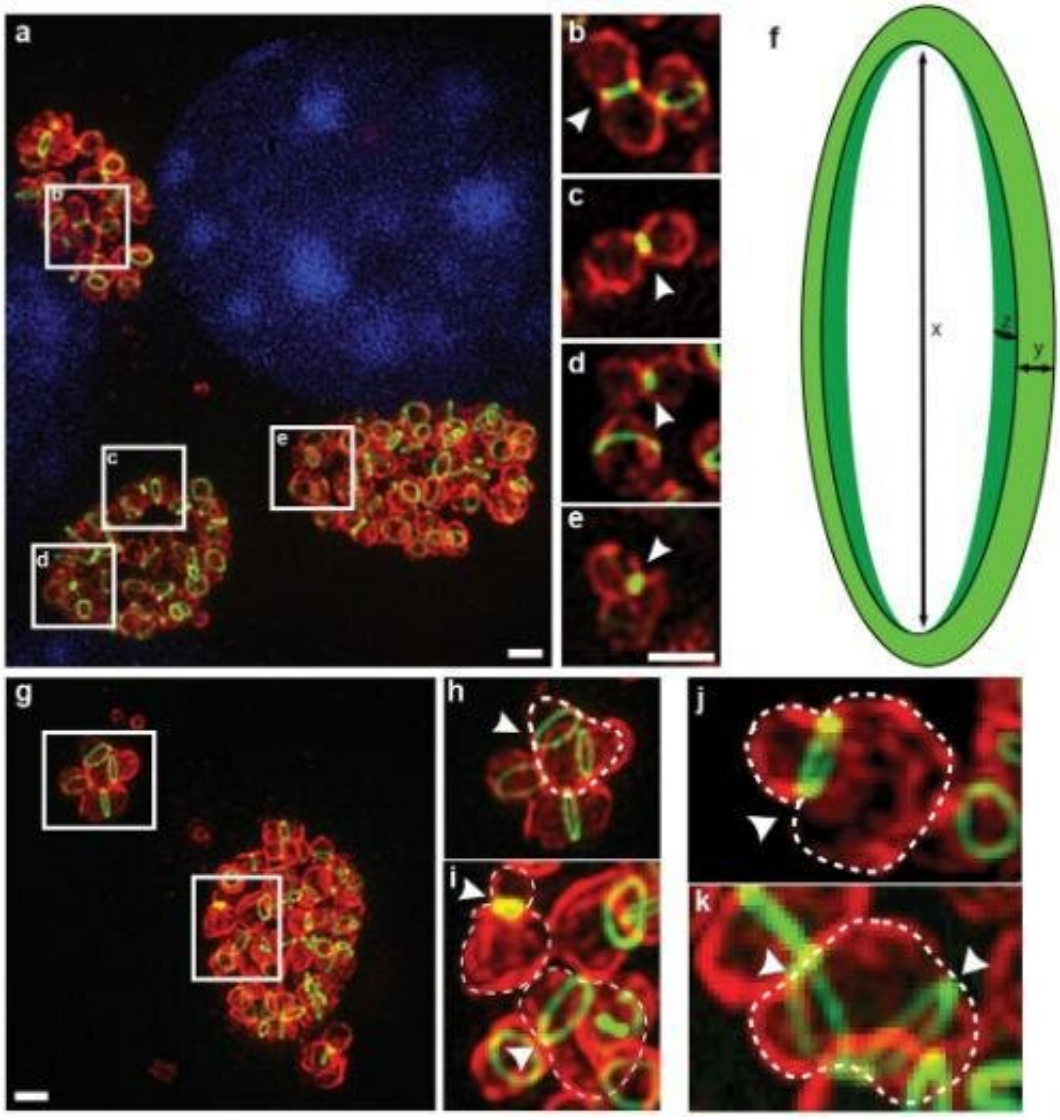
Chlamydia's covert reproduction

February 19 2020, by Delene Beeland



Credit: University of Florida

UF researchers have resolved a two-decade old mystery centered upon how the bacteria chlamydia divide and reproduce. Newly published results from the lab of Anthony Maurelli, a microbiologist in UF's College of Public Health and Health Professions and the EPI, reveal that how these parasitic pathogens replicate diverges from a nearly universal norm.

Best known as a sexually transmitted disease, chlamydia in the U.S. rose 3 percent between 2017 and 2018 for a total of 1.7 million cases—the highest ever reported to the Centers for Disease Control. It is caused by a unique bacteria so small and unusual that researchers first mistook it for a virus.

