Amy Gladfelter: Fungi with a streak of individuality

Gladfelter studies the behavior of nuclei and cytoskeletal structures in syncytia.

A syncytium is a cell that hosts multiple nuclei within a common cytoplasm. Such an arrangement may be expected to pose challenges for cellular organization. For example, nuclei within artificially created syncytia divide in sync with each other, suggesting the signals that trigger cell division are shared between them. But this isn't universally true; for example, the nuclei of the syncytial filamentous fungus *Ashbya gossypii* show strong streaks of individuality.

As a postdoc, Amy Gladfelter observed that *Ashbya's* syncytial nuclei divide asynchronously (1). Intrigued, she began investigating how syncytia compartmentalize themselves to organize their behavior. She has used *Ashbya's* unique biology to investigate the mechanisms guiding formation of higher-order septin structures (2, 3), and her work on asynchronous nuclear division in *Ashbya* (4, 5) has led to the discovery of a mechanism for cytoplasmic organization that Gladfelter suspects may be used by all kinds of cells. We called her at her office at Dartmouth College to learn more.

FRESH STARTS

Do you remember what career you wanted to have when you were a child?

When I was in elementary school I wanted to be a Rockette. I thought it would be really fun to get up in front of people and perform. But then in adolescence I realized I wasn't going to be tall enough.

I became interested in biology in high school, and I started out as a premed in college at Princeton, thinking I would probably become a doctor. But within my first year of college I realized there were people who studied science for a career. I had never been exposed to that idea before, so I hadn't even realized it was a possibility. When I stumbled into a lab experience in the summer of my freshman year, it completed the transformation for me.

Whose lab did you work in?

I joined the electrophysiology lab of Simon Lewis, a colleague of an old family friend, at the University of Texas Medical Branch in Galveston. Simon was a great mentor. He spent a lot of time with me, discussing my data and explaining things like the Nernst equation over coffee. He was my first scientific mentor. I really connected with the kind of one-on-one learning he offered me.

I spent the next summer with Toby Bradshaw doing plant genetics at the University of Washington, but, during the following summer and the rest of my time as an undergrad, I worked in Bonnie Bassler's lab at Princeton. It was my experiences with Bonnie that convinced me that I wanted to go to graduate school and get a PhD. She's a world expert on bacterial quorum sensing, so the science was great, but she also taught me that you can be both a scientist and a human being. She's funny and energetic and has a contagious attitude about how fun science can be.

INTELLECTUAL CONNECTION

Most undergrads don't get to experience so many kinds of science…

I've always had many interests, and I was curious about many different problems. I think I also get some stimulation from making a fresh start in a new setting. So I worked in mammalian cells, plants, and bacteria, and then for my PhD I studied morphogenesis in yeast. Even to this day my research program is pretty broad, and I like to branch out in different directions.

> "I like to branch out in different directions."

Of course, I also needed to make money during the summer to pay for books during the school year, so I went to labs that could pay me a stipend.

As a graduate student you worked in a different type of yeast than you do now…

My graduate work was done in *S. cerevisiae* and concerned how cells make the decision to go from being round to being polarized. I thought it was an interesting problem, but the reality is I chose my thesis lab because of my advisor, Daniel Lew. I joined his lab at Duke more because I enjoyed our interactions than because of my interest in the scientific problem, but it was a good decision because I ended up having a blast intellectually. He also does very rigorous science, so I learned a lot, enjoyed my work, and got a lot done.

PHOTO COURTESY OF ELI BURAK

Amy Gladfelter

Still, as a postdoc I wanted to do something different. I had been thinking about filamentous fungi such as *Ashbya gossypii* because they are a genetically tractable system in which one can ask broad questions. But, one can still find a unique question to study with these cells—something that's harder to do with *cerevisiae* because it is such a popular system to work with.

It was just by chance that I encountered my future postdoc advisor, Peter Philippsen, at a meeting, and he invited me to Switzerland. Within a couple weeks my husband also received a postdoc offer from someone in Switzerland, and we thought, "We're not superstitious, but how often does that happen?" So we went for it.

When I arrived there, I didn't have any clear idea of what I wanted to study, so I spent a lot of time watching peoples' movies of *Ashbya* cells. These are huge cells with a constitutively polarized pattern of growth. They form hyphae that branch out

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from their tips like a neuronal dendritic tree, but they're also a syncytium, with lots of nuclei sharing the same cytoplasm. While watching videos of *Ashbya*, I noticed that, even though they shared the same cytoplasm, neighboring nuclei divided out of sync with each other. That struck me as a really interesting organizational problem: How can the cell cycle be compartmentalized within a common cytoplasm?

