Talking TOR: a conversation with Joe Heitman and Rao Movva

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In 1991, Joe Heitman, Rao Movva, and Michael Hall (Figure 1) published a seminal paper identifying the targets of the immunosuppressive drug rapamycin and related compounds (1). Unlike others working toward understanding how these drugs blocked T cell activation, Heitman, Movva, and Hall focused on yeast and devised a genetic screen to isolate drug resistant mutants of *Saccharomyces cerevisiae*, also known as baker's and brewer's yeast. Their work identified FKBP12 as the direct binding partner for rapamycin and two previously undescribed proteins, which they designated TOR1 and TOR2 (for target of rapamycin), as essential for rapamycin toxicity. Subsequent work has shown that the TOR proteins are central for regulation of cell growth in response to environmental and nutritional cues, and alterations of TOR signaling have been implicated in many diseases, such as cancer, metabolic dysfunction, and neurodegeneration. Mike Hall discussed this work in November 2017 during an interview with the *JCI* (2), which can be viewed at www.jci.org/videos/cgms. *JCI Insight* recently had the pleasure of talking with Joe Heitman and Rao Movva to provide further insight into how this collaboration originated and the importance of partnerships between academic institutes and industry.

JCI Insight: How did each of you become interested in science?

Movva: I grew up in a small village in India. At the time, you either went to school or you worked on the farm, and I happened to like school more than the farm. I was interested in biology and medicine and pursued this education. I was influenced by seeing people die of snake bites and from simple skin cuts, as medical treatments were not readily available in my village. After I finished my university education in India, I received a fellowship to pursue doctoral studies at Stony Brook University. As a graduate student in the late 70s, I was fortunate to work with two excellent mentors, Bill Studier and Masayori Inouye, and used state-of-the-art genetic and molecular biology techniques to elucidate mechanisms by which bacteriophage overcome host restriction. These were the early days of recombinant DNA technology, and I was one of the few people who cloned and sequenced a gene (OmpA) at that time. When I finished my PhD, I was hooked and was very passionate about genetic and molecular biology tools. I was also keen on pursuing science with medical connections. I was lucky to be recruited by Wally Gilbert and Phil Sharp in 1980 to work at the newly started genetic engineering company, Biogen, in Geneva. When Biogen moved to Boston in 1987, I relocated to Basel to join a brand-new Biotechnology department at Sandoz (now called Novartis) and continued to work there until my retirement.

Heitman: I think people would describe me as a science nerd from day one. I grew up in the midwest in southwestern Michigan surrounded by fields and forests and lakes, with parents who were very supportive of diverse interests, and as a kid received as gifts: both a microscope and a telescope. I was also an amateur radio operator, and thus learned quite a bit about electronics. I attended a public high school, Portage Northern High School, which had an exceptional science and math curriculum, including courses in both inorganic and organic chemistry as well as an organic chemistry lab. My ninth and twelfth grade honors biology teacher, John Goudie, was one of the main teachers who inspired aspirations in science. I was fortunate to have formative research experiences as an undergraduate at the University of Chicago in chemistry, biochemistry, molecular biology, molecular genetics, and microbiology. I worked in Professor Joe Fried's lab learning synthetic organic chemistry and NMR, and two of his MD-PhD students, Pui Kwok and George Ebert, were critical in convincing me to apply for MD-PhD programs after college. I also worked with Kan Agarwal, an outstanding biochemist making some of the first oligonucleotides by hand at the time, and Malcolm Casadaban, a molecular biologist and bacterial geneticist, who turned my attentions from chemistry to genetics. In June of 1984, I joined the Rockefeller and Cornell MD-PhD program, where I had originally planned to work with the synthetic organic chemist Tom Kaiser. However, I decided that I didn't want to continue to be focused on a chemical approach to studying how proteins recognize specific DNA sequences. Dr. Kaiser recommended that I talk to Peter Model, who became my PhD advisor. I was able to work in the lab during the first two years of medical school. As I was studying bacterial genetics, my projects were amenable to being in the freezer during exams. In 1988, I went to Cold Spring Harbor and took the Yeast Genetics course. I fell in love with yeast as an experimental system and began reading more

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Figure 1. Mike Hall, Joe Heitman, and Rao Movva at the 2017 Lasker Award Ceremony. Photo courtesy of Joe Heitman.

and more. I decided that rather than going back immediately to complete medical school, I would take a leave of absence to do a postdoctoral fellowship. Mike Hall had moved to the Biozentrum in Basel in 1987 and I was impressed with his exemplary genetics pedigree. We started an exciting project with the goal of understanding how proteins with a nuclear localization signal are recognized and delivered through the nuclear pore; fortunately this project was supported by an EMBO long term fellowship.

