Orchestration of chitin synthesis: Could understanding this process be a deadly fungus kryptonite?

By: Maria Spyrou

Imagine being able to look into the microcosm of a living cell. You're a silent observer in a dark, cold room – tracking proteins, seeing them interact with each other and trying to decipher their behaviour. How do I do that you ask? Using live-cell fluorescence microscopy, which I consider to be the next best thing since sliced bread. And just like making bread, my work includes working with yeast. Not the delicious beer and bread making kind, but the nasty opportunistic fungal pathogen *Candida albicans*.

Since the early 1980s, fungi have emerged as major yet underappreciated culprits of human disease, particularly in people with weakened immune system. *C. albicans* can live quite happily inside our gut or genital tract without causing any issues. That is until it senses a weakness in the defence system of its host, compelling it to pursue a literal interpretation of a strategy commonly used in politics: divide and conquer.

Cell division is a fundamental process where a cell stretches until it splits in two cells. It's part of a cell's life cycle as it's essential for growth and enables spreading inside a host. As a fungal cell divides, it forms a barrier between the 'mother' and 'daughter' cell which we call 'septum'. This structure keeps the cells from bursting during the final stages of cell division right before they split from each other. Synthesis of the septum requires chitin, the second strongest material in the natural world which is found in a number of species excluding humans. Chitin is also an integral part of the wall surrounding the cell. The way I see it, chitin is to a fungal cell what a tree is to a tree house. This is what makes the study of chitin synthesis so appealing to me and my research: Without chitin, the cell would completely fall apart as it would lack its skeleton – meaning that the chitin/septum making factory could be a potential target.

Very little is known about the production of chitin in *C. albicans*. We know that it is made by four proteins called 'chitin sythases': Chs1, which is essential for septum formation, Chs2 and Chs8 which offer stuctural support when the cell is under attack by drugs and Chs3 which produces most of the chitin on the cell wall. All four proteins work together to make the septum and compensate for each other when needed. What we don't know is how or when they get to the cell division site. These are the two questions I aim to answer in my research.

To answer when these events happen, I tagged two Chs proteins in the same cell with different coloured fluorophores – those are smaller proteins that emit light under the right conditions. This way I am able to track them in living cells and see how they behave in

relation to each other. I still remember the first time I took a movie of the dividing cell. Saw the daughter cell growing, becoming equal to its mother and slowly pulling apart. Then suddenly, flashes of light appeared at the septation site. My tagged proteins were there! Beautiful bright rings right where the septum was about to form. While the septum was being made those rings slowly formed a spot in the middle of the septum and finally disappeared. I immediately went to my supervisors office for a crisp high-five. Now we knew that all four proteins were at the same exact location.

Making more cells with different combinations of tagged Chs proteins I also noticed that one of them goes there a few minutes before the rest. *Is it preparing something before the others arrive? Why are they all there at the same time? How do they get there?* Those are just some of the questions running though my head when I go back to my time-lapse movies trying to interprete the steps of the Chs proteins intricate dance.

But I also need to keep my eyes on the bigger picture. The ultimate goal of my project is this: Once we know when and how the Chs proteins go to the septation site, we can then use the information that we obtain against them. Interrupting cell division and ultimately replication of the fungus is surely an attractive antifungal strategy.

My research is still at its infancy. But that’s the exciting part - I will literally see it grow.