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INTERVIEW

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Sorting and Trafficking in the Endo-Lysosomal System

An Interview with Chris Burd, PhD

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Dr. Chris Burd is a professor of Cell Biology at Yale School of Medicine. He graduated from Northern Illinois University in 1987 with an M.S. He then pursued his Ph.D. at Northwestern University. Dr. Burd's research focuses on elucidating the mechanisms by which proteins and lipids in the endosome are packaged into transport carriers that bud from the endosome and ferry cargo to the Golgi apparatus and other organelles [1,2]. Defects in these pathways contribute to lysosomal storage diseases, cancer, Alzheimer's disease, and cardiovascular disease.

Can you talk about what your group is working on right now?

The lab investigates how molecules are moved in between different organelles of the cell. Because each organelle has a specific function that is endowed by its components, *e.g*. proteins, lipids, the sorting reactions underlie the basis of intracellular compartmentation. The lab works on the secretory pathway, trying to understand how proteins are selected to be secreted from the cell and how those sorting mechanisms work in the Golgi apparatus. A parallel interest is understanding how molecules in the endosomal system, which is initiated by internalization of part of the plasma membrane by endocytosis, brings nutrients and growth factors into the cells. Similarly, in the endosome, those molecules are either sorted into a degradative pathway that leads to the lysosome or exported from the endosome and sent back to the Golgi apparatus or the plasma membrane. Those pathways are implicated in a variety of neurodegenerative diseases. We're trying to understand how sorting of molecules within the endo-lysosomal system confers how those function to sort molecules and what are the mechanisms that are deficient in various diseases. Similarly, in the Golgi apparatus, we're trying to figure out how proteins are secreted from the cell and selected to be sorted, emphasizing the role of lipids that is the membrane where these membrane proteins exist. The lab is trying to develop new technologies that allow us to follow the lipids as they are synthesized and trafficked between different organelles of the cell. Not only signaling molecules like phosphoinositides (PIP) regulate some of these sorting reactions and fusion of transport carriers with different organelle membranes, but GTPases regulate the same sort of things as well. The lab is gaining a new appreciation for regulation by protein phosphorylation that we have recently discovered. We're trying to cover a lot of ground, but the underlying theme

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†Abbreviation: PIP, phosphoinositides.

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is how different molecules are sorted to different compartments/organelles of the cell.

Can you expand on your work on PIP and GTPase?

Signaling molecules, signaling lipids in particular, are low abundance lipids such as PIPs that are generated by PIP kinase that phosphorylate the head group of phosphatidylinositol at one of a number of different places. And there is a corresponding phosphatase that will also remove that phosphate and therefore terminate the signal. Those networks of PIP signal modules regulate the activities of different sorting molecules either by recruiting them to the endosome or the Golgi membrane by producing a lipid that's recognized by other proteins or by modifying the properties of the membrane itself to help it transport vesicles or carriers. Recently the lab has used genetic analysis to identify regulatory aspects of transferring molecules between the Golgi and the endosome. We found, for example, a cell cycle regulated kinase that phosphorylates a protein that is a subunit of a sorting complex that is called retromer [3]. Retromer complex functions at the endosome to select proteins to be exported from the endosome. We recently discovered that a subunit of retromer complex is phosphorylated by a cell cycle regulated kinase and this inactivates retromer, causing molecules that would normally be sent back to the plasma membrane now to proceed further in the pathway to the lysosome to be degraded. This way, during cell cycle, the abundance of those molecules that cycle on and off the plasma membrane is regulated.

How do you think these mechanisms are linked to cell cycle progression?

Our working hypothesis is rooted in the biology of the organism we used for our study, which is budding yeast or *Saccharomyces cerevisiae*. As budding yeast grows a new cell, it has to remodel an extracellular structure called a cell wall, akin to the extra cellular matrix. Because that is a very tough polymer, it inhibits growth. It has to be modified at the site where the new cell will form. This is tightly coupled to the cell cycle. We uncovered our findings through genetic analysis of mutants that are defective in growing new cells, and we identified a mechanism that controls targeting of these enzymes from intracellular organelles, endosome and Golgi, to the plasma membrane, where they are active and modifying the cell wall. And that's coordinated with the cell cycle to allow a new cell to form at the proper time and at the proper place.