But mistaken identity was just the beginning of many twists and turns in decoding its evolutionary story. The quirky biology of the genus Chlamydiae proved so intriguing that UF microbiologist Anthony Maurelli built a career upon teasing them out.

It wasn't only the pathogen's small size that initially confused microbiologists, it was also the fact that scientists tried and failed to demonstrate the presence of a cell wall in chlamydia. Nearly all bacteria make a bunker-like carapace, composed of a polymer called peptidoglycan, which offers protection from the environment.

"Peptidoglycan is what imparts structural integrity to the bacteria itself," explains Maurelli, who is a professor of environmental and global health in UF's College of Public Health and Health Professions and the Emerging Pathogens Institute. "Most other bacteria, such as E. coli, are basically encased in this peptidoglycan-wrapped shell. But chlamydia doesn't have this. In fact, for a long time people didn't think it even made peptidoglycan at all."

Maurelli's lab was involved in the basic research that turned this

assumption on its head. Since 2002, he has systematically focused on identifying key enzymes in the pathway for synthesis of a cell wall in chlamydia. Definitive proof came in 2014 when his lab published a seminal paper in *Nature*, which showed that chlamydia do in fact make peptidoglycan, albeit in minute amounts: it appears fleetingly during division at the point where the bacterium cleaves into two daughter cells. Two years later, Maurelli was the senior author to a paper published in *PLOS-Pathogens* which characterized how chlamydia make a small ring of peptidoglycan, labeled with flurorescent green proteins in the image below, that aligns with the division plane during replication and is then quickly disassembled when no longer needed, to avoid detection by the host's innate immune system.

Now, Maurelli's team has unlocked another of chlamydia's closely held secrets: which proteins govern its reproductive process.

Maurelli is the senior author of a new study, which published today in the journal *mBio*. Dev Ranjit, a postdoctoral fellow in the Maurelli lab, is the study's lead author. Both authors are affiliated with the Emerging Pathogens Institute. Maurelli came to UF in 2016 under the preeminence plan, after spending nearly three decades at the Uniformed Services University of the Health Sciences in Bethesda, Maryland. His research focuses on shigella and chlamydia bacteria, and how these microorganisms cause disease in their hosts. George Liechti, assistant professor of microbiology and immunology at the Uniformed Services University in Bethesda, MD was a key collaborator on the study.

"Chlamydia is a very important pathogen to public health. Despite all our medical advances, its incidence is increasing," says Ranjit, pictured above. "And for every bacteria that is studied, the most fundamental question is always, "How does this organism grow?" Because if we can figure that out, we can stop them from growing and dividing."

Quirky parasites

Of chlamydia's nine species, only C. trachomatis, is the causative agent of chlamydia, a common STD that infects human genital tracts. Left untreated, chlamydia infections can lead to severe health outcomes including ectopic pregnancies, pelvic inflammatory disease and sterility. Certain strains can also cause blindness.

But aside from the ability to cause disease, this genus harbors layer upon layer of oddities: for starters, these bacteria are parasites. chlamydia survive only by invading a host cell—usually within a mammal but sometimes in a bird—and quietly creating new versions of themselves before moving on. The technical term is "obligate intracellular parasite." Millions of years ago these parasitic bacteria swapped their independence in a harsh world for the safety of a host, and as a byproduct their genome shrank as it lost the genes for functions no longer needed.

Instead of making everything they required, chlamydia drew upon their hosts' resources. Tucked safely inside a eukaryotic cell, for example, they were exposed to fewer environmental stresses which made a peptidoglycan shell genetically cumbersome and energetically costly. Except, they still needed a tiny bit of structure for cell division to occur; hence the polymer forms only where and when necessary.

This was a pragmatic trade off easily explained by the raw efficiency of adaptive forces; but when researchers next discovered that chlamydia lacked a protein-encoding gene essential to reproduction in nearly every type of bacteria, it again stretched credulity.

Covert division, co-opted proteins

The mystery traces back more than twenty years, to when the genome of chlamydia trachomatis was first sequenced, and it was revealed that these bacteria lacked the normal genetic reproductive machinery employed nearly universally in all other bacteria. It was so unthinkable that chlamydia would not possess a gene called FtsZ, essential to division, that some researchers thought the sequencing results were in error.

"There was not only no FtsZ, there was not even anything that looked like it might encode a similar protein," Maurelli says. "It was simply unthinkable it would be absent. That's when we took it upon ourselves to look for ways that bacteria could divide without FtsZ."

