Environmental signaling through the mechanistic target of rapamycin complex 1
mTORC1 goes nuclear

Jason J Workman, Hongfeng Chen, and R Nicholas Laribee*

Department of Pathology and Laboratory Medicine and Center for Cancer Research; University of Tennessee Health Science Center; Memphis, TN USA

Mechanistic target of rapamycin complex 1 (mTORC1) is a well-known regulator of cell growth and proliferation in response to environmental stimuli and stressors. To date, the majority of mTORC1 studies have focused on its function as a cytoplasmic effector of translation regulation. However, recent studies have identified additional, nuclear-specific roles for mTORC1 signaling related to transcription of the ribosomal DNA (rDNA) and ribosomal protein (RP) genes, mitotic cell cycle control, and the regulation of epigenetic processes. As this area of study is still in its infancy, the purpose of this review to highlight these significant findings and discuss the relevance of nuclear mTORC1 signaling dysregulation as it pertains to health and disease.

Introduction and Overview of Cell–Environment Interactions

Eukaryotic cells, whether they be single-celled organisms such as yeasts or components of multi-cellular organisms such as humans, rapidly respond to changes in their environment, so as to readily adapt to such perturbations and maintain homeostasis. Nowhere is this process more apparent than the relationship between a cell's nutrient environment and the molecular signals controlling cell growth, proliferation, and development. A constantly changing nutrient environment (single-celled microorganisms) or changes in mitogen or growth factor availability (metazoans) necessitate the presence of complex molecular signaling pathways that interpret these environmental inputs and then propogate this information to the transcriptional and translational machinery responsible for mediating the appropriate cellular response. Deregulated signaling through these pathways negatively influences cell function and can directly contribute to many diseases, including cancer, cardiovascular disease, obesity, and diabetes.[1] Therefore, defining how environmental information is sensed and transmitted to impact cell growth will be key to understanding how axis malfunction can result in pathogenesis. The mechanistic target of rapamycin (mTOR) pathway is a key transmitter of nutrient information to the translational, transcriptional, and cell cycle-regulatory machinery and is highly conserved from yeast to man.[1,2] This pathway is fundamentally important to eukaryotic cell biology, yet how mTOR controls the numerous downstream processes necessary for cells to alter and adapt to their environment remains largely unknown. This review will briefly highlight the main components of the mTOR pathway, focusing specifically on the mTOR complex 1 (mTORC1) branch, as it is directly regulated by nutrient availability. It will then discuss in-depth, the relatively unappreciated roles for mTORC1 signaling in nuclear-localized processes, specifically focusing on transcription mechanisms governing ribosome biogenesis, its novel connections to mitosis, and the emerging links between mTORC1 and epigenetic regulation. Because the majority of the mTORC1 signaling pathway has been defined in budding yeast and mammalian cells, we will predominantly cite studies utilizing these 2 model systems. However, studies from other model systems will be included whenever appropriate.

Composition and Function of the mTORC1 Signaling Pathway

Tor protein kinases were originally identified as the molecular target of the immunosuppressant macrolide, rapamycin, derived from the Easter Island soil bacterium *Streptomyces hygroscopicus*.[3] Initial studies demonstrated that rapamycin could induce early G_1 cell cycle arrest in a variety of model systems, including the budding yeast *Saccharomyces cerevisiae* and mammalian

*Correspondence to: R Nicholas Laribee; Email: rlaribee@uthsc.edu

cell culture models, phenocopying the reversible cell cycle arrest observed in nutrient-deprived cells.[4,5] Subsequent yeast genetic screens identified 2 gene products as the target for rapamycin's anti-proliferative effects, and these genes were aptly named the target of rapamycin 1 and 2 (TOR1/2) genes.[6] Shortly thereafter, other laboratories confirmed the existence of a single, rapamycin-sensitive, kinase homolog in mammalian cells dubbed mTor (initially RAFT/FRAP).[7,8] Genetic, biochemical and genome sequencing studies have since verified the presence of Tor kinase homologs in all eukaryotic organisms and demonstrated their essential nature in promoting cell growth and proliferation in response to nutrients, growth factors, and energy levels. Although originally identified as a candidate antifungal, rapamycin has since been repurposed as an immunosuppressant and anti-cancer agent due to its conserved anti-proliferative activity in human cells (reviewed in Benjamin et al.).[9]

