## Rosa Puertollano: The importance of recycling cellular trash

Puertollano studies how lysosomes contribute to cellular homeostasis and disease.

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**Rosa Puertollano**

Rosa Puertollano grew up in Madrid in a family of lawyers, but her interests always turned to nature, animals, and science. She would watch documentaries from National Geographic or the Discovery Channel, eagerly awaiting whatever would come next. After earning her master's degree in molecular genetics and a PhD in molecular biology and biochemistry in Spain, she completed postdoctoral work at the NIH's National Institute of Child Health and Human Development in the laboratory of Juan Bonifacino, studying protein sorting in the endolysosomal system.

Now a senior investigator at the National Heart, Lung, and Blood Institute, Puertollano studies how defects in endolysosomal trafficking pathways contribute to human diseases such as the lysosomal storage disorders, a group of rare diseases in which enzyme deficiencies prevent the lysosomes from properly breaking down glycogen or other products for recycling, leading to a host of mental and physical ailments.

One of her early interests was understanding the molecular basis of mucolipidosis type IV (MLIV), a disorder characterized by severe neurological and ophthalmological abnormalities (1). She and her colleagues proposed that MCOLN1, the malfunctional protein in MLIV, releases calcium from the lysosome to the cytosol, facilitating fusion of lysosomes with different intraorganellar compartments.

More recently, her attention has turned to understanding how lysosomes help maintain cellular homeostasis and adaptation to stress. The identification of the transcription factor TFEB as a master regulator of lysosomal biogenesis suggests that cells monitor lysosomal function and can modulate lysosome number and activity, depending on environmental conditions. Puertollano's lab has investigated the function and regulation of TFEB and related transcription factors (2–5).

She spoke with *JCB* recently about her research, and what excites her about coming to work each day.

### STORING UP TROUBLE

***How common are lysosomal storage disorders?***

They are mostly rare diseases but there are many of them. There are mutations now in 50 or 60 different lysosomal proteins that induce lysosomal storage disorders. If you combine all of these diseases, probably 1 in 5,000 births is a person that is going to have one of those mutations.

In groups where those mutations are more predominant, like Ashkenazi Jews, many people are doing genetic testing to see if both parents carry a mutation, as these diseases are usually caused by recessive genes.

***Are they all very disabling?***

Yes, the symptoms are very similar, which is interesting because they can result from malfunctions of completely different proteins. In many cases, the babies seem healthy when they are born, but very early in development, when they are around six months old, they start to have some clear problems: They can have difficulty holding a spoon or a crayon and they have problems with their vision. As teenagers they are usually in a wheelchair. As adults they suffer mental retardation.

> "The beauty of this approach is that these transcription factors can eliminate the 'bad' lysosomes."

It seems in all of them that the regulation of lysosomes is not very efficient. You start accumulating a different type of substrate depending on the disease. Sometimes it's glycogen, like in Pompe disease, sometimes lipids. It could be protein aggregates, but the bottom line is that the cell reaches a point in which the lysosomes become swollen and nonfunctional. They probably cannot fuse very well with autophagosomes, late endosomes, or phagosomes. At this point, the cell cannot eliminate all its trash, the harmful products that have to be eliminated. Some cells seem to be okay, but others, like neurons, muscle cells, and retina cells, are extremely sensitive.

***What have you learned about Pompe disease?***

In Pompe disease, the enzyme that breaks down glycogen inside the lysosome does not work properly. So glycogen accumulates in lysosomes, predominantly in muscle, and the lysosomes become huge. Autophagosomes cannot fuse with them, and they accumulate, too. This accumulation is such a problem that it can affect muscle contraction.

We are currently investigating whether overexpressing TFEB or TFE3 can eliminate the abnormal lysosomes that are messing up everything in the cells. The beauty of this approach is that these transcription factors can eliminate the "bad" lysosomes, because they induce their fusion with the plasma membrane, sending all this storage material outside the cell. At the same time, they can induce lysosomal biogenesis.

