TOR and paradigm change: cell growth is controlled

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ABSTRACT **This year marks the 25th anniversary of the discovery of target of rapamycin (TOR), a highly conserved kinase and central controller of cell growth. In this Retrospective, I briefly describe the discovery of TOR and the subsequent elucidation of its cellular role. I place particular emphasis on an article by Barbet *et al.* from 1996, the first suggesting that TOR controls cell growth in response to nutrients.**

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In 1996, the fledgling journal *Molecular Biology of the Cell* (*MBoC*) published an important article (Barbet *et al.*, 1996) from our laboratory on the cellular role of target of rapamycin (TOR), much to our relief and everlasting gratitude. I thus readily accepted the current editors' invitation to write a Retrospective to commemorate both the 25th anniversary of the discovery of TOR and the 20th anniversary of that article. Why was Barbet *et al.* (1996) important, and why were we so relieved to have it published? Before I explain, I will set the scene by describing the discovery of TOR and events soon thereafter.

In the late 1980s, I was a fresh assistant professor at the Biozentrum of the University of Basel, Switzerland. We were studying nuclear protein import as an extension of my postdoctoral work at the University of California, San Francisco, during which I described the nuclear localization signal. Our work on nuclear import was not going very well, and we were getting desperate, so desperate that (as later described by the journalist Karen Hopkin) we turned to drugs—in this case the immunosuppressive drugs cyclosporin A (CsA) and FK506. Joe Heitman had just joined the lab as a postdoc after finishing the PhD part of his MD-PhD studies at The Rockefeller University and, given his medical background, was interested in how drugs worked. Another very fortunate circumstance was an ongoing collaboration with Rao Movva, who was a group leader at the Basel pharmaceutical company Sandoz (now Novartis). CsA was a blockbuster drug for Sandoz, and Rao was interested in determining its mode of action. Little was known about how CsA and FK506 worked, other than that they blocked nuclear import of a signal, possibly a protein, downstream of the T-cell receptor. Thus we thought we could use the drugs to probe novel signaling pathways or the nuclear import process. Something interesting had to come out, particularly since at the time no complete signaling pathway was known, other than two-component signaling pathways in bacteria. How a signal was transduced from the cytoplasm into the nucleus was then known as the "black box" of signal transduction. Unusual about our experimental approach was that we used yeast genetics to study drugs that were developed for use on humans. Indeed, some viewed our experiments as tantamount to giving aspirin to yeast—why would we do something so physiologically irrelevant? Today, it is not uncommon to see nonmammalian model organisms in biomedical research.

In our first experiments, CsA and FK506 had little to no effect on yeast cells. Rao then told us about rapamycin, a brand-new FK506-like compound that was not yet approved for use in the clinic or even commercially available. At the time, Sandoz was one of the few places in the world where one could obtain rapamycin. Unlike CsA and FK506, rapamycin blocked proliferation of yeast cells, and Joe quickly selected rapamycin-resistant mutants. Most of the yeast mutants were defective in the *FPR1* gene, which Joe had already characterized during his earlier work on FK506 (FPR1 stands for FK506-binding proline rotamase, also known as FKBP). The few remaining mutants were altered in one of two new genes, which we named *TOR1* and *TOR2*. Curiously, the *FPR1* mutations were recessive, whereas the *TOR* mutations were dominant. We published Joe's findings in August 1991 (Heitman *et al.*, 1991), soon after he returned to New York City to finish the MD part of his studies. Thus TOR was discovered in 1991, initially as genetic loci. However, we still did not know what the *TOR* genes encoded or why the *TOR* mutations were rare and dominant.

The rapamycin study was taken over by Jeannette Kunz, a Swiss PhD student, who was later joined on the project by Stephen Helliwell, a British PhD student. Jeannette and Stephen cloned and

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Abbreviations used: FKBP, FK506 binding protein; FRAP, FKBP-rapamycin associated protein; mTOR, mammalian target of rapamycin; RAFT, rapamycin and FKBP target; RAPT, rapamycin target.

