

# TOR, the Gateway to Cellular Metabolism, Cell Growth, and Disease

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Michael N. Hall is this year's recipient of the Lasker Basic Medical Research Award for the identification of the target of rapamycin, TOR. TOR is a master regulator of the cell's growth and metabolic state, and its dysregulation contributes to a variety of diseases, including diabetes, obesity, neurodegenerative disorders, aging, and cancer, making the TOR pathway an attractive therapeutic target.

In order to survive, differentiate, and grow, cells must evaluate their energy status and oxygen availability, take inventory of their surrounding nutrients, hormones, cytokines, and growth factors, and then integrate this information to decide what the cell's next step is. Amazingly, a signaling system has evolved that is capable of doing all of the above. The centerpiece of this pathway is the mTOR protein kinase.

## Rapa Nui

The study of TOR originated in the 1960s with an expedition to Easter Island (known by the island inhabitants as Rapa Nui), with the goal of identifying natural products from plants and soil with possible therapeutic potential. In 1972, Suren Sehgal identified a small molecule, from a soil bacterium Streptomyces hygroscopicus, that he purified and initially reported to possess potent antifungal activity. He appropriately named it rapamycin, noting its original source and activity (Sehgal et al., 1975). However, early testing revealed that rapamycin also had potent immunosuppressive and cytostatic anti-cancer activity. Unfortunately, rapamycin did not initially receive significant interest from the pharmaceutical industry until the 1980s, when Wyeth-Ayerst supported Sehgal's efforts to further investigate rapamycin's effect on the immune system. This eventually led to its FDA approval as an immunosuppressant following kidney transplantation. However, prior to its FDA approval, how rapamycin worked remained completely unknown.

#### The Discovery of TOR

An important outcome of the renewed interest in rapamycin was that large quantities of rapamycin were produced and made available to the academic community. Taking advantage of its anti-fungal activity, Michael Hall and Joseph Heitman designed a genetic screen in Saccharomyces cerevisiae to identify resistant mutants (Heitman et al., 1991). Three major hits from the screen provided the foundation for the identification of the target of rapamycin. First, the yeast ortholog of the cellular receptor for rapamycin, FKBP12 (FPR1), was identified. FKPB12 was known to be a cis-trans prolyl isomerase that bound rapamycin, but Stuart Schreiber and colleagues had demonstrated that inhibition of this activity was not linked to rapamycin's biological activity (Schreiber, 1991), so it remained unclear if FKBP12 was the cellular receptor mediating rapamycin's effects. Critically, Hall's genetics showed that mutants lacking FKBP12 were completely resistant to rapamycin, demonstrating that it is the drug receptor responsible for its activity. However, this alone did not provide insight into how rapamycin worked. That was to come from the identification of two novel and related mutants identified in the screen that were named TOR1 and TOR2, for targets of rapamycin. The TOR1 and TOR2 genes were later found to encode large kinases that resemble PI3 kinase (Kunz et al., 1993) and were the founding members of the atypical PIkinase-like protein kinase (PIKK) family. Importantly, the TOR mutations identified in the yeast genetics screen are in a single

codon that is required for FKBP12-rapamycin binding to and inhibition of TOR. The discovery of the *TOR* gene set the stage for 25-plus years of investigations into how TOR is regulated and signals, the biological consequences of its normal as well as its inappropriate regulation, and investigations into the therapeutic potential of targeting this pathway (Figure 1).

## **The TOR Pathway Takes Shape**

Following the Hall discovery, the use of rapamycin helped scientists identify the proteins acting both up and downstream of this novel kinase. In 1992, we and others discovered that rapamycin potently inhibited activation of S6 protein kinase 1 (S6K1) by growth-factor-stimulated receptor tyrosine kinases, the Src oncogene, heterotrimeric G proteins, tumor promoter phorbol ester (activator of protein kinase C, PKC), or stress stimuli, etc. We also demonstrated that rapamycin delayed S-phase entry linking TOR to G1 cell cycle progression. Inspired by the Hall paper, we demonstrated that the rapamycin receptor, FKBP12, was required for inhibition but that S6K1 was not directly inhibited by the drug (Chou and Blenis, 1995). However, rapamycin did not inhibit activation of ERK-MAP kinase and RSK, which we had shown to be activated downstream of Ras (Wood et al., 1992).

