Profile of Michael N. Hall, 2017 Albert Lasker Basic Medical Research Awardee: Target of rapamycin, cell growth, and translational control

Nahum Sonenberg[a,1]

Michael N. Hall (Fig. 1) is the winner of the 2017 Lasker Award "for discoveries concerning the nutrient-activated TOR proteins and their central role in the metabolic control of cell growth." His seminal and paradigm-shifting discoveries were attained using the yeast *Saccharomyces cerevisiae*. More recently, he made a number of breakthrough discoveries in mammalian systems wherein he showed that the target of rapamycin (TOR) plays a major role in regulating whole-body metabolism in multicellular organisms.

The TOR story began more than 50 years ago, when an expedition was sent by Ayerst Research Laboratories (now Pfizer, located 6 miles from my house) to isolate novel compounds from soil microorganisms. Their quest took them to Easter Island, and ultimately resulted in the isolation of *Streptomyces hygroscopicus*. In 1975, it was reported that this bacterium produces a compound with antifungal activity (1). The compound rapamycin was named after the indigenous name of the island, Rapa Nui. In the same year, an unrelated important finding was reported by several research groups, the discovery that eukaryotic messenger RNAs (mRNAs) contain the structure m^7GpppN (where N is any nucleotide) at their 5′ termini. The structure was termed "cap" (e.g., refs. 2 and 3). Hall was the first to mechanistically link these two discoveries, inferring that TOR controlled growth by effecting the initiation of translation (discussed below).

Fig. 1. Michael N. Hall, winner of the 2017 Albert Lasker Basic Medical Research Award, pictured in 2015 for the Gairdner National Program Lecture in Montreal. Image courtesy of University of Basel/Gairdner Foundation.

Over 25 years ago, Hall screened for yeast cells that become resistant to rapamycin. This monumental experiment led to the discovery of TOR, and transformed our understanding of cell growth control. TOR, which is a large, atypical serine/threonine kinase, is an evolutionarily conserved key regulator of eukaryotic cell growth. To reach this conclusion, Hall provided the first evidence that akin to stimulating cell division (i.e., increase in cell number by cyclin-dependent kinase), nutrients also stimulate cell growth (i.e., increase in cell mass), and this is chiefly achieved via TOR. He recently reflected on his journey leading to these pivotal discoveries (4). Briefly, Hall discovered two TOR genes in yeast (5) that, when mutated, conferred resistance to rapamycin. Hall and co-workers (6) went on to clone the yeast *TOR1* and *TOR2* genes, and deciphered their functions through painstaking and scrupulous work that led to the discovery and characterization of two multisubunit complexes, TORC1 and TORC2 (7). A single gene encoding the mammalian ortholog, which is referred to as mammalian/mechanistic TOR (mTOR) was cloned a year after the cloning of the yeast genes by several groups (8–11). Yeast and mammalian TOR complexes contain multiple subunits, and are strikingly similar in subunit composition, demonstrating their evolutionarily conserved role in regulating cell growth. For instance, TORC1 is sensitive to rapamycin and contains the core subunits of TOR, LST8, and raptor (Lst8 and Kog1 in yeast, respectively), while TORC2 is resistant to acute rapamycin treatment and contains LST8, rictor, and mSin1 (AVO3 and AVO1 in yeast, respectively), in addition to TOR (7, 12–14).

Notably, there was a long lag between TOR's discovery and cloning, and establishment that TOR is indeed a protein kinase. Even as late as 1995, in a review by Hunter (15) on the lipid/phosphatidylinositol 3-kinase–related kinase family, it was rightfully questioned whether TOR is, in fact, a protein kinase, despite having some of the conserved protein kinase catalytic motifs. Several papers suggested that TOR might also be a

[a]Goodman Cancer Research Center, McGill University, Montreal, QC, Canada H3A 1A3

This article is part of a series of articles in PNAS highlighting the discoveries and profiling the winners of the Lasker Awards. Information about the 2016 Albert Lasker Basic Medical Research Awardees can be found on page 13938 in issue 49 of volume 113.

Author contributions: N.S. wrote the paper.

