Q & A

Joseph Heitman

Joseph Heitman is James B. Duke Professor and Chair of the Department of Molecular Genetics and Microbiology at Duke University, and he studies model and pathogenic fungi as models to understand fundamental biological questions and unmet medical needs. He studied chemistry and biochemistry at the University of Chicago (1980–1984), trained in the MD–PhD program at Cornell and Rockefeller Universities, working with Peter Model and Norton Zinder on bacterial restriction–modification systems and DNA repair, and was an EMBO Fellow with Michael Hall at the Biozentrum in Basel, Switzerland. Collaborative studies by Heitman and Hall with Rao Movva at Sandoz/Novartis led to the discovery of FKBP12 and TOR as the targets of the immunosuppressive antifungal natural product rapamycin. Heitman joined the Duke faculty in 1992, and his research program focuses on the evolution of eukaryotic microbial pathogens via unisexual reproduction, and novel drug resistance mechanisms involving RNAi-dependent epimutations. He is a member of the American Academy of Microbiology, the American Academy of Arts and Sciences, and the National Academy of Sciences.

How did you become interested in science? I was born in Ohio, grew up in southwestern Michigan, and from an early age was interested in science. My parents were very supportive of early and explorative education; my mother was a specialist in teaching reading and my father was a mechanical engineer. I had a microscope re-gifted from an uncle, a telescope, a rock tumbler, and a rock collection, and then I gravitated to electronics, primarily shortwave radios, and became an amateur radio operator. Beyond this, I read and learned to build and repair things. I was also fortunate to have truly exceptional science teachers at my public high school, Portage Northern. My math and physics teachers were outstanding, and our school even had two remarkable chemistry teachers, one for inorganic and one for organic chemistry, as well as an organic chemistry lab section. But it was in my 9th and 12th grade AP honors biology classes, taught by John Goudie, where biology truly captured my imagination. After a lecture on the central dogma of how DNA makes RNA and RNA makes proteins, I was hooked.

What were your early scientific experiences and who were your mentors in college? Following high school, I matriculated at the University of Chicago and majored in chemistry. To this day, I believe that this was the most rigorous academic environment that I have ever experienced, forging scholarship into a highly honed art. I began working in an immunology lab, helping to take care of a mouse colony, until the entire colony was nearly wiped out by a viral infection and my skills in mouse husbandry were no longer required! During my second-year organic chemistry class, a few students were able to place out of the regular lab course and instead work in a research lab in the Department of Chemistry. Through this opportunity I joined Gus Fried's lab, working first with Pui-Yan Kwok, an MD–PhD student at the time and now a physician–scientist on the faculty at the University of California, San Francisco. My first project was dedicated to exploring the idea that fluorination modifications could prolong the bioactive half-lives of arachidonic acid derivatives as substrates for subsequent enzymatic conversion to prostaglandins and prostacyclins. This lab opportunity extended into the summer, when I worked with Jon Kremsky on the synthesis of a portion of a large macrocyclic compound, maytansine, which was then being explored as a possible cancer chemotherapeutic. It was a heady time for a science nerd undergraduate like myself, learning to carry out synthetic organic chemistry and operate the 500 mHz NMR instrument and collect spectra. My discussions with the two MD–PhD students training in Gus's lab, Pui-Yan Kwok and George Ebert, ignited an interest in MD–PhD training.

In the fall of my third year I moved to work with Kan Agarwal, a professor with a joint appointment in the Departments of Biochemistry and Chemistry. Kan had trained with Gobind Khorana and contributed to the development of approaches to synthesize DNA oligonucleotides. At the time, the lab was steeped in this tradition, and a PhD thesis could consist of synthesizing a single 18-base oligonucleotide. My project focused on the synthesis

of modified bases that would enhance base-pairing features for modified oligonucleotide synthesis. I became interested in applying these approaches to the synthesis of cAMP derivatives and studied their ability to activate the CAP transcriptional regulator in *Escherichia coli*, leading to expression of beta-galactosidase from the *lac* operon, thus mapping out features of the cAMP-binding site on the protein.