characterized the two *TOR* genes. The approach to cloning the *TOR* genes was to make genomic libraries from our dominant *TOR* mutants. The libraries were then transformed into a rapamycin-sensitive yeast strain, selecting rapamycin resistance. Such a function-based isolation of a gene in yeast is usually an easy task, but not in the case of the *TOR* genes. As we later found out, the *TOR* genes are among the largest in yeast and were thus underrepresented in our libraries containing inserts of conventional size. The mood in the lab was low as we tried to understand our nerve-wracking and seemingly endless cloning failures. We were finally able to isolate the *TOR* genes after making new libraries with larger inserts. We did not construct new libraries with larger inserts right away because we thought it unlikely that *both TOR* genes would be too big—something else had to be wrong. Once we had the two mutant *TOR* genes cloned, it was straightforward to obtain the wild-type versions of *TOR1* and *TOR2* by homologous recombination between a gapped plasmid-borne version of a *TOR* gene and the genome, so-called gap repair. On sequencing the two *TOR* genes, a task again made more difficult by the large size of the two genes, we discovered that they encode two highly homologous proteins that resemble lipid kinases (Kunz *et al.*, 1993; Helliwell *et al.*, 1994). The TORs turned out to be the founding members of a novel class of atypical protein kinases known as phosphatidylinositol kinase–related protein kinases (PIKKs; Keith and Schreiber, 1995). Jeannette knocked out the two *TOR* genes to reproduce the effect of rapamycin treatment, confirming that the TOR proteins are the physiologically relevant target of rapamycin. Mutations in *FPR1* and *TOR* conferred resistance because rapamycin acts by binding FKBP, and the FKBP–rapamycin complex then binds and inhibits TOR. The *TOR* mutations were dominant because they prevent FKBP–rapamycin binding without otherwise affecting TOR activity, and rare because they were confined to a single codon that specifies a critical residue in the FKBP–rapamycin binding site. Thus the article by Heitman *et al.* (1991) was key in the discovery of TOR, elucidation of the rapamycin mode of action, and identification of the FKBP–rapamycin binding site in TOR. Jeannette published her work in May 1993 (Kunz *et al.*, 1993). Shortly thereafter, the Schreiber (Brown *et al.*, 1994), Snyder (Sabatini *et al.*, 1994), Berlin (Chiu *et al.*, 1994), and Abraham (Sabers *et al.*, 1995) groups independently described the mammalian TOR orthologue, giving it the names FRAP, RAFT, RAPT, and mTOR, respectively. The field eventually chose the name mTOR based on the yeast precedent.

The race to discover TOR was fiercely competitive. According to lore, Eric Brown from Stuart Schreiber's lab flew to London to hand deliver their manuscript (Brown *et al.*, 1994) to the office of *Nature*, avoiding what has become known (since the advent of email) as snail mail. We were fortunate to win the TOR race, in large part because of the talented postdocs and students working on the project, but also because we worked with genetically tractable yeast. We made the assumption that the target of rapamycin is conserved, an assumption based on the fact that rapamycin is a natural product secreted by a soil bacterium, *Streptomyces hygroscopicus*, to inhibit other microbes such as fungi/yeast. Ironically, in the late 1980s, this was not widely appreciated—rapamycin was better known as a drug to treat allograft rejection in humans.

By 1994, it was clear that TOR is a highly conserved kinase and the in vivo target of rapamycin. However, the physiological role of TOR was unknown. Is TOR part of a signaling pathway, and, if so, what is upstream and downstream of the kinase? The general assumption at the time was that TOR controlled the cell cycle, that is, cell division. This assumption was based on the observation that cells treated with rapamycin, or yeast cells in which the *TOR* genes are knocked out, arrested in the G1 phase of the cell cycle. However, as first suggested by Barbet *et al.* (1996), the true role of TOR is to control cell growth (increase in cell size/mass) rather than cell division (increase in cell number). The misleading cell cycle arrest was an indirect consequence of a cell growth defect—cells arrest in G1 if they are not of adequate size to divide. Another general assumption at the time that prevented us from considering that TOR might be controlling cell growth was that cell growth is not controlled. As difficult as it may be to believe from today's perspective, conventional wisdom was that cell growth is a spontaneous process that just happens when building blocks (nutrients) are available rather than an actively regulated, plastic process. Thus the paradigm at that time gave us no reason to predict the existence of a regulator of cell growth. What led to a paradigm change? A very influential event was a seminar I gave at the Institute of Molecular Pathology (IMP) in October 1993. Kim Nasmyth, a leader in the cell cycle field and then Director of the IMP, invited me to Vienna to present our latest results and speculations on how TOR controlled cell division. I was pummeled—the question and answer session lasted longer than the seminar. The Nasmyth group was skeptical from the outset that TOR controlled cell division, and this skepticism was reinforced when they detected in my data what Thomas S. Kuhn would have called an "anomaly" (Kuhn, 1962). Yeast cell division cycle mutants, such as a *cdc28* mutant, arrest in G1 but continue macromolecular synthesis to become very large, up to four times as large as a normal cell. In contrast, as I presented during my seminar and as described in Barbet *et al.* (1996), *TOR* mutant cells arrest in G1 with a biphasic size distribution but do not continue to grow to become very large. Thus the anomaly was that the *TOR* mutants did not behave as expected for a bona fide cell cycle mutant. A lively discussion ensued on what TOR could be controlling if not the cell cycle. We arrived at the conclusion that TOR could be controlling cell growth (i.e., macromolecular synthesis), thereby inescapably accepting the notion that cell growth is actively controlled—a change in paradigm! Finally, we discussed what experiments could be performed to test the new paradigm. A high-priority experiment, also described in Barbet *et al.* (1996), was to measure incorporation of [^{35}S]methionine into protein in TOR-deficient cells to determine whether there was a defect in general protein synthesis. This experiment was done immediately upon my return to Basel and revealed that there was indeed a defect in translation. We next determined that the observed defect in protein synthesis is at the level of translation initiation and that this translation defect is responsible for the cell cycle defect, in agreement with our new paradigm that TOR controls cell growth directly and cell division only indirectly. We also determined by reciprocal shift experiments that the G1 arrest is in early G1, that is, actually a G0 arrest, as normally observed in starved cells. This led to the suggestion that TOR controls cell growth in response to nutrients. Thus, almost in one fell swoop, we learned what is upstream (nutrients) and downstream (cell growth) of TOR. We could now place TOR in context. It controls cell growth! Needless to say, we viewed this as an important advance.