The author declares no conflict of interest.

[1]Email: nahum.sonenberg@mcgill.ca.

lipid kinase because of its similarity to the authentic phosphatidylinositide (PI) kinases. It was not until biochemical work in 1997/1998 that the first compelling evidence emerged that mTOR is a protein kinase that can phosphorylate two of its major targets 4E-BP1 and S6 kinase (S6K) (16–18) (discussed below).

Although the regulation of TORC1 by nutrients is remarkably complex in mammalian cells, strikingly, many of the critical regulators are conserved from yeast to humans (19, 20). In the last two decades, Hall and others contributed seminal discoveries pertinent to the role of TOR in cell homeostasis and pathological conditions across all eukaryotic kingdoms. TOR plays a pivotal role in aging, and it is frequently dysregulated in many human disorders, including cancer, diabetes, and autoimmune diseases. In the mammalian brain, TOR complexes are critical for synaptic plasticity and memory formation, and their importance is underscored by a large number of neurological disorders in which TOR activity is perturbed (21).

It was established that mTORC1 is activated by extracellular stimuli, including growth factors and hormones, via the PI3K-AKT pathway (22–24), which is one of the most frequently hyperactivated pathways in cancer. Akt acts on tuberous sclerosis complex, for which there is no counterpart in budding yeast, upstream of Rheb and mTORC1. The activity of mTORC2 is also controlled by insulin, growth factors, and serum, and it phosphorylates AKT, thereby increasing its activity (25). These findings positioned mTOR as a prime target for the development of anticancer drugs. Indeed, rapamycin and its analogs (rapalogs) exhibit an optimal therapeutic window and are already in clinical use for a variety of cancers, including breast and kidney cancers. In addition, new drugs that directly target the kinase site of mTOR and thus inhibit both TORC1 and TORC2 (second-generation mTOR inhibitors), as well as those where targeting of the kinase site and allosteric inhibition are combined (third-generation mTOR inhibitors) (26), have recently been developed. Many of these drugs are currently being tested in a large number of clinical trials.

The identification of TOR's downstream effectors represented a turning point in our understanding of the molecular basis of TOR's function in the cell. I have been fortunate to collaborate with Hall throughout the years. Before our collaboration, regulation of cell growth and translation were studied as two separate processes. However, Hall first showed that rapamycin inhibits protein synthesis in yeast, and our seemingly independent lines of research then started to converge to ultimately position TOR as a major regulator of mRNA translation. Key to the understanding of the mechanism by which TOR regulate protein synthesis was the discovery of the 4E-binding protein (4E-BP) (27, 28). Cap-dependent mRNA translation is mediated by the eukaryotic initiation factor 4F (eIF4F) complex that binds to the mRNA 5′-cap structure to facilitate the recruitment of the ribosome to the mRNA. The eIF4F complex consists of the cap-binding subunit eIF4E, the RNA helicase eIF4A, and the scaffolding protein eIF4G (29). It is thought that eIF4A unwinds the secondary structure present in the 5′ UTR of the mRNA to promote the binding of the ribosome and scanning of the 5′ UTR (29). The 4E-BPs exist in all eukaryotes, ranging from yeast to human (with the exception being *Caenorhabditis elegans*). In mammals, the 4E-BPs constitute a family of small-molecular-weight proteins that bind to eIF4E and

