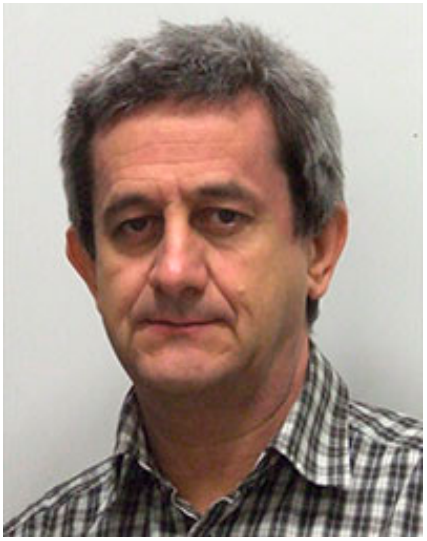
Cayetano González: Mothers, daughters, stemness, and cancer

González studies centrosomal inheritance, asymmetric cell division, and cancer.

If you've come across a diver wearing a dry suit in the sea south of Spain, you may have run into Cayetano González. A dry suit might be overkill for the warm waters of the Mediterranean, but González uses one because he first learned to dive in the cold, dark lakes around Heidelberg, Germany, where a dry suit is a necessity.

While he was exploring the depths of European lakes, González and his lab were also striking out into uncharted waters of centrosomal biology (1)—and toward a confluence between asymmetric cell division and cancer (2, 3). Studies conducted by the González lab in *Drosophila* have informed us about the basic biology of both centrosomal inheritance (4) and cancer (5). But now it's time to dive deeper, González told us when we reached him at his office at the Institute for Research in Biomedicine in Barcelona, Spain.



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Cayetano González

THE PROPER TOOLS

When did you decide on a research career?

I come from a working-class background, and my father was determined that my sisters, brother, and I should all study hard and get university degrees. So, I started telling everyone that I wanted to be a medical doctor when I was seven years old. But when I didn't make it into medical school, I decided to study biology instead. Later, once I had done my first year of studies and passed my exams, I was admitted to medical school after all. [*Laughs*] But by then I had realized that I was really much more interested in biology.

Where did your interest in cell division come from?

After my graduate studies I got an offer to join Pedro Ripoll's lab. His was one of the very few Spanish labs that, at that time, had international recognition, so this was an opportunity I could not miss! The project I chose to work on in Pedro's lab was the study of cell division in the fly.

Later, when I was looking for a postdoc, I learned that David Glover had recently returned to England from Stanford with fantastic expertise in molecular biology. When I joined his lab I brought my know-how on classical genetics. It was a great partnership, and I worked with David both as a postdoc in his lab in London, and then as a joint principal investigator when he became chair at Dundee University. I spent four happy years working in Dundee, where my daughter Laura was born, but I really wanted to have complete independence and my own lab, so I took a position as group leader at the European Molecular Biology Lab in Heidelberg, where my son Andrés would be born a few years later.

One of the topics your lab first focused on was centrosome biology…

Actually, this was an old interest of mine. Some of the mitotic mutants I had identified during the course of my PhD had phenotypes that suggested they might impact centrosome behavior. We had observed structures we thought were monopolar spindles. But at that time we didn't have the tools we needed to investigate this problem.

> "What we were seeing made no sense whatsoever."

The first antibodies that would allow visualization of centrosomal components by immunofluorescence were developed while I was a postdoc in David's lab. Around the same time, David's group also cloned Polo and Aurora kinases, which are important regulators of the centrosomal maturation cycle. So, this was definitely one of the things I wanted to continue investigating in my own lab.

We started out recording videos of centrosomal behavior in dividing cells. But at first, we had markers for the pericentriolar material (PCM) but not the centriole, and we spent a long time being completely baffled by what the videos were showing us. What we were seeing made no sense whatsoever.

Why?

When you stain for PCM in a typical mammalian tissue culture cell—a symmetrically dividing cell—you will see one signal, a dot, of PCM that splits in two at the start of mitosis. These dots then migrate away from each other toward opposite spindle poles. The original centrosome, which is composed of two centrioles plus the PCM has duplicated and then split. But we were studying cell division in *Drosophila* neuroblasts, which divide asymmetrically to produce one self-renewing neuroblast stem cell and one differentiating cell. In neuroblasts, staining for PCM showed one signal that never split in two. During mitosis, a second PCM signal appeared all of a sudden at the other side of the cell. That was really puzzling.

