By Beth Staples

Battle lines

A UMaine biomedical lab looks for answers in the transformation of a peaceful yeast to fatal fungus

LIFE-AND-DEATH battles rage in Robert Wheeler's lab at the University of Maine.

The combatants — zebrafish and *Candida albicans* — fight to the bitter end in glass-bottom microplates.

Similar perilous battles are being fought inside humans. The *C. albicans* fungus is a leading cause of hospital-acquired infection that annually kills several thousand patients nationwide.

During the staged scuffles in Wheeler's lab in Hitchner Hall, anesthetized zebrafish are injected with *Candida* and placed in a gelatinous material called agarose.

A laser microscope captures and magnifies the struggles inside the zebrafish blood vessels in real time in high-definition color detail.

The microplate clashes provide the assistant professor of microbiology with the ability to view how immune cells fight the microbe, identify genes involved in virulence, test new drugs and learn how gene perturbations affect host-pathogen interaction.

"We're using zebrafish to ask really specific questions that cannot be answered another way," Wheeler says. "These questions have been inaccessible for a long time. We hope to be able to better utilize existing therapies and be able to develop better therapies."

In March 2012, Wheeler received a three-year, more than $421,600 grant from the National Institutes of Health to ask and answer these questions in the project: "Genetics & Visualization of Innate Host Response to *Candida albicans* Infection In Vivo."

The goal is that the resulting answers will save human lives.

The grant is the most-recent funding Wheeler has received during his 13-year quest to unravel the mysteries of *Candida.*

MILLIONS OF *C. albicans* live peacefully in digestive tracts of people with healthy immune systems. Despite being the culprit of pesky vaginal infections in adults and oral infections in babies, for the most part, "the organism has evolved to coexist rather than constantly attack," Wheeler says. "It's part of our natural microflora."

Battle lines

But when a person's immune system is compromised — as occurs with organ transplant patients and people with cancer and human immunodeficiency virus — *Candida albicans* transforms from peaceful yeast to an invasive, potentially fatal fungus that infects vital organs.

Candida's Jekyll-to-Hyde conversion proves deadly for about one-third of people afflicted with bloodstream infections of the pathogen. It's clear, says Wheeler, that better diagnostics and therapies are needed.

Wheeler also uses mice to study immunity to *C. albicans*. The research is beneficial, he says, but limited because the live mammals aren't see-through and don't fit under a microscope.

But transparent tropical fish larvae measuring a few millimeters fit the bill. And the ability to conduct experiments in vivo — "within the living" zebrafish — have been and continue to be elucidating.

Zebrafish also have backbones, share many of the same genes as people, and have the ability to respond to infections and vaccinations in ways similar to humans, Wheeler says.

For the life-and-death battles in microplates, Wheeler uses engineered zebrafish with green fluorescent immune system cells and fluorescent red *C. albicans* fungus. A laser scanning confocal microscope captures layered, 3D images of the skirmishes blow-by-blow, in real time.

On Wheeler's iPad, a battle that lasts for hours is condensed into a time-lapsed movie that can be viewed in minutes. The movie of green zebrafish immune cells gobbling up red *C. albicans* resembles a Pac-Man arcade game.

The Pac-Man reference is one example of how Wheeler explains the fungal host-microbial pathogen interaction in ways that make sense to nonscientists.

He also compares sugar layers of the fungus cell wall using everyday objects and terminology, including M&M candies and GORE-TEX.

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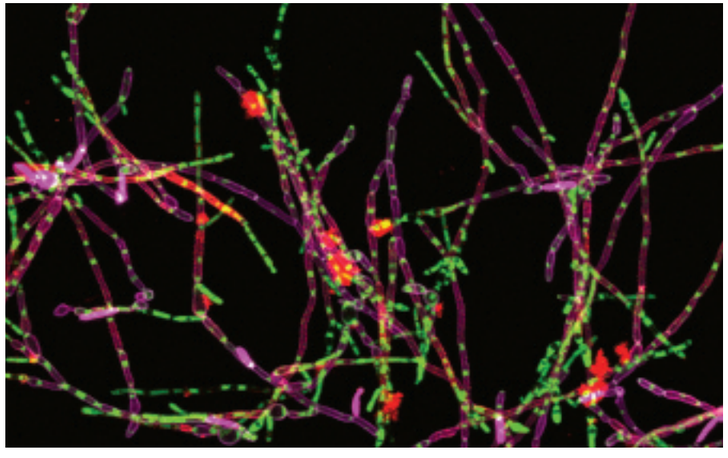

ZEBRAFISH AND fungi are familiar foes with apparent elaborate knowledge of each other's respective arsenals.