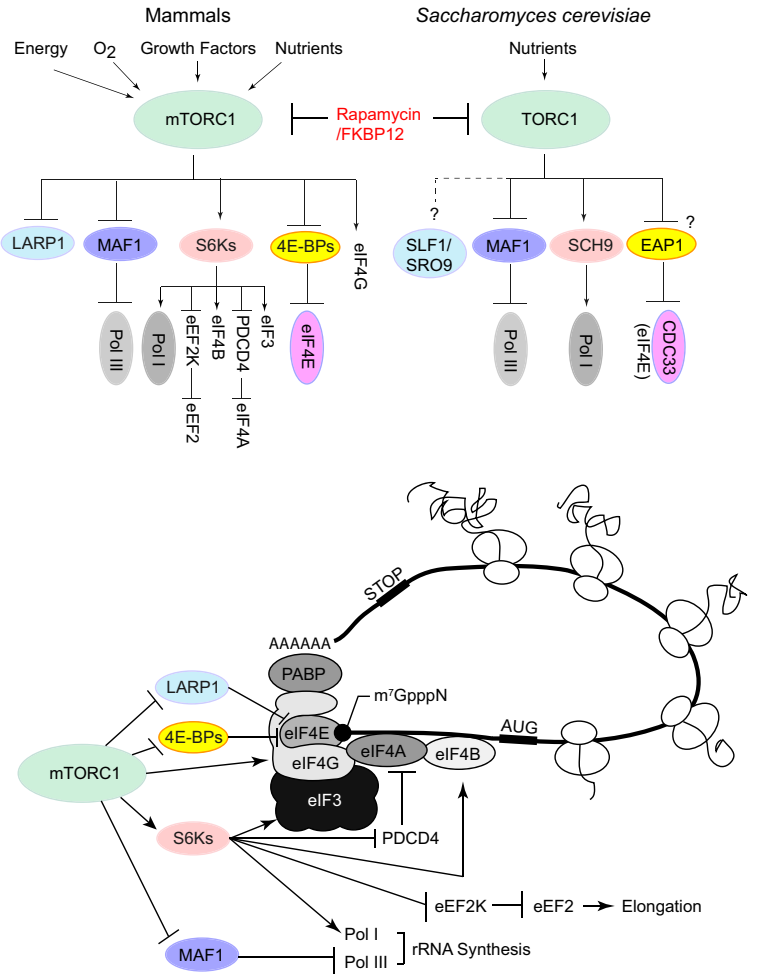

Fig. 2. Regulators of mRNA translation downstream of TORC1/mTORC1. Rapamycin in a complex with the immunophilin FKBP12 (12-kDa FK506-binding protein) inhibits TORC1/mTORC1 activity. Many targets of *S. cerevisiae* TORC1 and mTORC1 are orthologous translation factors. In mammals, in addition to 4E-BPs, many other components of the translation machinery are regulated by mTORC1 (details are provided in ref. 34). For example, an inhibitor of eIF4A, PDCD4, is phosphorylated by S6 kinase (S6K; an mTORC1 target), which leads to its ubiquitination and degradation by the proteasome. In addition, the synthesis of all of the components of the ribosome, including rRNAs (via regulation of RNA polymerases and phosphorylation of transcription factors) and ribosomal proteins [via phosphorylation of LA-related protein 1 (LARP1]), is controlled by mTOR (35, 36). LARP1 in mammals contains the La-motif, an RNA-binding domain found in all eukaryotes. It controls translation of mRNAs containing a terminal oligopyrimidine sequence at their 5′ termini (36). SLF1 (SMC5–SMC6 complex localization factor 1) and SRO9 (suppressor of RHO3 protein 9) are paralogous RNA-binding proteins that contain the highly conserved La-motif and control translation. MAF1 is a repressor of RNA polymerase III. S6Ks (ribosomal protein S6K1 and S6K2) are mammalian AGC (named after protein kinase A, G, and C) family protein kinases, which phosphorylate, in addition to S6, several initiation factors (eIF3 and eIF4B), and eukaryotic elongation factor 2 kinase (eEF2K) (35). SCH9 in *S. cerevisiae* is the functional ortholog of mammalian S6K. The 4E-BPs and EAP1 are described in the main text. CDC33 is the *S. cerevisiae* eIF4E. The sizes of the translation factors are not to scale. Question marks indicate that further work is required to support the data.

compete with eIF4G for eIF4E binding, resulting in the blockage of eIF4F complex assembly.