Missing in our study was a molecular mechanism to control translation initiation. Fortunately, parallel to our study, Nahum Sonenberg and John Lawrence discovered the eIF-4E–binding protein 4E-BP (then also known as PHAS-I), which controls translation initiation in response to insulin (Lin *et al.* 1994; Pause *et al.*, 1994). These findings not only suggested a mechanism by which TOR (mTOR in this case) controls translation initiation, but they also suggested that the biology underlying our observations was conserved from yeast to mammals. The discovery of 4E-BP also bolstered our confidence in our findings. We eventually collaborated with Nahum Sonenberg to demonstrate that mTOR indeed controls translation initiation via 4E-BP (Beretta *et al.*, 1996).

With enthusiasm befitting our new findings, we submitted a manuscript to a high-impact journal in July 1994. Much to our dismay, it was rejected. Indeed, it was rejected a total of seven times from several journals. The exasperation in the lab was palpable. We had what we thought was an important set of findings but could not get our message across despite extensive telephone conversations and rewrites to address the reviewers' misunderstanding. One editor wrote, "I must say that a paper of this complexity does not make life easy. I am not sure many readers will take the time to digest such a tome." Perhaps the reviewers and editors were not ready for the new paradigm. To make matters worse, we had never had an article rejected before. We were shell-shocked. Finally, in late 1995, we submitted our manuscript to *MBoC*, where, fortunately, it landed on Lee Hartwell's desk. Lee Hartwell was one of the few at that time who was thinking about cell growth and cell division as distinct, regulated processes (Hartwell, 1993). He was more than ready for the "new" paradigm. The manuscript was accepted in short order and appeared in press in January 1996 (Barbet *et al.*, 1996). Our painful publishing nightmare was over.

Over the next years, we continued to develop the concept of TOR as a central controller of cell growth (Thomas and Hall, 1997; Schmelzle and Hall, 2000; Hall *et al.*, 2004; Wullschleger *et al.*, 2006), often explaining to audiences that cell growth and cell division are distinct processes each with its own regulator. Work from our and others' labs has now shown that TOR in yeast and mammals activates several anabolic processes (e.g., ribosome biogenesis and protein, lipid, and nucleotide synthesis) and inhibits catabolic processes (e.g., autophagy) to control cell growth (Laplante and Sabatini, 2012; Shimobayashi and Hall, 2014). Of importance, in 1998, Joe Avruch demonstrated that nutrients—amino acids in particular—activate mTOR in mammalian cells (Hara *et al.*, 1998). It is now well established that TOR is a central controller of cell growth in response to nutrients.

Reflecting a more chivalrous, bygone era in science, shortly after Barbet *et al*. (1996) appeared, we received a very kind, hand-written note from our competitor, Stuart Schreiber: "Dear Mike, Congratulations on your recent work on the mechanism of TOR signaling. I found your paper in Mol Biol Cell to be illuminating, and satisfyingly in congruence with your work with N. Sonenberg that appeared in EMBO J. These represent major steps forward in the study of a fascinating family of proteins. Hope to see you in the near future. Sincerely, Stuart." In 2012, in celebration of *MBoC*'s first 20 years, members of the Editorial Board picked Barbet *et al*. (1996) as a favorite from the previous two decades. Mark Ashe wrote, "I remember reading the paper and being struck by how comprehensive the story was; the authors outlined the basic mechanism of translational regulation and detailed downstream consequences in terms of cellular physiology" (Ashe, 2012). All's well that ends well.

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