"For each mode of host immunity, the challenger has designed a defense, which, in turn, leads the host to devise a new avenue of attack," according to Wheeler.

For instance, innate immune cells recognize surface molecules of the deadly fungus, including sugar β-glucan. When the immune cells recognize the *Candida* cell wall, the immune system goes on high alert and responds to eliminate the threat.

In some instances, the *Candida* fungus covers up the β-glucan with a thick protective cell layer, thereby blocking the immune system's response. Wheeler compares this dense sugar coating to GORE-TEX clothing. Just as GORE-TEX doesn't let water penetrate, this outer layer prevents immune molecules from touching the β-glucan.

The switch to drug-containing media causes *Candida* filaments (green fluorescence) to lose its GORE-TEX outer coat and expose the immune-stimulating sugar β-glucan (red reactivity). The surface-label (purple) marks old growth without drugs, where β-glucan is masked.

Researchers in the Wheeler lab include, left to right, Sarah Barker, a postdoctoral research fellow from Yorkshire, England; Xiaojie Ji, first-year biomedical science doctoral student from China; and Remi Gratacap, a postdoctoral research fellow from Grenoble, France.

Some pharmaceutical drugs make *Candida* more recognizable. In addition to killing fungi, one antifungal drug has a side effect of uncovering the β-glucan, Wheeler says.

"If we're able to expose the β-glucan, the immune system goes crazy," says Remi Gratacap, a postdoctoral research fellow from Grenoble, France. "You see almost a threshold where the immune system is able to cope and if you go just past that, suddenly (β-glucan) can't cope anymore."

The UMaine group, says Wheeler, is also trying to better understand how *Candida* gets from one place to another in the body. Since *Candida* cannot move independently, Wheeler seeks to discover if the pathogen is carried in immune cells.

Candida, Wheeler says, can change shapes, from bunches of yeast to long filaments. Both shapes serve it well: *Candida* travels easily in the blood in yeast form and penetrates tissues best as a filament.

Gratacap says that Wheeler, a proponent of re-examining long-standing scientific concepts accepted as true, is "ridiculously clever."

WHEELER COMES naturally by it. His paternal grandfather is John Archibald Wheeler, a theoretical physicist who collaborated with Albert Einstein and is heralded for his contributions to gravitation and quantum mechanics. The former Princeton professor, who coined the terms black hole and wormhole, died in 2008.

Robert Wheeler earned degrees from Harvard and Stanford universities and did his postdoctoral work at Whitehead Institute, a nonprofit research institution in Cambridge, Mass., where biomedical researchers seek to improve human health.

Wheeler, a sought-after expert in the field, is slated to lecture this semester in Germany and France.

Wheeler lauds UMaine colleagues Carol Kim, professor of microbiology and director of the Graduate School of Biomedical Sciences and Engineering, and Clarissa Henry, associate professor of biological sciences, for their help, collegiality and excellent research with zebrafish. The Zebrafish Research Facility, run by Mark Nilan, is only a few steps from Wheeler's lab.

"It's hard to overstate how instrumental the well-run facility has been," Wheeler says. "I really don't know if I could have done this work anywhere else."

His research group has already made a significant breakthrough discovery regarding *C. albicans*.

After receiving grants in 2008 and 2009 from the Maine Agricultural and Forest Experiment Station and the National Institutes of Health, he and students Kimberly Brothers and Zachary Newman started viewing interactions between fungi and immune cells. They showed for the first time that NADPH oxidase is required for regulation of *C. albicans* filamentation in vivo.

These observations, first made by Brothers, implied the deadly fungus might spend more time inside zebrafish phagocytes — immune cells that ingest microorganisms, other cells and foreign particles — than researchers had believed.

This, Gratacap says, demonstrates that examining these microscale battles in different hosts can lead to striking insights. ■