Hall was the first to report that rapamycin inhibits mRNA translation at the level of initiation in yeast. The important findings were phenocopied by depleting TOR1 (30). This decrease in translation coincided with a reduction in cell growth, which led to a cell cycle defect. As such, in yeast, TOR directly controls cell size by augmenting protein synthesis and increasing cell mass in response to nutrients, which then has a secondary impact on cell cycle progression. These findings led to a new paradigm. First, cell growth is regulated and, second, TOR controls cell growth directly and cell division in an indirect manner. While the molecular mechanism to explain the role of translational control in this model was lacking at the time, Hall predicted that the cap-binding protein eIF4E was at play. Consistent with his prediction, together, we showed that mTOR controls translation initiation via 4E-BPs (31). Phosphorylation of 4E-BPs is inhibited by rapamycin, which fosters the 4E-BP–eIF4E binding, thus inhibiting cap-dependent translation. In another study, we showed that 4E-BP1 undergoes hierarchical phosphorylation that is mediated by mTOR (32). These findings demonstrated that TOR is an evolutionarily conserved master regulator of protein synthesis and uncovered the precise molecular mechanism underlying this process.

While Hall's discoveries relating TOR signaling to cell growth and translation were revolutionary, editors and reviewers did not immediately appreciate the significance of the findings, as Hall reminisced in a recent retrospective in the journal *Molecular Biology of the Cell* (4). In fact, the article that rigorously documented the findings that yeast TOR primarily regulates cell growth in response to nutrients was rejected a total of seven times. As Hall relays in his retrospective, one editor went as far as to write, "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."

Hall and I continued to collaborate to discover a novel eIF4E-BP in yeast that we coined eIF4E-associated protein (Eap1), which inhibits translation by binding to eIF4E (33). Eap1 competes with eIF4G for binding to eIF4E and inhibits cap-dependent translation. Targeted disruption of the EAP1 gene resulted in a temperature-sensitive phenotype and also conferred partial resistance to growth inhibition by rapamycin. These data indicated that Eap1 plays a role in cell growth and implicated this protein in the *S. cerevisiae* TOR signaling cascade. Unfortunately, we could not show that Tor1/2 can phosphorylate Eap1. Perhaps today's state-of-the-art MS could provide a more conclusive result. TORC1/mTORC1 controls several additional translation factors via direct or indirect phosphorylation (Fig. 2).

It is striking that the rapamycin-sensitive and -insensitive TOR signaling pathways in yeast and mammals are conserved, and that TOR is a major controller of translation and cell growth in both. In mammals, the inputs of mTOR, especially mTORC1, have expanded dramatically because of the need to coordinate cell growth and division while sensing growth factors and hormones, as well as energy, oxygen, and nutrient availability.

Hall's extraordinary achievements are the culmination of gaining insightful answers to basic questions using powerful yeast genetics combined with classical biochemistry. The fundamental new knowledge of TOR has already engendered revolutionary understanding of general cell and organismal physiology, with critical implications for human health and disease.

1 Sehgal SN, Baker H, Vézina C (1975) Rapamycin (AY-22,989), a new antifungal antibiotic. II. Fermentation, isolation and characterization. *J Antibiot (Tokyo)* 28:727–732.
2 Furuichi Y, Morgan M, Muthukrishnan S, Shatkin AJ (1975) Reovirus messenger RNA contains a methylated, blocked 5′-terminal structure: m-7G(5′)ppp(5′)G-MpCp-. *Proc Natl Acad Sci USA* 72:362–366.
3 Wei CM, Moss B (1975) Methylated nucleotides block 5′-terminus of vaccinia virus messenger RNA. *Proc Natl Acad Sci USA* 72:318–322.
4 Hall MN (2016) TOR and paradigm change: Cell growth is controlled. *Mol Biol Cell* 27:2804–2806.
5 Heitman J, Movva NR, Hall MN (1991) Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* 253:905–909.
6 Kunz J, et al. (1993) Target of rapamycin in yeast, TOR2, is an essential phosphatidylinositol kinase homolog required for G1 progression. *Cell* 73:585–596.
7 Loewith R, et al. (2002) Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. *Mol Cell* 10:457–468.
8 Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH (1994) RAFT1: A mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell* 78:35–43.
9 Brown EJ, et al. (1994) A mammalian protein targeted by G1-arresting rapamycin-receptor complex. *Nature* 369:756–758.
10 Sabers CJ, et al. (1995) Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells. *J Biol Chem* 270:815–822.
11 Chiu MI, Katz H, Berlin V (1994) RAPT1, a mammalian homolog of yeast Tor, interacts with the FKBP12/rapamycin complex. *Proc Natl Acad Sci USA* 91:12574–12578.
12 Kim DH, et al. (2002) mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* 110:163–175.
13 Hara K, et al. (2002) Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell* 110:177–189.
14 Kim DH, et al. (2003) GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. *Mol Cell* 11:895–904.
15 Hunter T (1995) When is a lipid kinase not a lipid kinase? When it is a protein kinase. *Cell* 83:1–4.
16 Brunn GJ, et al. (1997) Phosphorylation of the translational repressor PHAS-I by the mammalian target of rapamycin. *Science* 277:99–101.
17 Hara K, et al. (1997) Regulation of eIF-4E BP1 phosphorylation by mTOR. *J Biol Chem* 272:26457–26463.
18 Burnett PE, Barrow RK, Cohen NA, Snyder SH, Sabatini DM (1998) RAFT1 phosphorylation of the translational regulators p70 S6 kinase and 4E-BP1. *Proc Natl Acad Sci USA* 95:1432–1437.