JCI Insight: Can you tell me more about how your collaboration came about?

Movva: During my time at Biogen, my work focused on large biological proteins and their medical use. In 1985, one of our chemists came back from a scientific conference and was reporting about an unusual, 11-amino acid cyclic peptide that inhibited T cell activation. That was my first introduction to cyclosporine A, which revolutionized transplant medicine. At that time, the precise details of cyclosporine A's mechanism of action were not known and I became very interested in such small molecule natural products that are exciting medicines. Soon after I joined Sandoz in 1987, I initiated my efforts to understand the mechanism of action of cyclosporine A . Much of the work at that time in pharmaceutical companies and academic labs was naturally focused on mammalian cells and was highly competitive. For this reason, I decided to pursue a novel and different approach, consistent with my passion —genetic and molecular biology strategy— in microorganisms. I began to test cyclosporine A and its derivatives that were available at Sandoz for growth inhibition in *E. coli* and *S. cerevisiae*, but none were found to be growth inhibitory; a requirement that would facilitate the genetic identification of targets through mutation studies. Handschumacher and colleagues (3) had shown that many species, including *S. cerevisiae*, have the cyclosporine-binding protein, cyclophilin, and I reasoned that such an evolutionarily conserved and abundant protein must have important basic biological functions in the cell. Therefore, I felt it would still be a good fundamental basic science story, even if we couldn't immediately make the direct connection to T cells, which was foreseen to be solved by directly working with immune cells. Many of my Sandoz colleagues, who were actively working with cyclosporine A in mammalian cells, generously supported my exotic efforts with compounds, technical support, and resources. At Sandoz, we purified, completely sequenced the yeast

cyclophilin protein, cloned the yeast cyclophilin gene within a year of starting the project, and published our results in the journal *Gene* in 1989 (4). We subsequently deleted the cyclophilin A gene in yeast strains and to our disappointment, these deletion strains were viable, showing that the cyclophilin A gene was not essential for growth in the laboratory. Naturally, there were also many colleagues at Sandoz that were skeptical and critical about the value of working on yeast with a T-cell immunosuppressant. As I was passionate about this approach, I was able to convince the organization to have my freedom to pursue this research. Mike Hall had joined the Biozentrum in Basel around the same time I joined Sandoz. I had never met him before, but was familiar with his work because of our common previous research interests on bacterial outer membrane proteins. He was doing genetics of nuclear transport in yeast at the Biozentrum with a dedicated group. I approached Mike and engaged him as a consultant to access the research tools, such as strains and plasmid libraries that would facilitate my mechanistic pursuits with yeast at Sandoz. While Mike was busy with his own projects, he generously provided help, even dissected my first cyclophilin gene deletion yeast strains in his lab, and provided guidance to name the yeast cyclophilin gene. Our real collaboration came later with Joe joining forces with me to do genetic studies on the next immunosuppressant FK506.

