Thomas Pucadyil: Piecing together membrane fission

Pucadyil investigates membrane fission using novel model membrane systems.

Molecules from outside the cell, or those produced inside the cell, can be packed into membrane-bound vesicles to facilitate their transport to different cellular compartments. This is a highly regulated process that remains poorly understood, even at the most basic level; it is still unclear, for instance, how vesicles form and bud off from their parent membrane.

Having become interested in membrane biology during his graduate work on receptor biology (1), Thomas Pucadyil first cut his teeth on the problem of membrane fission as a postdoc, where he studied the function and regulation of dynamin (2, 3), a GTPase essential for vesicle fission from the plasma membrane. Pucadyil says that to comprehend vesicular trafficking, it will be necessary to isolate and study the inherent capabilities of proteins that drive membrane fission. We called him at his laboratory at the Indian Institute of Science Education and Research (IISER) in Pune to hear about the strategies (4, 5) he's pursuing for this work.

ENVIRONMENTAL INFLUENCE

Did anyone influence your decision to pursue a career in research?

I would attribute my leanings toward science largely to the environment I grew up in. My father, who's now retired, was a plasma physicist, so I was exposed to science at a very early age.

What did he do to foster your interests?

My parents never explicitly stated a desire for me to go into science, but I remember that his colleagues would often visit and invariably we'd end up having dinner and discussing their work, or more generally talking about where the world was headed, Indian science policy, and things like that. I think this underlying current made it easier for me to go down this path. My dad is also an avid reader and made sure that there was plenty to read at home. I was exposed to a lot of science fiction novels early on.

Other than that, I think the most enjoyable parts of my childhood were spent with my older brother. We were both—and honestly, still are—tremendously interested in LEGO®. My son Nikhil has serious competition from his dad when it comes to LEGO®. [*Laughs*]

I also liked gardening a lot—probably because of my mom, who was and continues to be very fond of gardening. She passed the gene down to me. Right now I'm cultivating some of the exotic plants collected by my IISER colleagues in my back yard.

Do you still enjoy science fiction?

My favorite author is Arthur C. Clarke. It's remarkable how Clarke proposed so many things in his writing that eventually ended up coming true. My favorite novel is Clarke's *Childhood's End*, which I think is really pertinent these days. It's about civilization on Earth coming of age, leaving aside petty squabbles in the face of a monumental challenge.

THE FUTURE WITHIN REACH

What led you into a research career in biology?

My bachelor's and master's degrees were in biochemistry. I had some exposure to bench work during my master's dissertation project, and I think that's when I learned that the ground beneath you is always shaky when it comes to research; I made that transition from taking everything that the textbooks said for granted to actually going out there and testing those ideas.

> "All of a sudden I realized science was actually within reach."

I did a summer rotation in Amitabha Chattopadhyay's laboratory at the Centre for Cellular and Molecular Biology in Hyderabad. I would eventually end up doing my PhD there, as well. That summer, I tagged along with a postdoc in Amit's laboratory named Kaleeckal Harikumar, who was very patient and just a fantastic mentor at the bench. We worked on understanding how a G protein–coupled receptor would respond to covalent modification. These proteins are extremely difficult to express and handle in a laboratory setting, so we would start with a tissue where the protein was expressed, then work our way down through a fairly elaborate biochemical isolation technique to get a more enriched preparation. It was a fairly short project, but it resulted in my first publication.

PHOTO COURTESY OF JOSEPH PUCADYIL

Thomas Pucadyil

That was an eye-opener for me. All of a sudden I realized science was actually within reach. Students are raised by reading textbooks, research articles, and reviews, and for some reason, this can all seem very distant. I recall reading articles and looking at the affiliations of the authors in places I'd only heard of. There was no real connection. It seemed so far away. But it was a remarkable experience to see my name with my institution in print. My PhD thesis evolved from that work, studying how membrane lipids influence receptor function.

So you were interested in membrane physiology even before your postdoc?