19 Wolfson RL, Sabatini DM (2017) The dawn of the age of amino acid sensors for the mTORC1 pathway. *Cell Metab* 26:301–309.
20 González A, Hall MN (2017) Nutrient sensing and TOR signaling in yeast and mammals. *EMBO J* 36:397–408.
21 Costa-Mattioli M, Monteggia LM (2013) mTOR complexes in neurodevelopmental and neuropsychiatric disorders. *Nat Neurosci* 16:1537–1543.
22 Dibble CC, Cantley LC (2015) Regulation of mTORC1 by PI3K signaling. *Trends Cell Biol* 25:545–555.
23 Gingras AC, Kennedy SG, O'Leary MA, Sonenberg N, Hay N (1998) 4E-BP1, a repressor of mRNA translation, is phosphorylated and inactivated by the Akt(PKB) signaling pathway. *Genes Dev* 12:502–513.
24 Hay N, Sonenberg N (2004) Upstream and downstream of mTOR. *Genes Dev* 18:1926–1945.
25 Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 307:1098–1101.
26 Rodrik-Outmezguine VS, et al. (2016) Overcoming mTOR resistance mutations with a new-generation mTOR inhibitor. *Nature* 534:272–276.
27 Pause A, et al. (1994) Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5′-cap function. *Nature* 371:762–767.
28 Lin TA, et al. (1994) PHAS-I as a link between mitogen-activated protein kinase and translation initiation. *Science* 266:653–656.
29 Gingras AC, Raught B, Sonenberg N (2001) Regulation of translation initiation by FRAP/mTOR. *Genes Dev* 15:807–826.
30 Barbet NC, et al. (1996) TOR controls translation initiation and early G1 progression in yeast. *Mol Biol Cell* 7:25–42.
31 Beretta L, Gingras AC, Svitkin YV, Hall MN, Sonenberg N (1996) Rapamycin blocks the phosphorylation of 4E-BP1 and inhibits cap-dependent initiation of translation. *EMBO J* 15:658–664.
32 Gingras AC, et al. (2001) Hierarchical phosphorylation of the translation inhibitor 4E-BP1. *Genes Dev* 15:2852–2864.
33 Cosentino GP, et al. (2000) Eap1p, a novel eukaryotic translation initiation factor 4E-associated protein in Saccharomyces cerevisiae. *Mol Cell Biol* 20:4604–4613.
34 Bhat M, et al. (2015) Targeting the translation machinery in cancer. *Nat Rev Drug Discov* 14:261–278.
35 Iadevaia V, Huo Y, Zhang Z, Foster LJ, Proud CG (2012) Roles of the mammalian target of rapamycin, mTOR, in controlling ribosome biogenesis and protein synthesis. *Biochem Soc Trans* 40:168–172.
36 Lahr RM, et al. (2017) La-related protein 1 (LARP1) binds the mRNA cap, blocking eIF4F assembly on TOP mRNAs. *eLife* 6:6.