Heitman: Mike Hall had moved to start his independent research lab at the Biozentrum in 1987 and I was impressed with his impeccable background and experiences. He completed his PhD with Jon Beckwith and Tom Silhavy at Harvard and had worked at the Pasteur Institute. He was then a post-doctoral fellow with Ira Herskowitz, a leader and pioneer in yeast genetics, at UCSF. Mike had an exciting view of studying the nucleus and its structure and its function. He had also published a couple of the very first papers identifying the nuclear localization signals that target proteins for transport from the cytoplasm into the nucleus. We thought we could harness this knowledge to identify how this signal operates to take a protein to the nuclear pore and into the nucleus. Very little was known about this in any detail at the time. We developed a yeast genetic screen using a fusion protein that we thought was jamming the nuclear pores and isolated resistant mutants. Very quickly, we realized that we were identifying elements of the yeast pheromone response pathway, which was already being studied by other labs. The further I progressed with the project, the clearer it became that it was not taking us in the desired direction. Around that time, I thought, "Well, maybe, I should just go back to medical school. This has been a good learning experience, but science can be very hard. You don't always succeed." But then I started thinking, "Well, what else can I do?" I was in the library toward the end of 1989 and came across a paper that had just been published in the journal *Nature* by Max Tropschug (5) who was working in Walter Neupert's lab in Munich, Germany. Their study focused on the immunosuppressive drug cyclosporine in the fungi *Neurospora* and *Saccharomyces*. They had isolated mutants of *Neurospora* that were resistant to cyclosporine. I went back to the lab and said, "We should be working on this. This is amazing." Normally, Mike would say, "Okay, that's a crazy idea. Maybe you should think a little bit more before you abandon your other project." Instead, Mike said, "You know, there's this investigator at Sandoz named Rao Movva. He's hired me as a consultant and is interested in exactly the same thing." Sandoz was just a bike ride away from the Biozentrum. I called Rao, we got together, and it was just amazing because he had purified cyclophilin from *Saccharomyces*, cloned the gene, and made mutants. But cyclosporine didn't have activity against the yeast strains that he had tested or that we had in the lab at that time.

Movva: After cyclosporine A**,** FK506 was seen as the next hope in clinical transplantation and was actively pursued by the Japanese pharmaceutical company Fujisawa and the clinical transplant surgeon Tom Starzl in Pittsburgh. With a strong commercial franchise in transplantation, Sandoz was keen to pursue any and all molecules in this area to understand their mode of action. Naturally, at Sandoz, I focused my FK506 efforts in yeast. Like cyclosporine A, FK506 also did not inhibit yeast growth, but with the tools and biochemical expertise at Sandoz, we continued the same path that we pursued with cyclosporine A. Again, in collaboration with Peter Hiestand's team, we purified the FK506-binding protein (FKBP12) from yeast, identified its complete amino acid sequence, and noted its high homology to that of the mammalian protein that was also recently reported. I met Joe for the first time around this time and was impressed with his enthusiasm for these studies. We immediately hit it off and started working together on FK506 and its genetics in yeast.

Heitman: The experimental plan that we set up from that very first meeting was to pursue studies in yeast as a genetic model. Because cyclosporine, FK506, and rapamycin are all natural products of microbes that live in the soil, our hypothesis was to study their intrinsic antifungal activities to determine their targets and mechanisms of action. The overarching hypothesis was that these targets would be conserved from

yeast to humans, and thereby provide insights into how these drugs suppress the human immune system. Our specific plan was to first use the information from the FKBP12 protein sequence to make degenerate oligonucleotide pools and use these pools to clone the gene and then make mutants lacking this drug-binding protein to see what their phenotypes might reveal. I tried to clone the gene by colony hybridization from a yeast genomic library using an end-labeled pool of degenerate primers, but this wasn't successful. Finally, I decided to try to use the primer pools for PCR such that the region between the primers would have perfect homology. This sounds very simple now, but back then this was state-of-the-art. The Biozentrum had bought a Perkin Elmer PCR machine that no one was using and was still packed in a box. No one had any idea what temperature to use for annealing for this degenerate PCR, so I empirically tried 25°C, and remarkably this worked. The first gel stained with ethidium bromide did not reveal any PCR product, until it was realized that the very short PCR products could be revealed by gently destaining the gel. This strategy was successful and with this probe we cloned and sequenced the FKBP12 gene, and then disrupted the gene by transformation and homologous recombination with a selectable marker interrupting the open reading frame. One copy of the gene was disrupted in a diploid strain, and the heterozygous diploid mutant sporulated and dissected, revealing that the haploid FKBP12 deletion mutant was viable. Thus, the first important conclusion was that this incredibly conserved enzyme, a peptidyl-prolyl cis-trans isomerase thought to play an important role in protein folding, was not essential for cell viability.

