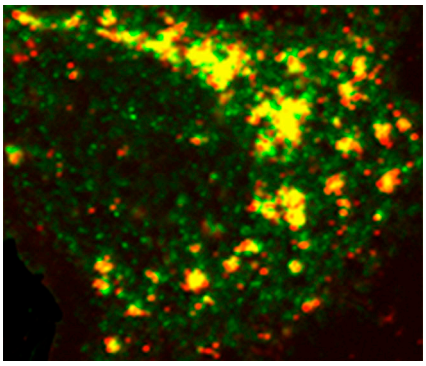

IMAGE COURTESY OF NIHAL ALTAN-BONNET

Coxsackievirus-induced replication platforms containing Arf1 (green) and viral replication enzymes (red) in an infected human cell.

phosphatidylinositol-4-phophate (PI4P) and viral replication enzymes.

We initially did this work with enteroviruses, like poliovirus and Coxsackievirus, but soon discovered that hepatitis C virus was also replicating on PI4P-enriched membranes. These are all important human pathogens, and it was very surprising how much membrane remodeling was going on and that these viruses all depended on this particular lipid, PI4P. Our paper was the first to bring forward this idea that viral replication depends on specific lipids and that manipulating those lipids could potentially be a pan-viral way to treat infections.

TRACKING TRAFFICKERS

Viruses are sneaky…

Yeah, they don't have much to work with. These RNA viruses make at most 10 or so proteins, including their capsid proteins. So they hijack molecules from the host cell—Arf1, PI4K, PI4P—that can give them access to many more molecules. Indeed, on those replication platforms, you also have PI4K- and PI4P-binding host proteins that facilitate replication.

How did the 2015 viral transmission study come about?

It came about from just wanting to understand how enteroviruses are assembled and get out of the cell. These viruses had always been classified as "non-enveloped." In other words, they do not have a membrane around their capsids, and so it was thought they could only leave by lysing the cell.

We decided to use noninvasive imaging to look at the dynamics of the exit process. We watched viruses being assembled at replication platforms, saw capsids coming off those structures and going into the cytoplasm and then disappearing from the cytoplasm. But the cells still looked intact, not lysed!

We discovered the assembled viruses were being captured by autophagosomes whose outer limiting membranes then fused with the plasma membrane, and the inner membrane containing the viral particles was dumped outside the cell.

What's the implication of these virus packets for transmission?

That was most exciting because this paper showed for the first time that viruses could travel collectively between cells. These packets being released were really large, 300–500 nm, and they contained hundreds of viral particles.

Viral RNA polymerases don't have error proofreading, so there is tremendous genetic diversity even within a single RNA species. Some of these viral quasi-species will be better at replicating over others and some will be better at dealing with host innate immune defenses. So if they are traveling and infecting a cell together, you could envision that there might be cooperation and complementation among those viral quasi-species in the next round of infection.

> "We have to rethink viruses, not as lone soldiers infecting cells, but coming in as an army… of genomes."

We showed that, indeed, traveling this way is very beneficial for the virus. Viruses that go into the cell collectively have much higher infection efficiency than viruses that go in at the same titer, but individually.

So we have to rethink viruses, not as lone soldiers infecting cells, but coming in as an army, as a collection of genomes that cooperate. This could have real implications for how drug resistance emerges.

DEMANDING DAYS

What was the biggest hurdle in going from a postdoc to independent investigator?

The biggest challenge during those early years was trying to establish a place for myself within a new field. Most new investigators start their laboratories on projects that they developed and published while they were postdocs. Starting out I had no footprint in virology—I had not published any papers, I had not even gone to any virology meetings. I was a completely unknown quantity. So it was very difficult the first three or four years as an independent investigator to be recognized and get a grant to continue to do this kind of work.

Your husband, systems biologist Grégoire Altan-Bonnet, has been at Memorial Sloan Kettering Cancer Center in New York for two years while you've been at the NIH. How do you make that work?

The good news is that he's moving to the National Cancer Institute this year, so it's going to work much better. The last two years have not been easy—the kids (we have a 13-year-old son and 10-year-old daughter) and I were in Bethesda while he was commuting from New York on the weekends. But we never considered this to be a long-term situation. If he had not been able to move, I would have looked for positions in the New York area.

I tell young scientists to find a good partner who is going to support you. I have been very lucky in finding someone who supports and encourages me to do my best science, and I strive to do the same for him. We don't count or quantify things at all and that's very important for a successful relationship. Things are not ever an even 50/50.

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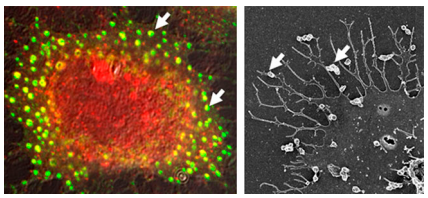

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(Left) Poliovirus particles packaged in autophagosomal vesicles (arrows) ready to exit the infected cell. (Right) Poliovirus containing vesicles (arrows) that have already exited the cell.