People & Ideas

Pier Paolo Di Fiore: Plumbing the depths of endocytosis

Di Fiore specializes in fishing out connections between the endocytic machinery and cancer.

Pier Paolo Di Fiore

Pier Paolo Di Fiore likes to describe his scientific successes as bits of happenstance—that as he and his colleagues pursued the next logical step in a sequence of experimental inquiry, luck often handed them something even more interesting to run with. Others would surely describe it as the payoff for perseverance that has led Di Fiore from studying cancer to endocytosis and back to cancer again. Along the way, his group was largely responsible for a mini-explosion of new ideas about how endocytosis functions well beyond its traditional role of intracellular trafficking to act as an all-around signaling platform that controls key cellular processes such as the cell cycle.

As a "restless" medical student in Italy, Di Fiore began studying retroviruses, at the time the preferred model for cancer research. In 1984, he arrived in the US as a postdoc to study growth factor receptor tyrosine kinases, such as EGFR, at the National Cancer Institute (NCI) in Bethesda, Maryland, where he would eventually become a section chief.

At the start of the 1990s his laboratory identified EPS15 as a substrate for the EGFR's tyrosine kinase activity (1). Further probing in Di Fiore's and other laboratories revealed that EPS15 plays a vital role in endocytosis, exhibiting a new form of signaling involving monoubiquitination (2). Recently, his group has shown that EGFR signaling gets prolonged or shut down depending on which endocytic pathway engulfs the receptor (3).

In addition, Di Fiore has become an adept wrestler of the new and mysterious "beasts" that come his way from fishing among cell signaling pathways. One such pursuit led to startling connections between the cell fate determinant Numb, endocytosis, breast cancer, and the tumor suppressor p53 (4, 5).

Di Fiore returned to his homeland 14 years ago to direct the Experimental Oncology department of the European Institute of Oncology in Milan. In 2001, he was asked to head up IFOM, the FIRC Institute of Molecular Oncology. Also a professor at the University of Milan, he stepped down from running IFOM in January 2009 to resume being a "full-time scientist." He shares his thoughts on research funding in Europe, how the endocytic system is like a Roman road, and why he misses American food.

CHANGING THE TEXTBOOKS

The old view of endocytosis was that it attenuated signaling by engulfing cell surface receptors and dooming them to degradation. How did that view change during your career?

People were wondering whether endocytosis had a role in signaling because the receptors were still active in the endocytic compartment. The real question was, "What is the productive part of signaling? Does it only happen at the plasma membrane or is what happens in the endocytic route also important?"

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Everything changed when Sandy Schmid's laboratory published their 1996 *Science* paper on the dominant-negative mutant of dynamin. She showed that when endocytosis was inhibited, certain signaling pathways were severely impaired. That's the moment that even old-fashioned signalers like me started thinking that maybe endocytosis played a major role in signaling. Today, we think that endocytosis is the framework on which signaling is written—basically they are two sides of the same coin.

What makes endosomes good platforms for signaling?

When we thought that all signaling was from the plasma membrane, the simple fact that another compartment could work for signaling was pretty novel. But endosomes make very unique signaling stations because they have some peculiar characteristics. Endosomes are small and so they concentrate proteins on the membrane. They are rich in certain molecules not on the plasma membrane, such as PI3P, a signaling lipid, and p18, an anchoring protein for ERK signaling. Endosomes are acidic, which is key for a number of signal transduction pathways.

I could go on and on, but the concept today is that it's not just another station for signaling, but it is a privileged platform where a number of things happen that could not occur on the plasma membrane.

THUMBING A RIDE

You've likened endocytosis routes to the Roman road system, which was developed to move armies, but then quickly used for communication and commerce as well. How did endocytosis get repurposed?

Well, it's an infrastructure. And once the infrastructure is there, the cell is normally "smart" enough to figure out ways to use it for different things. The initial selective

Text and Interview by Kendall Powell
kendall2@nasw.org

pressure to develop an endocytic system was most likely to gulp down other cells and get easy access to nutrients—at least that's what a lot of evolutionary biologists think.

You have to endocytose membrane and then you have to somehow put membrane back on the plasma membrane, and so you start developing an endocytic and exocytic cycle. At a certain point—regardless of the evolutionary reason—you find yourself with a very powerful network that can move things around the cell.

And you've extended the road analogy to include hijackers and hitchhikers?

Yes, so now the question is: Are other functions going to parasitize this infrastructure? Probably, yes—we know already that there are pathogens that parasitize the endocytic system. Of course, somebody from the outside doesn't need to be very careful—he can just hijack the entire system and use it for his own purpose.

But, if the cell wants to use the system, it has to do so without destroying it. The first step might have been molecules that hitched a ride on the system—to move faster, or reach other destinations, or to be segregated. Those hitchhikers, as I call them, might have "learned" to do something for endocytosis as well. They might have learned how to pay their fare.

Of course, this idea might turn out to be wrong. We pay a lot of attention to how things are, but I do believe that we also have to pay attention to how things came into being. It's important because we can learn from that. But, I wouldn't want my scientific career to be remembered for a hypothesis… unless it turns out to be a smashing success!

Your group showed that Numb—a protein normally associated with cell fate determination—is an important player in breast cancer. How did you make this connection?

We screened for proteins that interacted with EPS15, and one of the proteins that came out was Numb. What is Numb? We'd never seen it before. This is what happens when you do signaling—you're always fishing out a different beast that you have never seen or worked with. It's very interesting but also frustrating because you're working in an area in which a lot of people are better than you are.

But, on the other hand, you bring a novel outlook to the area. We did the obvious experiments to show that Numb was a bona fide endocytic protein. Then we got lucky with the tumor connection. One of the first things I did when I came back to Italy was to set up a molecular pathology unit that would routinely screen human tumors for all the proteins we were fishing out from our work. And it turned out that Numb was lost in 50% of breast cancers.

Di Fiore and family, visiting the Chateau d'Amboise in France, the burial place of Leonardo da Vinci.

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FOR THE LOVE OF MONEY AND FOOD

What is the current funding atmosphere like in Europe for scientific research?

The economic downturn is hitting everybody of course, but there are two levels to consider in Europe: the national level and the European Commission. At the EC level, the situation has actually improved. Two years ago, they introduced individual European Research Council (ERC) grants, which are very similar to the US RO1 grants. These are very good grants.

But at the national level, at least in Italy, the matter is really problematic. The money for Italian research has never been much and has become less and less with the economic crisis. I'm lucky because our institution is well funded through private foundations and charities, but you cannot run the research of an entire nation through those. But, I'm not much for complaining, and I think in difficult times, you roll up your sleeves and your character comes out.

You were at the NCI for 12 years: what do you miss about the States?

When asked this question, I give an answer and everybody laughs: I miss the food. And you will say, "Food is fantastic in Italy!" Yes, but it's Italian. In the States you are exposed to so many different cuisines.

There's a deeper meaning to what I'm saying. What I miss about the States is the multiculturality, and the fact that there is a little piece of something for everybody in America. America belongs to everybody; Italy belongs to the Italians. I never felt like a stranger in the States, from the moment I first set foot there.

What type of cuisine did you enjoy most?

I love everything. I got introduced to sushi in the States, and I can easily run up a $200 bill at a sushi restaurant with my wife. In the Washington area there were all kinds of fantastic food: Indonesian, Chinese, Indian, Thai—you're exposed to everything. Ethiopian food—eating with your hands, now that's a very rewarding experience.

I knew we were going to end up talking about food!

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