Movva: Following the success of FK506 as a transplant drug, rapamycin became the next promising agent for transplantation, a drug pursued by Wyeth-Ayerst. It was recognized quickly through structural similarity that rapamycin also bound FKBP12, but had a different mode of immunosuppressive action in mammalian cells. As soon as rapamycin was isolated at Sandoz, I did my favorite growth inhibition halo test and to my delight, for the first time, I had a compound that produced a large zone of inhibition in yeast. I immediately shared my excitement with Joe as we could now really do classical genetics in yeast with an immunosuppressive molecule and we prioritized this effort over the FK506 studies.

Heitman: One day Rao arrived to the lab with a small vial. I still remember this day. The tube was the size of an Eppendorf and had a screw cap and contained about one milligram of rapamycin. Sandoz had the rapamycin-producing organism (*Streptomyces hygroscopicus*) and purified this batch of the drug following fermentation. The way I remember it, Rao swooped into the lab and said, "Okay, this is the world's supply of rapamycin. Think very carefully about the next experiments you're going to do. Don't blow it because this is all we have."

Movva: I also remember telling him that I had given him the "world's precious supply," because I didn't know when additional material would be made again, which was not trivial. I remember Joe asking me what concentration he should use for growth inhibition and I told him that he had to titrate and find that out. Joe brilliantly and diligently pursued this work.

Heitman: I really had no idea how much rapamycin to use in making media. I made one or two Petri dishes containing rapamycin in the media based on the amount of FK506 I was using in other experiments, and then about a dozen different experiments were tested simultaneously on different sectors of those few Petri dishes. We immediately saw that the WT yeast strains were exquisitely sensitive to rapamycin and the mutants lacking the FKBP12 drug binding protein were completely resistant. This single experiment revealed that both the drug and the binding protein were required for the antifungal activity; providing evidence that the FKBP12-rapamycin complex is the active agent in the cell. This was a critical finding because at the time many in the field thought that because FKBP12 was an abundant protein expressed in cells and tissues throughout the body, that it was unlikely to mediate the activity of rapamycin. This yeast experiment showed definitively that FKBP12 was required for the activity of rapamycin in yeast. To identify the target(s) of the FKBP12-rapamycin complex, we next selected spontaneous rapamycin-resistant mutants. From this approach, we isolated 18 mutants, 15 of which had recessive loss of function mutations in FKBP12. The other 3 turned out to be partially dominant or dominant mutations in two novel genes, which were named TOR1 and TOR2, which stand for target of rapamycin. Tor is also the German word for door or gateway, so TOR was named in homage to the Spalentor, one of the historic gates into the city of Basel that is still standing, and to commemorate the city in which TOR was discovered.

JCI Insight: Of course, we now know that TOR signaling is conserved in all eukaryotes and regulates several facets of cell growth and metabolism. Your initial screen in yeast really paved the way for characterization of mTOR signaling. Can you speak to the importance of yeast and other model systems?

Movva: Everybody has to find their niche. Yeast was our niche here and gave us an advantage in understanding the basic biology, which, as we know, is conserved from DNA replication to transcription and translation. Nobody should be surprised that understanding the fundamental nature of how things work, no matter in what system, helps solve the bigger puzzles in the long run. There is still so much waiting to be discovered. What we know is very little compared to what we should know and the quest will continue forever.

Heitman: It's incredible to see the myriad contributions based on *Saccharomyces* as a model system. I think it's often been said, "if you can study it in *Saccharomyces*, you should." That was certainly the case for the discovery of FKBP12 and TOR as the targets of rapamycin, and the subsequent discovery of the role of TOR as a nutrient sensor and the elucidation of the TOR signaling pathways and of the two TOR complexes (TORC1 and TORC2). We now appreciate how closely related fungi and animals are to each other in the eukaryotic tree of life, but at the time, this was much less clear, the yeast genome was not sequenced, and the idea of studying immunosuppressive drugs in a yeast was thought heretical. It is quite amazing to see just how conserved FKBP12 and TOR are over the billion or more years of evolution separating yeast and humans from their common ancestor. There are areas of biology that you can't study in *Saccharomyces* as they do not have a biological clock, light sensors, or RNAi. But other fungi do have biological clocks, can sense light, and have RNAi pathways, and fungal genetics is therefore a rich area for discovery.

