Graça Raposo: Melanosomes, more than skin deep

Raposo studies the biogenesis of melanosomes as exemplars of lysosome-related organelles.

Tiny, membrane-bound packets of tinted protein, called melanosomes, dictate the color of human skin. Deficiencies in melanosome production underlie pigmentation diseases, so it is important to understand how melanosomes are generated. But melanosomes are just one example of a larger family of lysosome-related organelles, many of which are associated with pathologies when they are functionally impaired.

Graça Raposo, from the Institut Curie in Paris, France, is—quite literally—taking a close look at the generation of melanosomes. Her training in electron microscopy (EM) and protein trafficking during her graduate work (1) and postdoctoral studies (2) has been essential to her work. Combining EM with other approaches, her lab has demonstrated the endosomal origin of these organelles (3) and has begun dissecting the machinery needed for their construction (4, 5). We called her to get a close-up view of both melanosomes and of her career.

PHOTO COURTESY OF CNRS

Graça Raposo

FIRST LOOK

You're the daughter of a Portuguese Admiral…

Yes, so my family traveled quite a lot when I was a child. I was born in Lisbon, but my father was immediately sent to Angola, in Africa. We lived there until I was two years old, then, after a brief time in Portugal, we moved to France. My first schooling was in France, from age three to six, and something about that time really stuck with me. Even though we subsequently moved several more times, including to Mozambique, I always wanted to return to France. Later, it turned out that my father was nominated to the Portuguese embassy in Paris just after I finished high school, so I had the luck to start my university studies in France.

My father was posted to the Azores Islands three years later—which was great because I could go there on my holidays. But even though it was difficult to be separated from my family, I stayed in Paris, living in a tiny apartment with very little money and my best Portuguese friend, who is now a professor at Paris VI University.

What did you study in university?

I studied biochemistry and immunology because I had loved biology in high school. In my courses at Paris VII University, I was introduced to EM, and, even though it was not very fashionable anymore, I really liked it and the beautiful images it could produce. I felt that putting cells in a test tube and smashing them up was not for me.

I decided to do a master's in membrane biology, and when I applied there was one lab on the list that had expertise in EM. It was led by a fantastic Italian professor, Lucio Benedetti. When I first contacted him, he said he did not have room in his lab for anyone else, but he talked it over with his wife and close collaborator, Irene Dunia, and they finally agreed they could take me. I was very happy in the lab and managed to get a paper published before I finished my master's degree, so I decided to stay and do my PhD there as well. It turned out to be a good choice for personal reasons, too, because one of Lucio's sons was always coming around to visit the lab, and eventually I married him. [Laughs]

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THE RIGHT CHOICE

EM has remained a key part of your work throughout your career…

Lucio had been trained by Wilhelm Bernhard in Paris. I was Lucio's last PhD student, and when he passed away in 2009 I inherited all his books on electron microscopy and cytology and original reprints from Palade and Novikoff. I was determined to keep working with EM, but there were very few places in Europe that specialized in the technique. So the first postdoc I did was in Jean Davoust's lab in Marseille. He was building confocal microscopes, and I joined his lab because I had become interested in intracellular trafficking in specialized cells such as those of the immune system.

I started trying to characterize the compartments where antigen processing takes place and where MHC class II molecules are paired with fragments of antigens. I was enjoying my work, but at the same time I knew that, if I wanted to continue working with EM, the place I should really be was in Utrecht. Hans Geuze's lab was one of the best EM labs in Europe, and when I met him at a meeting I asked if he had any space in his lab. He was excited to get someone who was already experienced in EM because almost no one in my generation had training in it anymore.

This is why I feel so lucky in my choice of PhD lab and husband: when I told my husband I wanted to move to Utrecht, even though we had a two-year-old son and my husband would have to take a sabbatical from his position at CNRS to do a postdoc in a new field, he still agreed to do it. So we moved to Utrecht, and I continued my work on the trafficking of MHC class II molecules.

Is that when you started working on exosomes?