My plan to this point had been to train in chemistry and pursue PhD training in synthetic organic chemistry, with an interest in studying DNA–protein interactions. However, during my senior year of college I spent a lot of time visiting friends working in Malcolm Casadaban's lab, including Eduardo Groisman, Joe Yost, and Alfonso Martinez-Arias. One thing led to another, and I ended up moving to Malcolm's lab and working there for six months. In contrast to my original plan, research in Malcolm's lab was dedicated to microbial genetics, and the group was combining new molecular biology approaches with classical genetics techniques. It was exciting, and I began working on restriction–modification enzymes and developing ways to study and understand their exquisite DNA-binding specificity. Malcolm had trained with Stanley Cohen as a postdoctoral fellow, and during this time a plasmid encoding the EcoRI restriction endonuclease and DNA methyltransferase had been identified in which there was a temperature-sensitive mutation in the methyltransferase gene. Introduction of this plasmid into *E. coli* at lower growth temperatures was tolerated, but growth at higher growth temperatures was lethal due to expression of the EcoRI endonuclease without the protective methyltransferase, which had been heat inactivated. This opened the door to thinking about how to conduct genetics on the system and ways to isolate mutants of the restriction endonuclease with altered DNA-binding specificity.

These successive, formative research experiences as an undergraduate forged in me a life-long interest in biomedical research. At the same time, I was completing a combined BS/MS chemistry–biochemistry training program and was largely taking graduate-level and medical school classes taught by outstanding instructors, including Lucia Rothman-Denes, Nick Cozzarelli, Ferenc Kezdy, Bob Heinrikson, and Michael Johnston, among others.

Why did you decide to pursue MD–PhD training, and what were your experiences? I was genuinely interested in both basic and biomedical research, and the opportunities afforded by the combined MD–PhD training program seemed the best way to encompass the widest spectrum of research opportunities. I joined the MD–PhD program at Weill Cornell Medical College and Rockefeller University because I was excited about the unique aspects of graduate training at Rockefeller that stressed independence and scientific discovery. I joined the lab of Peter Model and Norton Zinder, and my PhD thesis focused on how proteins and enzymes recognize specific DNA sequences as well as how DNA nicks and double-strand breaks are repaired in bacteria. Both Peter and Norton stressed independence, scientific rigor, and application of genetics to questions of central biological importance. Their lab was a fast-paced and exceptionally stimulating environment in which to learn genetics and molecular biology — model systems in the lab allowed us to set up experiments in the evening and interpret results the next morning. While it was challenging to juggle both medical training and graduate school, the lab provided me with an environment for intellectual stimulation throughout medical school.

I understand that you took a scientific sojourn to Basel, Switzerland. That must have been interesting! During graduate school at Rockefeller I took the yeast genetics course at Cold Spring Harbor in the summer of 1988 and fell in love with yeast as an experimental system. I decided to take a leave of absence from medical school, and after defending my PhD and receiving

Joseph Heitman (right) with Neil Gow (left) at Cold Spring Harbor Laboratories.

an EMBO long-term fellowship I moved to Basel, Switzerland, to study yeast genetics with Mike Hall, a new assistant professor at the Biozentrum of the University of Basel. At that time, Mike was interested in the assembly of the nucleus and using yeast as the model system to study the nuclear import of proteins. Back then, little was known about the detailed structure of the nuclear pore or the proteins that recognized nuclear localization signals and directed proteins to the nucleus that Mike had been involved in discovering. My project was focused on genetic screens to identify proteins involved in nuclear import, but they were oddly yielding mutants with defects in the yeast pheromone response pathway and mating, and this was not what we were hoping to discover. Fortunately, however, this led to an interest in signal transduction pathways, how cells recognize extracellular cues, and the pathways that allow appropriate physiological outcomes. I had an idea that there might be some inhibitors or drugs that could be employed to study intermediate steps in signal transduction pathways that connect receptors on the cell surface with transcription factors in the nucleus.

How did you end up working on immunosuppressive drugs in yeast? I was reading in the library and came across a paper by Max

Tropschug about studies on the immunosuppressive drug cyclosporin in the filamentous fungus *Neurospora crassa*. I knew from medical school that cyclosporin was a gold standard drug for organ transplant recipients to prevent and treat organ rejection. I returned to the lab waving a Xerox-copy reprint and saying that we should be working on this in yeast as a model system. Mike Hall suggested that I contact Rao Movva at Sandoz, who had hired Mike as a consultant for yeast work, studying the cyclophilin proteins known to bind to cyclosporin. Rao and I met and discussed several projects and began collaborating that day to study the possible role of the FKBP12 protein in the action of another natural product immunosuppressive drug, FK506. We later turned our attention to the related natural product rapamycin, which also binds to FKBP12. We discovered that FKBP12 is not essential for yeast growth but is essential for rapamycin toxicity. Our genetic studies led to the discovery of two novel proteins named TOR1 and TOR2 as the targets of rapamycin in yeast. Later studies from several groups identified the mTOR protein as the mammalian ortholog of the yeast TOR proteins. Thus, the mechanism of rapamycin antifungal and immunosuppressive action is conserved from yeast to humans.