JCI Insight: This discovery of TOR was made possible through your strong collaboration. How important are such partnerships?

Movva: Collaborations are always important. By having multiple scientists focus on the same problem, answers come much faster, because no one person has all the tools. Pursuing science is largely a team effort, even if the vision and dreams start with one person. It is important that industries and academics collaborate as they bring different skills and perspectives. There should even be enhanced collaboration within each group to get the answers faster as there is an unmet medical need now.

Heitman: Our collaboration was an amazing turn of events, and I was in exactly the right place at the right time. I was a young postdoctoral fellow and Mike and Rao were both early in their independent careers. It was an amazing time, because we really worked more like partners and colleagues than professor and postdoc, or mentor and student. I think that sometimes science can be hierarchical, and other times it's a much more level playing field, and that contributes to advance science as well. I used to visit Sandoz at least one day a week and would spend that day working in Rao's lab and discussing science; it was very stimulating. I can't imagine this happening so easily today. Now, there are logistics and paperwork that create a barrier. While there are definitely collaborations that happen between academic and pharma or biotech labs today, I don't know that the kind of collaboration that we had for this project would happen as readily today.

JCI Insight: Any advice for trainees on finding a topic/field that can sustain research throughout an entire career?

Movva: Follow your passion. Be aware of the differences between your observations and the conventional wisdom. Let the experiments teach you and drive you. In industry, I have had the privilege to pick a subject that I enjoyed, learn the essentials of it, work toward a breakthrough observation with competent and collaborating colleagues, and then move on to the next project of interest. You don't need to worry about wanting to be famous, as long as you can follow your passion. Believe in yourself, and make sure your heart is in the right place in supporting the unmet medical needs and drug discovery. Another important aspect is to surround yourself with talented colleagues who not only challenge you, but also support you to realize the shared dreams and vision. As they say, success has many parents, and your prize is the inner satisfaction that you made a contribution for the common good.

Heitman: My advice is to not be lulled into complacency that everything important has already been discovered. There's so much that remains to be discovered and I think trainees should be fearless in thinking about that. Second, it is important to have a robust model system. You need the right system to ask and answer questions. Finally, select something that you're really passionate and curious about. There are a lot of things we attempt that are not successful, so choosing something that might be hard to tackle, but is exciting is important to provide a buffer against the challenges of experimental science.

JCI Insight: Any closing thoughts on the 2017 Lasker Basic Medical Research Award being awarded to Mike Hall for "discoveries concerning the nutrient-activated TOR proteins and their central role in the metabolic control of cell growth"?

Movva and Heitman: It is very exciting to see that the discovery of TOR, based on pioneering studies conducted in yeast, catalyzed so many subsequent studies that together established the conservation of the TOR pathways throughout eukaryotes, spanning yeast to humans and beyond. The elegant and facile yeast genetic studies enabled rapid elucidation of the pathways and targets of this master cellular regulatory network. We are delighted that the role of the TOR pathways in growth control was recognized with the Lasker Award to our collaborator Mike Hall, who continued focusing on the TOR pathway and further dissected its role with many talented colleagues in his group at the Biozentrum in Basel. To have played key roles in the inception of this project is both gratifying and humbling. As we look to the future, given the central regulatory roles of the TOR pathways in many conserved physiological pathways governing nutrient sensing, cell growth, autophagy, and life span, these discoveries will continue to drive biological insights and drug development in many areas with unmet medical needs.

Postscript by M. Hall

The early work on TOR —in other words, the seminal work of Joe Heitman and Rao Movva that led to the discovery of TOR and much more—was a perfect convergence of fortuitous circumstances. As the reader will appreciate from the above, the right people came together at the right time and in the right place and with a common interest. This happens rarely, if ever, in the career of a scientist. Joe, Rao, and I can be grateful it happened to us. It was, indeed, an exciting time scientifically, and was enhanced by good camaraderie. I am indebted to Joe and Rao for introducing me to rapamycin and for everything that followed (6).

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