Amit was really instrumental in getting me excited about membranes, and during my

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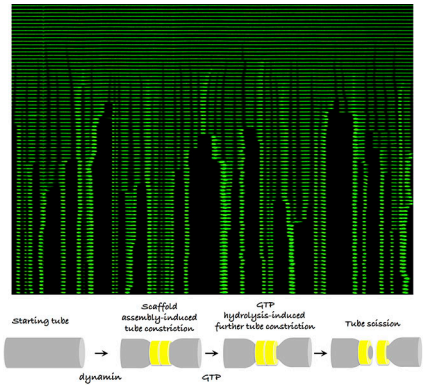

IMAGE COURTESY OF THOMAS PUCADYIL

Supported membrane tubes (SMrT) undergoing multiple cuts by dynamin.

PhD I was already working with membrane preparations isolated from the hippocampus. These were rather complex membranes, and I guess at some point I realized that I needed to strip down that complexity if I wanted to clearly understand what was going on. I needed to be able to reconstitute membranes so that I could know, for example, precisely which kind of lipids or proteins were present. So, toward the end of my PhD, I started working on model membrane systems: liposomes and supported lipid bilayers. I had already decided by then that for my postdoc and later career I wanted to move away from receptor biology and into an area where I could make more original contributions. That's when I got excited about vesicular transport, and decided to join Sandra Schmid's laboratory at Scripps.

BUILDING BETTER MODELS

In Schmid's lab you pioneered a new technology to study membrane fission…

I was determined to come up with an assay to study dynamin-catalyzed membrane vesiculation. But I had been working on supported bilayers for a fairly long time in Sandy's laboratory—maybe two years—and wasn't making headway until a paper came out from Jennifer Hovis' group that just put everything in order. Conventional supported lipid bilayers are formed from lipid vesicles that fuse to cover the surface of a silica bead. They're fairly taut membranes with little reservoir to bud vesicles from. But her group showed that if you had negatively charged lipids and played around with salt concentrations, you could generate bud-like features or membrane tubes emanating from the beads' surfaces. The biochemist in me took over and I started testing these conditions until I managed to pack much more membrane onto a bead than is required to cover its surface. This additional membrane reservoir was very useful for studying proteins that deform membranes.

Then I teamed up with another postdoc in Sandy's laboratory, Rajesh Ramachandran. He worked on a lot of the molecular details about how dynamin binds membranes and how its GTP hydrolysis cycle affects its assembled state, while I perfected assays to allow us to observe regulated vesiculation reactions. We identified some of the key residues important for dynamin's enzymatic attributes and membrane fission activity, and this gave us some ideas about how the protein might manage membrane fission.

> "I needed to strip down that complexity… to understand what was going on."

Then you returned to India to start your own lab…

I always knew I wanted to come back to India, and at that time there was a lot of buzz about a new set of institutes being set up by the Indian government as part of the Indian Institute of Science Education and Research Initiative.

My laboratory is a little different from a typical laboratory in the States, perhaps, because most laboratories in India are graduate student–heavy. It's a lot of fun and a lot of effort working with students! They also seem to have a lot going on in life outside of work. They keep me up to date on movies and music. [*Laughs*] I spend a lot of time with my group members outside the laboratory.

Do the approaches you used in Schmid's laboratory still figure in your work today?

Oh, absolutely. Our emphasis is largely on trying to understand vesicular transport, starting with a reconstitution approach. One of our aims is to uncover the intrinsic capabilities of molecules involved in vesicular transport reactions. If we take out the layers of regulation that are built around these processes, can we understand how they are achieved?

We've been working quite a bit on clathrin polymerization on membranes, and how this molecule promotes vesicle budding. We've set up assays to observe the polymerization reaction taking place in real time under a microscope, that also allow us to interrogate the functions of different clathrin adaptor proteins. There's a huge variety of clathrin adaptors and they're not functionally interchangeable, so we've begun looking at how different adaptors affect clathrin membrane recruitment.

In addition to that, we're working on assay systems to study membrane fission. We had a paper come out recently in *Nature Cell Biology* describing a new generation of model membrane system that we call supported membrane tubes, or SMrT. This technique is fairly straightforward to use and allows us to approach membrane fission from a variety of angles.

Dynamin gets a lot of attention but it's restricted to the plasma membrane. Surprisingly, Pietro De Camilli's laboratory published that cells lacking all three dynamin isoforms are viable in culture for a few weeks. That means there must be other molecules that catalyze membrane fission, both at the plasma membrane and elsewhere in the cell. We're taking a candidate-based approach, revisiting other molecules that have been suggested to function in membrane fission.

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The Pucadyil laboratory, at the cutting edge with model membranes and culture.