Chris Burd, MS, PhD, Professor of Cell Biology, Yale University

Can you expand on your approach of harnessing genetics/genomics approach and live cell fluorescence microscopy?

We do a lot of live cell microscopy of cultured human cells as well as yeast cells that express various types of fluorescent proteins. The reason we use that was because, although one draws schematic of a cell in a very simple way where we just started to draw circles for different organelles, what we now appreciate is that there is a very rapid, and dynamic exchange of macromolecules between organelles. In order to understand those dynamics, one has to look in live cells where one can follow the fate of individual organelles and individual proteins on organelles and see how those change over time.

For example, in the endosomal system, a so-called early endosome will mature into a late endosome and that maturation process involves the removal of proteins from the endosome membrane as well as the acidification of the interior of that organelle. As a result, proteases and lipases and other degradative enzymes that function at acidic Ph optimum are activated during that maturation process. In order to understand this maturation process, one has to follow that as it occurs inside the cell. This is why we use live cell microscopy.

We also are developing ways, both live cell imaging and also with fixed cells, that allow us to follow the trafficking of different lipids in the cell – that's something very new for us and for the field [4]. This is enabled by recent breakthroughs in chemical biology that provided new tools that allow us to feed cells metabolic precursors to different lipids, which will be incorporated into native lipids. Some of these groups, using a type of chemistry called "click chemistry", allow us then to, after fixing the cells, attach "click-on" of fluorescent groups to the lipids that incorporated that precursor. We know where these lipids were made and over time we can follow where

these lipids are transporting to. Again, this is something very new for us and for the field because we have always been very focused on following proteins in between different organelles. It turns out that there are quite a few diseases, in particular neurodegenerative diseases, that we now understand severely affect the lipid composition of organelles, particularly in the endo-lysosomal system. Understanding how that contributes to disease is a goal for the lab.

So, this "click-on" technology works like a pulse-chase system?

Yes, that's exactly right. We use CRISPR-mediated gene editing to create cell lines that will use a particular lipid precursor that we provide in the medium. We gene edited these cells lines so that they can only use it in one particular lipid biosynthetic pathway. We can then do a pulse, where we do a short labeling period with that precursor, it's taken up, incorporated into newly synthesized lipid. And then we can wash away that lipid and begin a chase period, where we can follow at a different timepoint, fix the cells, click on a fluorescent group and ask, "where does the lipid go after being synthesized?" For example, we can watch a particular class of lipids called sphingolipids that are initially synthesized in the endoplasmic reticulum and the Golgi, but overtime they are transported to the plasma membrane, where they reside.

So, the main advantage of fixed cell imaging over live cell imaging is your ability to trace lipid as oppose to protein?

That's exactly right. In the past, the field has used genetically encoded lipid sensor: for example, proteins that recognize the head group of a particular lipid, *e.g.* PIP. You're going to express that in the cytoplasm as a fusion to green fluorescent protein. In live or fixed cell imaging, you can determine where that lipid resides and where it's the most abundant within the cell. Of course, the use of a large protein, which is actually much larger than the lipid you're trying to look at, might perturb the localization and the function of that lipid. The advantages of some of these new tools I just described is exactly as you mentioned: we look directly at the lipid. Moreover, the chemical modifications that allow the visualization of that lipid through click chemistry are quite small or minor. For many of the enzymes and the lipids we're studying, as far as we can tell, they don't distinguish between our chemically modified precursors and natural precursors. We think to a large degree these precursors faithfully report the endogenous lipids and that's a big advantage for our studies.

What do you think can be improved for this technology?

We still have to feed cells these precursors. We need to put large amounts of them into the cell medium. They have to find their way to the enzymes that use them. Typically, these enzymes are in the endoplasmic reticulum which means these precursors need to get across the plasma membrane, and find their way to the endoplasmic reticulum. The extent to which this reflects the natural process we really don't know. Of course, it's also possible that these lipid precursors do not faithfully report how their natural counterparts function. These are the good new tools that we have the best right now, so we're using them.

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