Two years later, we found that PI3-kinase was required for growth factor- and insulin-dependent activation of S6K1 and that this activation was also potently blocked by rapamycin (Chung et al., 1994). This suggested that a mammalian





# Figure 1. From Rapamycin to the mTOR Pathway and Disease

(A) The core components of mTOR complex 1 (mTORC1) and mTORC2. mTORC1 is acutely sensitive to rapamycin, whereas mTORC2 is occasionally observed to have reduced signaling upon long-term treatment. mTORC1 is regulated by multiple inputs. This information is deftly integrated to promote mTORC1-dependent regulation of a variety of growth-associated biological processes. mTORC2 is regulated downstream of PI3-kinase, and ribosomes participate in its activation.

(B) Over the past 25-plus years since the discovery of yeast TOR, the molecular details of mTORC1 regulation have been investigated. This has resulted in the discovery of multiple novel mechanisms for signal transduction, as well as revealing potential next-generation therapeutic targets and biomarkers. In red are putative or known tumor suppressors, where loss of expression/function occurs in cancer. In green are positive regulators of mTORC1 that are often activated by mutation or overexpression in cancer. mTORC1 has been estimated to be activated in 60%–80% of all cancers. For detailed description of the regulation of the pathway components see Saxton and Sabatini (2017) and Shimobayashi and Hall (2014).

TOR was mediating signaling from PI3K to S6K1. The following year, Akt/protein kinase B (PKB) was shown to be regulated downstream of PI3K and upstream of S6K1 by Coffer and Tsichlis (Chan et al., 1999). Since Akt was also not directly inhibited by rapamycin, there remained a gap between PI3K/Akt and S6K1. Based on the work of Hall and the above studies, we believed that a mammalian TOR was acting upstream of S6K1 and downstream of Akt, although how was unclear. Moreover, our studies suggested that TOR and S6K1 participated in a novel growth (G1 cell cycle) promoting pathway (Figure 1B).

## **Mammalian TOR**

To realize the importance of Hall's discovery of yeast TOR, it was essential to demonstrate its conservation in higher eukaryotes as he had predicted. In 1994, the mammalian homolog of TOR was biochemically purified and found to be the ortholog of yeast TOR by Snyder, Schreiber, Berlin, and Abraham (Saxton and Sabatini, 2017; Shimobayashi and Hall, 2014). Although initially assigned several names, mTOR or mammalian TOR was accepted in recognition of its discovery in yeast by Hall (now also referred to as mechanistic TOR). As mentioned, rapamycin was known to suppress cell proliferation, and once discovered, mTOR was linked to cell cycle progression by regulating cell growth, a process where cells essentially double their cellular material as a result of dramatic and coordinated changes is cellular metabolism before proceeding to cell division (proliferation). The Hall lab suggested a link between yeast TOR and cell growth and also provided evidence that this was related to the ability of TOR to regulate protein synthesis (Barbet et al., 1996). mTOR was then shown to phosphorylate and inhibit the repressor of CAP-dependent translation, 4EBP1, and to phosphorylate and contribute to the activation of S6K1 (Burnett et al., 1998), major players in translational control.

## mTORC1 and mTORC2

What was to follow in the early 2000s were several elegant studies utilizing biochemistry, cell biology, and genetics, predominately by the Hall and Sabatini labs, to demonstrate that mTOR existed in two highly conserved, large molecular complexes, termed mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (Figure 1A). mTORC1 consists of mTOR and the core components Raptor (Kog1) and mLst8 (Lst8), and mTORC2 consists of mTOR and Rictor (Avo3), Sin1 (Avo1), and mLst8 (Lst8). Rapamycin: FKBP12 binds to and acutely inhibits mTORC1, but not mTORC2. Interestingly, long-term treatment with rapamycin can also suppress mTORC2 in some cell types, and this is possibly due to sequestration of mTOR in the inhibited mTORC1 complex and its decreased availability for incorporation into new mTORC2 complexes. Additional unique components for these complexes have also been identified that likely contribute to specific functions in yeast versus mammals (Saxton and Sabatini, 2017; Shimobayashi and Hall, 2014). Initially, the main targets for mTORC1 were S6K1 (Sch9) and 4EBP1, and for mTORC2, the main targets were AGC kinases Akt. SGK (Ypk1), and PKC. We now know that there are many potential mTORC1 and mTORC2 targets based largely on the recent mass-spectrometry-generated phosphoproteomes from the Hall lab, the Sabatini lab, and my lab (Shimobayashi and Hall, 2014). These datasets are likely to reveal new functions of mTORC1 and mTORC2. Indeed, several of these targets have led to new insights into how mTORC1, in particular, is linked to negative feedback loops, metabolism, transcription, mRNA processing, and translation.