Why a focus on infectious diseases? I returned from Switzerland to complete medical school and then moved to start a lab as an assistant professor at Duke University in 1992. Initially, the focus was on yeast as a model to study immunosuppressive drugs. However, given the serious risks that transplant patients face from infectious disease due to immunosuppression, I was thinking more and more about infectious diseases. Shortly thereafter I received a phone call from John Perfect, a physician–scientist studying infectious diseases. John was interested in a fungal pathogen called *Cryptococcus* that frequently infects the central nervous system in transplant patients. He drew attention to several papers that had demonstrated the antifungal activity of cyclosporin against *Cryptococcus in vitro*, and yet cyclosporin increased the risk of cryptococcal infection in patients. We began collaborating to understand the molecular and genetic basis for this conundrum, and this led to the discovery that calcineurin, the target of cyclosporin and FK506, is broadly required for the pathogenesis of myriad eukaryotic microbial pathogens. These studies are continuing in the lab in two general directions: the first is to understand how calcineurin orchestrates responses to stress that are required for infection of the host, and the second involves efforts to develop analogs of FK506 with reduced immunosuppressive activity for application as novel antimicrobial therapeutics.

Where did an interest in sexual reproduction originate? At the time, much of our focus was on developing tools to facilitate studies on *Cryptococcus* as a model human fungal pathogen. We became interested in the mating-type locus and sexual cycle through June Kwon-Chung's work that linked mating type and pathogenesis as well as identified part of the mating-type locus. Around this time, I was invited to serve on the PhD thesis committee for Tim James, one of Rytas Vilgalys's graduate students. Their expertise in mycology with respect to evolution, taxonomy, and transitions in modes of sexual reproduction inspired us to consider new research directions from an evolutionary perspective.

Another inspiring colleague was the medical mycologist Arturo Casadevall, who often draws an analogy of active bench scientists to cartographers, as we each map a restricted part of a field. He further encourages one to consider how research and discoveries can be fit into a broader synthetic perspective analogous to tectonic plate movement, in which it was realized that all the continents of the Earth could be fit into a greater landmass, like pieces of a puzzle. In one of many conversations, he intoned that, of all the work ongoing in the lab, the study of sexual reproduction had the greatest potential to be generalizable across disciplines. That served as impetus for us to think more deeply about the unusual sexual cycle of *Cryptococcus* and this led to the discovery of unisexual reproduction.

A central conundrum in the field was why, despite the discovery of a sexual cycle involving isolates of opposite mating types (**a** and alpha) by June Kwon-Chung, the vast majority of clinical and environmental isolates were exclusively alpha mating type. The dogma then was that this pathogenic microbe had largely given up on sexual reproduction in nature and was reproducing clonally by asexual mitotic division. Even more perplexing was the discovery of a developmental pathway called haploid fruiting, or monokaryotic fruiting, during which isolates of the alpha mating type would respond to nutrient limitation and undergo a dimorphic transition from yeast to hyphae, ultimately producing basidia and spores. It had been concluded that this was strictly asexual, mitotic, and clonal. However, because *Cryptococcus* was known to have a sexual cycle involving isolates of opposite mating types, and given that haploid fruiting shared many features with sexual reproduction, we hypothesized that this might represent an unusual sexual cycle in which a partner of opposite mating type was not required.

We tested this hypothesis and discovered the process that we named same-sex mating or unisexual reproduction. This work was led by a talented fellow in the lab called Xiaorong Lin, who is now a distinguished mycology professor at the University of Georgia. Key experiments in the discovery of unisexual reproduction included demonstration that the key meiotic gene *DMC1* was critical, and *dmc1* mutants produced very few viable spores, and analysis of a heterozygous alpha/alpha diploid strain showing that independent chromosomal assortment and meiotic recombination occurred, resulting in production of haploid spores. These studies revealed a novel type of selfing, which is called homothallism in fungi. Back then there were only three known mechanisms for fungal homothallism (mating-type switching, mating-locus fusion, and presence of both mating-type loci), and unisexual reproduction

represented a novel mode of selfing. The discovery of unisexual reproduction in *Cryptococcus* and its impact on population structure, the production of infectious propagules, and both the generation and the maintenance of genetic diversity turned out to be of general importance for other eukaryotic microbial pathogens including fungi, as well as parasites that infect humans. Moreover, the discovery of unisexual reproduction provides insights into how sexual reproduction might have first evolved in the last common eukaryotic ancestor (LECA) and suggests an evolutionary epoch in which there may have been sex before there were sexes.