Nearly two decades later, Maurelli's team is confident they have found two genes in chlamydia—shape-determining genes which are common in most bacteria—which chlamydia co-opts to mediate cell division. Maurelli's team identified two chlamydial genes, known as mreB and rodZ, which produce proteins that cooperate to form the structural scaffold upon which division occurs.

"We have shown how chlamydia are using these two proteins to carry out the role that is normally carried out by FtsZ," Maurelli says.

The finding is highly unusual because MreB and RodZ are typically proteins which help determine a bacterium's shape and size, and contribute to cell wall growth. They have never before been shown to replace FtsZ in bacterial division but the chlamydial versions of these proteins appear to carry out the FtsZ function.

The study did not address why the bacteria use this unusual process.

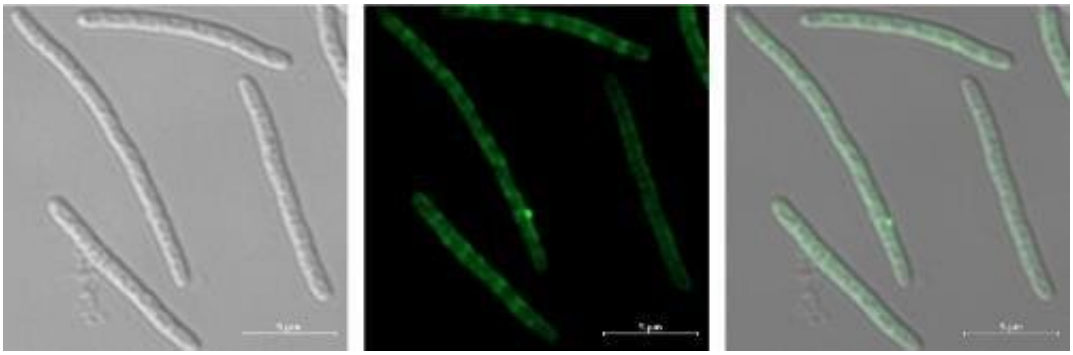
"Did they drop FtsZ from their genome, or did they never have it in the first place? It has always been a chicken or egg kind of situation," Ranjit

says. "But one little guess is that when chlamydia adopted an intracellular lifestyle, they began getting rid of all the genes that were not necessary and underwent genome reduction."

Chlamydia are difficult to study because they carry out their reproductive processes inside a host cell. A suitable surrogate is found in E. coli, which Maurelli's team manipulated to suppress this bacteria's own cell division.

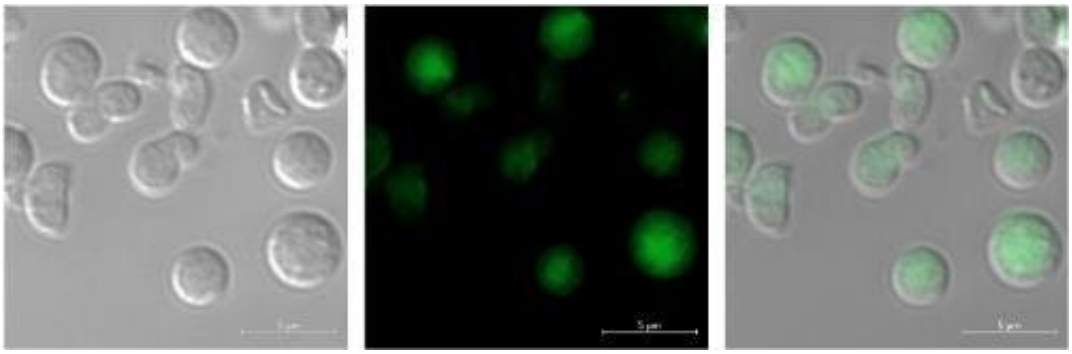
"Because chlamydia can only grow inside other cells, this imposes limitations in terms of the genetic tools that are available to manipulate the organism," Maurelli says. "This is why we used E. coli as a model to study genes that direct cell division in chlamydia."