During this time, the convergence of human genetics associated with the rare autosomal dominant disorder tuberous sclerosis complex, combined with genetic studies in mouse, yeast, Drosophila, C. elegans, mammalian cell biology, and biochemistry revealed the complex relationship between the TSC1 (hamartin) and TSC2 (tuberin) tumor suppressor complex, its GAP activity toward the Rheb GTPase, and the ability of Rheb to activate mTORC1 and promote cell growth. Another key finding during this time was the demonstration that Akt directly phosphorylated TSC2, antagonizing its ability to suppress mTORC1 signaling and providing the link between mitogen signaling and mTORC1 (Saxton

and Sabatini, 2017; Shimobayashi and Hall, 2014). Additional insights into mTORC1 selective sensitivity to FKBP12rapamycin, its regulation, and substrate specificity came from structural analysis of mTORC1 from the labs of Drs. Sabatini, Pavletich, Hall, and colleagues (Saxton and Sabatini, 2017). The activation of mTORC2 has remained more enigmatic; however, recent studies suggest a role for ribosomes and PI3K signaling (Liu et al., 2015; Zinzalla et al., 2011).

### A Signaling Platform for mTORC1

The importance of spatial and temporal regulation of several signaling pathways, such as the Ras/ERK-MAP kinase cascade, had been previously established, raising the question of whether the mTORC1 pathway was similarly regulated. Since the Rheb GTPase is prenvlated, one guess was that it was regulated at intracellular membranes, similar to other Ras and Rho family GTPases. We now know, from the work of Sabatini, Kun-Liang Guan, and others, that the lysosome is the major intracellular membrane location for activation of mTORC1. Furthermore, as demonstrated by Sabatini and others, the lysosome serves as the landing pad for the complex aminoacid-dependent Rag GTPase activation, resulting in the recruitment of mTORC1, where it is activated by the lysosome-associated Rheb GTPase (Saxton and Sabatini. 2017: Shimobavashi and Hall, 2014). Critically, the TSC1-TSC2-TBC1D7 tumor suppressor GAP complex, which inactivates Rheb, dissociates from the lysosome following Aktdependent phosphorylation of TSC2, allowing Rheb to assume a GTP-bound state and activate mTORC1 (Manning and Toker, 2017). Furthermore, we are still learning how the combination of amino acids, nutrients, energy and oxygen status, stress, mitogens, and other inputs are sensed by specific mechanisms and coordinated to exquisitely regulate mTORC1 signaling (Figure 1B).

# Beyond Basic Research: mTOR in Physiology and Disease

Due to its complex regulation, it was anticipated that mTOR would have a significant role in controlling metabolic homeostasis at the organismal level. This has been confirmed through the use of rapamycin in preclinical and clinical investigations and by the generation of a variety of tissue-specific mouse genetic models, where mTOR or its regulatory subunits have been manipulated. These exciting investigations are ongoing, particularly in the areas of development, diabetes, obesity, neurodegenerative diseases, aging, and cancer (Saxton and Sabatini, 2017; Shimobayashi and Hall, 2014).

With regards to cancer, as illustrated in Figure 1, many proto-oncogenes and tumor suppressors participate in mTOR signaling. Thus, activating mutations, overexpression, or loss of function or expression of many positive or negative regulators of the mTOR pathway result in inappropriate activation of mTORC1 signaling. These include several growth factor receptors, cytoplasmic tyrosine kinases, PI3-kinase, Akt, mTOR, folliculin-FNIP, PTEN, LKB1, TSC1, TSC2, etc. Indeed, it is estimated that between 60%-80% of all cancers have activated mTORC1 signaling. In the last 10 years. rapalogs have received FDA approval for renal cell carcinoma, neuroendocrine carcinomas, angiomyolipomas associated with tuberous sclerosis complex and lymphangioleiomyomatosis. advanced breast cancer, and some rare children's brain cancers, with other cancers being investigated in monotherapy and combination therapy approaches and investigations into various dosing options. Furthermore, preclinical and clinical validation of additional pathway inhibitors is being pursued in academia and the pharmaceutical industry. As happened with rapamycin, the availability of small molecule inhibitors to these pathway participants will hasten the characterization of these candidate drugs and identify potential side-effects, feedback, or compensatory mechanisms. Such thorough studies are needed for the continued evolution of drug development and therapeutic strategies. Rapamycin is an on-going poster child for such an effort.

By identifying the rapamycin target, mTOR, and playing a major role in defining its function in cell signaling and disease, the legacy of Michael Hall's original discovery is being further defined as mTOR inhibitors and inhibitors of other pathway components are winding their way through preclinical and clinical trials for cancer, diabetes, obesity, heart disease, neurodegenerative disorders, and aging. We are already witnessing some limited success in cancer with rapalogs, but additional progress will continue to be realized as the mechanisms of feedback control, metabolic dependencies, compensatory mechanisms, and combination therapies are identified and incorporated into therapeutic strategies.

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