QnAs with Christine Jacobs-Wagner

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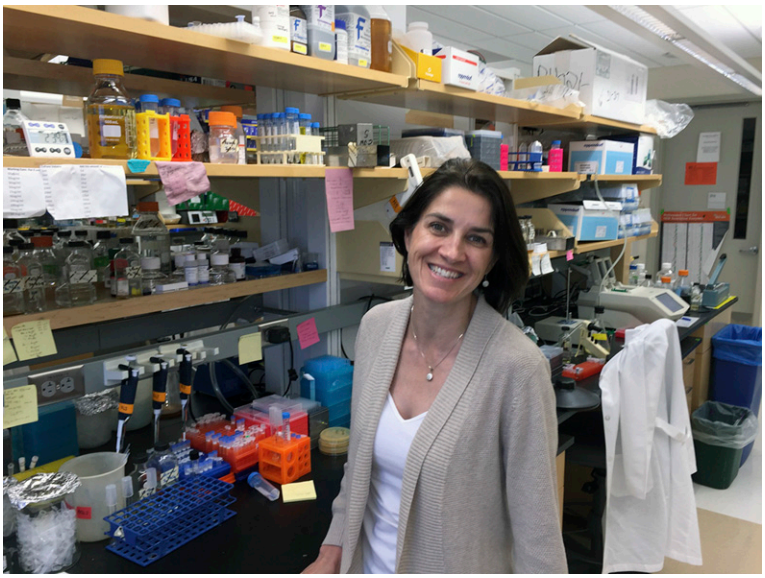
In the bacterial kingdom, the essence of life can be distilled down to one simple principle: divide and conquer. Bacterial cell division is remarkable, not just because one cell produces two, but also because the process, despite its complexity, almost never fails, says Christine Jacobs-Wagner, a member of the National Academy of Sciences since 2015.

This phenomenon is "amazing" when one thinks of the many biological processes that must unfold in a replicating cell, adds Jacobs-Wagner, a Howard Hughes Medical Institute investigator and a professor of molecular, cellular, and developmental biology at Yale University, where she also directs the Microbial Sciences Institute.

A bacterial cell must grow, replicate its genome, double all of its cellular components, and then physically split in half. However, each division successfully produces two daughter cells that themselves self-replicate. Through this process, bacteria proliferate, sometimes in host organisms, where they can serve as agents of health or disease. Jacobs-Wagner's Inaugural Article (1) ventures into uncharted territory, offering the first glimpse of cell replication in the Lyme disease bacterium, *Borrelia burgdorferi*. PNAS asked her to paint a portrait of cell growth and division in this pathogen and describe how the findings may improve treatments for Lyme disease.

PNAS: Bacteria have been studied for far longer than eukaryotic cells, yet researchers know less about bacterial growth and replication in bacteria than in eukaryotic cells. Why is that?

Jacobs-Wagner: In part because we've been studying bacteria as if things were randomly distributed in the cell. Bacteria lack organelles, such as nuclei, mitochondria, Golgi, ER [endoplasmic reticulum]. They're basically made of a membrane that is surrounded by a cell wall, and inside you have everything: DNA, RNA, proteins, metabolites. It looks incredibly simple. This led to the assumption that bacteria are just tiny little vessels of jumbled macromolecules. But in the last 15 years or so, we and others realized this assumption is completely incorrect. Most bacteria do not have internal organelles, but they do exhibit quite sophisticated cellular organization. For so long, we lacked a crucial understanding of bacterial cell function because we weren't considering this level of organization.



Christine Jacobs-Wagner. Image courtesy of Christine Jacobs-Wagner.

PNAS: Why is spatial organization so important?

Jacobs-Wagner: For example, the cytoskeleton, which is made of protein filaments, was thought to be a feature of eukaryotic cells. We now know that bacteria possess a cytoskeleton, and that protein filaments form at specific cellular locations where they achieve critical functions, such as maintaining the size and shape of the cell. We also know that certain proteins and other factors act at specific cellular addresses: for example, at the end of the cell. This organization is critical for various aspects of cellular function. Even more exciting, this organization can change over time, particularly in the context of the cell cycle. As the cell progresses through the cell cycle, it shifts its organization, in part to accommodate DNA replication and cell division.

PNAS: Little is known about cell replication in *Borrelia burgdorferi*. What makes this pathogen so mysterious?

Jacobs-Wagner: There is beautiful work being done on *Borrelia*, but we still don't understand some of the very basic biology: for example, how these cells grow and divide. Part of the reason that *Borrelia* are not as well studied as other bacteria is that they are tough to

This is a QnAs with a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 9162.

work with. They grow slowly. Doing genetics on them is feasible but not easy.

Despite these challenges, *Borrelia* and related spiral-shaped bacteria, called "spirochetes," are fascinating, not only because some are important human and animal pathogens, but also because their features differ from what you'd find in other bacteria. For example, *Borrelia* have very long, slim cells, while most other bacteria are shorter and fatter. And the *Borrelia* genome is highly segmented, with over 20 genetic elements, many of which are linear. Most other bacteria have a single, or occasionally two, circular chromosomes. These differences mean that *Borrelia* have different biological and physical constraints than other bacteria.

PNAS: How did you tease out the mechanisms of cell growth and replication in *Borrelia*?

Jacobs-Wagner: The first thing to keep in mind is that when you ask how bacteria grow, you're really asking how the bacterial cell wall expands. For bacterial cells to grow, they have to expand the wall that surrounds their membrane and cytoplasm. The major component of the wall is a material called peptidoglycan. If you use a fluorescent probe to see where *E[cherichia] coli* incorporates new peptidoglycan blocks into its wall, you would see signals almost everywhere along the cell body. That's because *E. coli* grows and expands by incorporating new wall material all around its cell. The other major way bacteria elongate is by adding new material at one end of the cell. So when we examined how *B. burgdorferi* was elongating, we expected to see one of those two standard modes of growth.

PNAS: But you saw something completely different.

Jacobs-Wagner: Yes, it was very unexpected. We found that new cell wall material is incorporated into very discrete zones along the cell body. We realized *Borrelia* primarily starts growing from a zone at the middle of the cell. Later in the cell cycle, as it expands, the cell gets longer and forms second and third zones at one-quarter and three-quarter positions along the cell body. To establish these zones of growth, *Borrelia* does not actually "measure" the absolute length of its cells. Rather, *Borrelia* cells "sense" the relative locations of the one-quarter, middle, and three-quarter positions. The one-quarter and three-quarter positions become the sites of division for the next generation of cells. So, the mother cells determine where their daughter cells will divide. This is unusual because in most bacteria, this determination is made by the daughter cells themselves.

PNAS: What might this mean for drug development?

Jacobs-Wagner: Our study shows that *Borrelia* growth differs from other growth in other bacteria. Once we understand the molecular mechanisms, this might lead us to strategies for killing these spirochetes and treating the disease without harming the entire human microbiota.

Understanding how *Borrelia* grow and multiply can also reveal why it's so difficult to treat the symptoms of Lyme [disease] when the infection is not treated right away. The broad spectrum antibiotics that are currently used for treating Lyme disease work very well, though they also kill the "good" bacteria in your body. However, when the infection is not treated early enough—in some cases, people aren't treated right away because they think they have the flu—you can develop serious, long-lasting complications that may not be resolved by conventional therapies.

1 Jutras BL, et al. (2016) Lyme disease and relapsing fever *Borrelia* elongate through zones of peptidoglycan synthesis that mark division sites of daughter cells. *Proc Natl Acad Sci USA* 113:9162–9170.