The authors suppressed FtsZ and MreB in the E. coli. They found that E. coli which lack MreB lost their distinctive rod shape and transformed into pudgy ovals; and E. coli that lacked FtsZ function grew into ever-longer rods that never divided, like this:

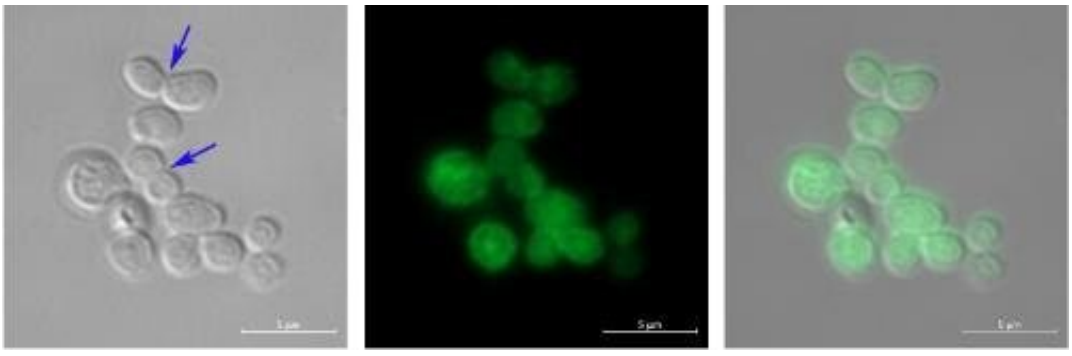


Credit: University of Florida

The team then introduced the genes for chlamydial MreB and RodZ into a mutant E. coli and made several different findings. When chlamydial MreB was overproduced in E. coli and FtsZ function was suppressed, it lost its rod shape and instead grew in a spherical or coccoid shape but did not divide, like this:

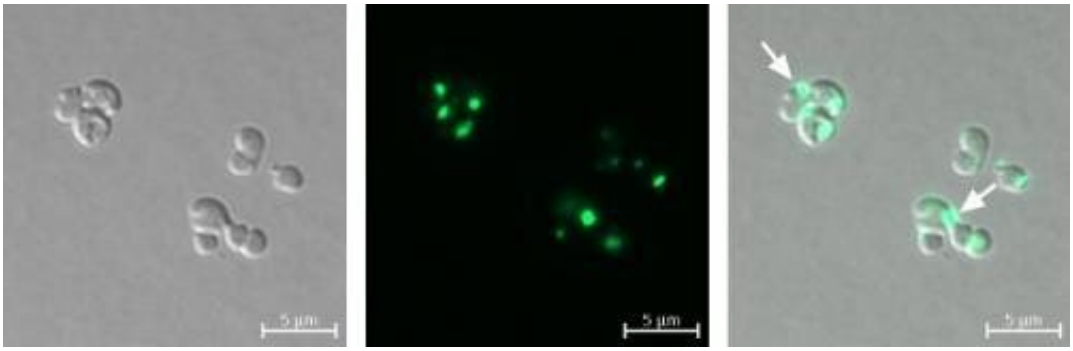


But when both chlamydial MreB and RodZ were introduced to the mutant E. coli, it grew spherically and it divided, like this:



"Just having the one gene that makes MreB or RodZ by itself was not enough to substitute for the suppressed FtsZ function," Maurelli says. "They both had to be provided together."

The researchers showed that chlamydial RodZ acts like a guide that directs chlamydial MreB to the plane in the cell wall where the bacterium will divide into daughter cells. In the image below, the chlamydial MreB is tagged with fluorescent protein labels and shown localizing to the division plane, noted with arrows, on the manipulated E. coli which have suppressed FtsZ, MreB, and RodZ. The chlamydial MreB then assists in assembling a new cell wall at this site that slowly constricts and eventually leads to separation of the two daughter cells from one another. The authors also suspect that chlamydial MreB also supports peptidoglycan synthesis around the spheres before division occurs.



The findings show how chlamydia uses MreB, which normally plays a role in determining shape in rod-shaped bacteria, to partner with RodZ

and carry out cell division, too.

"The beauty of biology is that there is such variety," Maurelli says. "We've shown the novel function of a few proteins that have been characterized in other organisms, but they are being employed differently in this significant human pathogen. This could open the way to discovery of novel inhibitors or blockers of these essential functions."

More information: Dev K. Ranjit et al. Chlamydial MreB Directs Cell Division and Peptidoglycan Synthesis in Escherichia coli in the Absence of FtsZ Activity, *mBio* (2020). DOI: 10.1128/mBio.03222-19

George Liechti et al. Pathogenic Chlamydia Lack a Classical Sacculus but Synthesize a Narrow, Mid-cell Peptidoglycan Ring, Regulated by MreB, for Cell Division, *PLOS Pathogens* (2016). DOI: 10.1371/journal.ppat.1005590

Provided by University of Florida