AN INDEPENDENT STREAK

Several of your papers have mentioned the concept of molecular noise as a driver of this asynchrony…

We think there are two pieces to the puzzle of asynchronous nuclei. First, there has to be something that makes timing variable so that the division cycle in different nuclei takes different amounts of time, even though they're in the same cytoplasm. Second, we think there has to be a mechanism to insulate the nuclei, to make them autonomous.

Molecular noise is one potential source of variation in timing, particularly in the regulation of the G1 phase of the cell cycle. It can actually become a dominant source of variation when key components are limiting. For example, we've done experiments that suggest G1 cyclins can be limiting in the system because when they're overexpressed they drive modest increases in local synchrony.

But there's another source of variation, which is that each nucleus in the syncytium creates a functional cell within a cell. We had assumed that transcripts could diffuse evenly throughout the cytosol, but we now have evidence that certain transcripts remain somewhat local. We found that one of the ways this can happen is that transcripts for the G1 cyclin, *CLN3*, bind to an RNA-binding protein that has a large polyQ expansion and is therefore prone to aggregation. If the cyclin transcripts don't bind this protein they become randomly distributed throughout the cytosol, and nuclear divisions become more synchronous. We're really excited about the idea that these low-complexity sequences in RNA-binding proteins, which have been associated for a long time with P-bodies or stress granules in other organisms, have very restricted diffusion. Therefore, they serve as a means to capture cyclin transcripts and keep them local to the nucleus. I think this mechanism of cytoplasm organization could be quite general. We just had to study this large cell with a quirky cell cycle in order to find it!

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Gladfelter (back), her children, and lab members convene with Peter and Ursula Philippsen (front right) at Woods Hole.

How could this principle manifest in other syncytia, such as muscle?

That's a question I'm really interested in looking at in the future. Muscle nuclei don't divide, but decades ago it was shown that different muscle nuclei express different transcripts. We would really like to test whether proteins with low-complexity regions keep these transcripts locally restricted.

> "I think this mechanism of cytoplasm organization could be quite general."

Part of your lab focuses on septins…

I first started studying septins towards the end of my PhD when I found a Cdc42 mutant with defects in septin organization. I didn't work on them as a postdoc, but, when I came to Dartmouth and was discussing potential projects with my first graduate student, Brad DeMay, I spontaneously mentioned we could do something with septins. That's what he decided to do. We hadn't looked at them carefully in *Ashbya* at all, and it turned out that they form higher-order assemblies that are very different from those we see in *cerevisiae*. In fact, one *Ashbya* cell can host two distinct higher-order structures on adjacent spans of membrane. So again we were faced with the problem of how cells specify particular structures within a common cytoplasm.

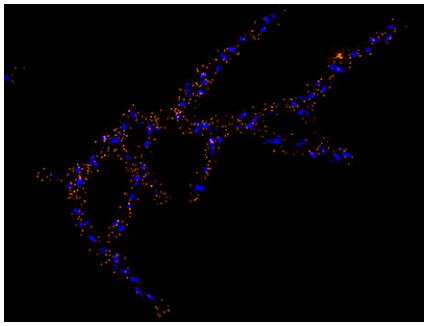

IMAGE COURTESY OF CHANGHWANG LEE

FISH shows the restricted localization of the G1 cyclin transcript CLN3 (orange) near nuclei (blue) in *Ashbya* cells.

A couple years later I started working together with Rudolf Oldenbourg, a staff scientist at the Marine Biological Lab in Woods Hole, to study septin structures using polarization microscopy. For the past six years I've been going to Woods Hole every summer to work with Rudolf, Christine Field, Tim Mitchison, and Tomomi Tani. Together we're pushing polarization microscopy to the single-molecule level and combining it with TIRF microscopy to look at the orientation and dynamics of individual septin molecules within higher-order structures.

Woods Hole has become an important part of my life and my family's life. I have two kids, six and eight, and they both think it is normal to go to Cape Cod every summer. [Laughs] Even my parents came to love this science village when they came along to care for the kids as babies. My mother, who just passed away, always encouraged me to find a career path where I supported myself, engaged my mind, and found pleasure. I would not be in this privileged place, exploring life, were it not for her and my father's continual support of my dreams and my work.

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