What was an unexpected turn in your scientific trajectory? The discovery of epimutations evoked by RNAi that confer antimicrobial drug resistance was unexpected. This began as a summer project for a rotating graduate student. The idea was to isolate mutants of the basal fungus *Mucor* that were resistant to the antifungal immunosuppressive natural product FK506. We had previously studied this in *Saccharomyces*, *Cryptococcus*, and *Candida*, and it seemed reasonable to expect that we would find Mendelian mutations in the genes encoding the known drug targets FKBP12 and calcineurin. By the end of the summer we could write a paper that would be solid, if not earth-shattering. Sure enough, the student working on this project, Cecelia Wall, rapidly found drug-resistant isolates but also discovered that some of the drug-resistant isolates were unstable. Rather than discard these unstable isolates, we decided instead to study them, and this began a scientific odyssey that spanned eight years.

Both Cecelia and a later fellow, Silvia Calo, ultimately discovered a novel type of reversible, unstable, antimicrobial drug resistance in which the target gene was silenced by RNAi, known as epimutation. This appears to be a largely spontaneous process that occurs in some small percentage of nuclei in this filamentous fungus during growth and confers drug resistance. In parallel to the discovery of the unstable RNAi epimutants that revert to sensitivity in the absence of drugs, we found stable, Mendelian mutations occurring in the genes encoding FKBP12 and the two subunits of calcineurin. The epimutants appear to be strictly epigenetic; once they have reverted, the frequency with which they again become drug resistant is no different from the original wild-type isolate. What is particularly interesting about this discovery is that it illustrates two different evolutionary trajectories occurring side by side, one that is fully reversible and the other that requires a permanent genetic change. From the recent work of Robin Allshire, we now know of an example of epimutations occurring in fission yeast that result from gene silencing due to heterochromatin, and we anticipate that there may be many other examples of epimutations throughout the fungal kingdom.

Epimutants are also known in plants and animals, including the nematode *Caenorhabditis elegans*, and even in humans. In the nematode, small RNA sequencing studies by Peter Sarkies and colleagues revealed that RNAi-dependent epimutations occur throughout the genome, in the absence of selection, arising and disappearing during culture. Thus, natural selection has a broader palette to act upon in a population of variants that arise due to mutations or epimutations than we had previously appreciated.

What are you excited about now? I am very excited about current advances combining genetics and epigenetics, and genomics and epigenomics. The advances in sequencing technology — combining Illumina, PacBio, and Nanopore short- and long-read technologies — are truly revolutionary. It's clear that many different types of antimicrobial drug resistance are happening in nature beyond those caused by Mendelian mutations, and this rampant genotypic and phenotypic plasticity is both fascinating and challenging to study.

I am excited about the communities of scholars who are focusing on mycology, fungal genetics, and infectious diseases, and the impact that they will have on science and on both human health and disease. The next generations of scientists that we are training continue to amaze and inspire me, as they are the future. And many of them are as adept at the bench doing experiments as they are at the computer analyzing data and testing hypotheses. Our department as well as the vibrant and talented faculty we have been fortunate enough to recruit are also very exciting to me. I am eager to see what they will discover next. Furthermore, I am excited about the program 'Fungal Kingdom: Threats & Opportunities' that is funded by CIFAR (the Canadian Institute for Advanced Research) and the opportunity that this provides to work collaboratively with an extremely talented and diverse cadre of colleagues over a sustained period to tackle problems and opportunities of global importance involving the fungal kingdom.

I am also inspired by how people are thinking creatively about utilizing fungi to address some of the planet's greatest issues, despite the devastating effects of fungi in many sectors. For example, on the issue of global warming, fungi are a threat via plant diseases and wood degradation destroying a proportion of the Earth's carbon sinks, and yet fungi are integral to the health of trees and forests in association with plant roots, and thus we may be able to harness fungi to sequester more carbon dioxide. Already, people are creatively using fungi to produce more environmentally friendly packaging, furniture, and even meat alternatives. In the context of human disease, fungi are obviously a threat to public health, and yet studying fungi as model organisms has provided tremendous advances in our understanding and treatment of human diseases. It is both impressive and inspiring to see all the ingenious ways in which people continue to think about and utilize fungi to both understand and shape our world. I very much look forward to seeing how these clever and creative ideas manifest as tools and solutions to improve the world.

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