We wanted to study how MHC class II molecules reach the plasma membrane. These molecules traffic through late endosomes/

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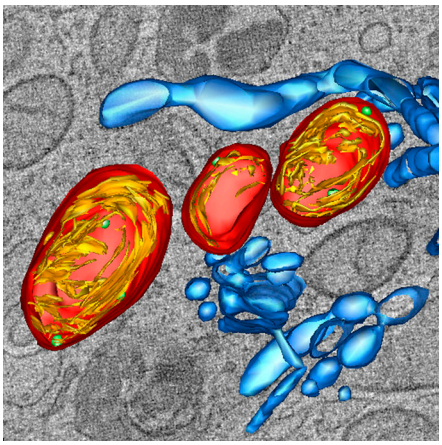

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Melanosomal membrane (red) enclosing fibrillar Pmel1 protein (yellow) is shown connected to endosomal membrane (blue) in this tomographic reconstruction.

lysosomes, which are degradative compartments, so how were they still able to stimulate T cells after that? In the electron microscope, I started seeing that some late endosomes are full of tiny vesicles and that these so-called "multivesicular bodies" could fuse with the plasma membrane. It looked like a degranulation process, so we decided to see if there were MHC class II molecules on these vesicles and if they could stimulate T cells. For almost two years I was doing almost exclusively biochemistry because I really needed to isolate, fractionate, and do Western blots and pulse-chases to answer this question. Finally, in collaboration with Cornelius Melief, we showed that these vesicles, which are called exosomes, could indeed stimulate T cell proliferation.

CLOSER EXAMINATION

Are you still working with exosomes today?

I'm still working with exosomes and multivesicular bodies but now in the more general context of biogenesis and secretion. There are several organelles—including MHC class II compartments, mast cell granules, platelet-dense granules, and melanosomes—that are generated by very different cells for specialized functions but that are all related to lysosomes. We wanted to know whether these organelles all share a common biogenetic process, so, in collaboration with Michael Marks from the University of Pennsylvania, we started looking at melanosomes. These organelles are exclusively found in specialized cells called melanocytes, but, although they were known to be important for skin pigmentation, very little was known about their biogenesis.

You are studying the origin of melanosomes…

In 2001, we had a paper where we showed for the first time that melanosomes originate from the endosomal system. In 2009, we published a follow-up to that paper where we showed that there are structural connections between endosomes and melanosomes. We are now working intensely on how these endosomal domains are created, how they are maintained, and, finally, why they can be contiguous with melanosomes. So far, we have made some progress on how these tubular domains are created, the role of the kinesin KIF13A in this process, and how small GTPases of the Rab superfamily are able to control and regulate these domains.

We wanted to know whether these organelles all share a common biogenetic process.

Does the formation of melanosomes require special proteins?

The molecular machineries—adaptors, Rabs, SNARE, and BLOC complexes—that help drive melanosome formation are ubiquitous, but I think that in order to get formation of any specialized lysosome-related compartment what you really need is the specialized cargo that's found in these compartments. Maybe in melanocytes you have certain interactions that are favored because of the specialized cargo and that cooperate to deliver melanosomal enzymes, pre-melanosomal proteins, and other proteins to the right place at the right time.

For example, when we express a major melanosomal protein, Pmel, in HeLa cells, we are able to create premelanosomal-like compartments that show fibrillar structures just like premelanosomal compartments in melanocytes. But still, these compartments are missing a lot of other proteins that are important for pigmentation and for promoting melanogenesis. So HeLa cells have been useful, especially for our studies on minimal requirements for melanogenesis or Rab proteins. But I'm bored by HeLa cells because they are "empty." When you look at either primary melanocytes or pigmented melanoma cell lines, these cells are crowded with melanosomes and have a well-developed biosynthetic and endosomal system.

People also forget that the melanocyte is never alone. Melanocytes in the skin are in contact with keratinocytes, to which melanosomes are transferred. A lot of our research is now aimed at developing co-culture systems and in vitro skin models to understand how the endosomal domains that interact with melanosomes may be modulated by the interactions between these two cell types. As we develop and adapt techniques such as improved correlative light electron microscopy, we should be able to get better insights into these questions.

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Raposo and her lab enjoy the view from their building near the center of Paris.