When we overexpress TFE3 in myotubes from a mouse model of Pompe that was created by Dr. Nina Raben, we can see that in two days all those big, ugly lysosomes

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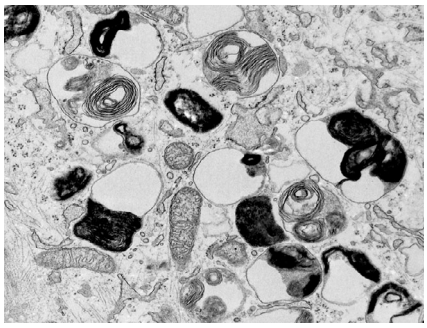

IMAGE COURTESY OF ROSA PUERTOLLANO

**Storage materials accumulate in fibroblasts isolated from patients with mucolipidosis type IV.**

full of glycogen are gone. The glycogen is no longer in the cell, and we start to see all these new healthy lysosomes populating the cell. It's really amazing.

Of course there are still a lot of caveats. As a basic cell biologist, I always feel worried about patients. I don't want them to think that we have a cure ready to go. But, at the same time, the results from our group and others are very promising, and I think we need to keep assessing the use of TFEB and TFE3 as therapeutic targets.

## STRESS RESPONSE

***What else are you working on?***

We know that TFEB and TFE3 are very important to facilitate cellular adaptation to starvation stress. If you don't have enough nutrients, cells activate these transcription factors, which now promote autophagy induction, formation of new lysosomes, and adjust cellular metabolism. These changes are critical for cell survival. Now we are trying to understand if TFEB and TFE3 are also involved in adaptation to other types of stress. We are starting to have more of a global vision of the role of those transcription factors in cellular stress responses.

I think that this increase in lysosomal biogenesis and autophagy is going to be just one arm of the stress response regulated by TFEB and TFE3.

***What do you ultimately hope to find?***

Well, the goal will be to connect the dots. First we have to collect and understand a few more pieces of the puzzle. In science we tend to be very specialized in a particular field. It's how it has to be, because the fields move so fast that it's almost impossible to keep track of everything that's going on.

But we are starting to see some intriguing links between different diseases. For example, there are clear connections between chronic inflammation and obesity. At the same time, obesity can be related to neurological disorders and some cancers. It's therefore clear that those processes are connected, and here we have a very interesting family of transcription factors that can regulate metabolism, cytokine production, autophagy, and clearance of aggregates.

By understanding these transcription factors, I think we are going to be able to get a global picture of how these different processes are related, and they are going to give us a great opportunity to identify some therapeutic targets. It's critical to learn how to modulate the activity of these transcription factors, because I think this has the potential to be used for a plethora of different diseases.

> "I think we are going to be able to get a global picture of how these different processes are related."

## EXCITED FOR THE FUTURE

***What excites you about coming to work each day?***

Seeing the results of the experiments we are doing is very exciting. We are studying very basic cell biology, really to understand at the molecular level how these diseases are related, but with the hope that it's going to have an application to human disease.

I don't know if I'm being too optimistic, because these things obviously take a lot of years, but I think it's good to keep this goal in mind. We are trying now to treat animals (mice and zebrafish) with lysosomal disorders with various small molecules and activators. I don't know if it's going to work or not, and it's probably going to take a while to optimize the conditions, but it's really exciting to think that there could be a real life application for our basic research. I will be happy even if we get a hint that things are going in the right direction.

We have lab meetings every Wednesday, and everybody gives me a heads up of what they've been doing. I really look forward to that. I'm always hoping they are going to give me some great data.

***What do you do to unwind after work?***

You mean if I had time? [*Laughs*] As soon as I leave the lab I just go home. I have a six-year-old son who obviously requires a lot of attention. My husband is also a scientist, so after work and on weekends we just spend time with him, reading books and talking to him and getting this face-to-face time that kids obviously need. Then, once he goes to sleep, we come back to the computer and keep working for a little while.

We also love to travel. Whenever we can, we go back to Spain at Christmas and spend time with the family, or some weekends we go to the mountains and walk around. It's really fantastic. And we love to take my son to the theater or to museums. He loves to go to art museums. He has decided to be an artist and a scientist when he grows up; it is not a bad combination.

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4. Martina, J.A., et al. 2014. *Sci. Signal.* 7:ra9.
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**Puertollano spends time with her son, Christopher.**