When we finally obtained markers for the centriole, we realized that when centrioles split in *Drosophila* neuroblasts, one of them lacks PCM, and this one migrates away to the other side of the cell before it begins rebuilding its PCM. It took us years to work out if this behavior is correlated with centriolar age, but eventually we saw that the centriole that moves away is the mother centriole,

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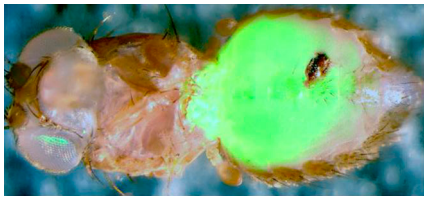

IMAGE COURTESY OF FABRIZIO ROSI

A malignant tumor (green) arising from the defective asymmetric division of *Drosophila* neuroblasts can be propagated indefinitely.

the older one of the pair. The one that remains decorated by PCM throughout the cell cycle is the daughter centriole in this cell type.

BENEATH THE SURFACE

Is there a relationship between centrosomal inheritance and stemness?

Inheritance of centrosomes is not random within a given lineage of asymmetrically dividing cells. In the case of *Drosophila* neuroblasts, the daughter is inherited by the neuroblast, which is the stem cell. The same applies most of the time in female germline stem cells. But in the case of male germline stem cells, the mother centriole is the one that is retained by the stem cell. Therefore, there does not seem to be any general principle for whether the mother or the daughter will be retained by the stem cell of the lineage. Nor is there any clear rule for whether the mother versus daughter has a particular functional role, such as the ability to organize interphase microtubule arrays or to drag along certain proteins or RNAs. These properties vary by species, and even in different cell lineages within the same species. The more we learn, the more it seems there are no general principles in biology.

Are there markers to distinguish neuroblast mother centrosomes from daughters?

The mammalian protein centrobin (Cbn) localizes asymmetrically to daughter centrioles. We cloned the *Drosophila* homologue of Cbn, fused it to GFP, and observed that Cbn also bound to only the daughter centrosome in the fly. Then we asked whether the fact that it is asymmetrically localized could be functionally relevant. We found that if we forced localization of Cbn on the mother, which doesn't normally have it, the mother now behaves like a daughter. It stays at the apical side of the cell and can organize microtubules. Conversely, when you remove Cbn from the daughter, it now behaves like a mother, becoming motile and unable to accumulate PCM. We also found that Cbn is a phosphorylation substrate of Polo, which is a major regulator of centrosomal maturation; Cbn phosphorylation by Polo during interphase is essential for organizing neuroblast microtubule asters. We're now looking more closely at the features of this regulatory pathway.

You know, it's funny: until now, Polo has never been a main subject of my research, but this protein just keeps coming back to me. It may have something to do with the fact that my wife is the first author on the paper that described its cloning. [*Laughs*]

"The more we learn, the more it seems there are no general principles in biology."

DEEP UNDERSTANDING

You've also used neuroblasts as a model for cancer…

Disruption of asymmetric division could lead to faulty cell fate specification and could produce malignant behavior, for example by creating a cell that fails to enter the differentiation program and continues dividing. We found that disrupting genes that determine neuroblast cortical polarity creates daughter cells with malignant behavior. We've transplanted these mutant cells into a new host, an adult fly, and shown that the cells divide hundreds of times and can be serially propagated into new hosts. We've maintained these tumors in this way for more than 10 years. These cells are immortal. They also acquire the capability to migrate and invade other parts of the fly, and some of them exhibit a significant level of chromosomal and centrosomal instability.

Completely separate from our work on the polarity mutants, we're studying another mutant fly isolated decades ago by Elisabeth Gateff. A few years ago, we showed that brain tumors that grow in these flies express dozens of germline proteins that are normally found only in the testes or ovaries. This is very reminiscent of the expression of "cancer-testes antigens" in certain human cancers. We showed that a subset of these germline genes is actually required for the tumor to grow in the fly. Now we are setting up a gigantic screen to look for other proteins required for tumor growth in these flies, with a focus on genes known to be upregulated in human cancers.

Has the cancer community embraced the fly as a model for tumor development?

Our work with the fly tumor lines has been generally well received. Of course, there will be some processes or aspects of malignant growth that we just cannot see in the fly. But other aspects are amenable to study in flies. The success of this organism as a model will ultimately be measured by whether we make a difference in terms of human health.

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González dives deep.