Tor kinases belong to a family of atypical serine/threonine kinases that exhibits homology to the PI3 lipid kinase and includes ATM (yeast Tel1), ATR (yeast Mec1), DNA protein kinase (DNAPK), and human suppressor of morphogenesis in genitalia-1 (hSMG-1) (reviewed in Lovejoy et al.).[10] Identification of direct Tor kinase substrates has been hampered significantly by the lack of a known consensus phosphorylation sequence. However, in recent years a number of critical mTORC1 targets have been identified, and their contribution to downstream TORC1-regulated processes are currently being defined. In yeast, the Sch9 protein kinase (functionally homologous to S6K1 and Akt/PKB kinases) is the best-characterized mTORC1 substrate. mTORC1-dependent Sch9 phosphorylation leads to its activation and the regulation of some, but not all, mTORC1 downstream functions.[11] These processes include key aspects of ribosome biogenesis, transcription, translation, and cell cycle regulation.[11-13] The Tap42 subunit of the protein phosphatase PP2A is also phosphorylated by mTORC1, yet its role as a downstream effector of mTORC1 remains poorly understood.[14-16] Studies in mammalian cell culture models have been more successful in identifying direct substrates. While enumerating the roles of these specific factors in the TORC1 pathway is outside the focus of this review, a number of these substrates, including S6K1, 4E-BP1, and the transcription factor STAT3, regulate key aspects of growth, proliferation, and differentiation.[17-19]

The Tor kinases function as members of 1 of 2 distinct complexes, mTORC1 or mTOR complex 2 (mTORC2).[20] mTORC1 and mTORC2 are highly conserved, both structurally and functionally, from yeast to mammals; however, only mTORC1 is directly inhibited by rapamycin.[20] Mammalian mTORC1 includes the mTor kinase, Raptor, mLst8/GβL, and the unique, non-conserved inhibitory subunits, DEP domain containing mTOR interacting protein (DEPTOR) and proline-rich Akt substrate of 40-kDa (PRAS40).[1] In yeast, mTORC1 consists of Kog1 (Raptor), Lst8 (mLst8/GβL), Tco89 and either of the 2 paralogous kinases, Tor1 or Tor2.[2] While mTORC1 is directly regulated by nutrient signals (discussed below), mTORC2's nutrient responsiveness is only indirect and depends on prior mTORC1 activation. Regarding mTORC2, the reader is referred to a recent review concerning its biological functions.[21]

Cell growth and division is an energetically taxing process requiring the commitment of significant cellular resources to faithfully duplicate not only the genome, but all of the other essential constitutents necessary for cell viability, such as mitochondria, lysomes/vacuoles, as well as all the necessary anabolic machinery (i.e., ribosomes). An inability to coordinate the commitment to cell division with the availability of nutrients and energy states necessary to sustain biomass accumulation could have disastrous downstream consequences on cell viability. Accordingly, cells have evolved complex mechanisms to synchronize growth and division with their nutrient environment, and a central regulator of this process is mTORC1.[22] mTORC1 is activated by a number of stimuli, including mitogens, growth factors, amino acids, and energy states, while it is also repressed by environmental stressors such as hypoxia and genotoxins.[1] We will briefly describe amino acid and mitogen/growth factor signaling, as these are both the most topical and best-characterized mTORC1 stimuli.

mTORC1 activation by amino acid sufficiency

The mechanisms by which amino acids stimulate mTORC1 have only recently been identified, but a remarkable degree of conservation has already been observed between yeast and mammals. In yeast, the amino acid-sensing EGO complex localizes primarily to the vacuolar surface and consists of the scaffold protein Ego1, a homodimer of Ego3, and a heterodimer of the Rag family GTPases, Gtr1 and Gtr2.[23,24] Under adequate amino acid levels, the guanine nucleotide exchange factor (GEF) activity of Vam6 is stimulated, and the GTPase activating protein (GAP) activity of the recently described SEACIT complex is inhibited, resulting in a GTP-bound Gtr1 and a GDP-bound Gtr2.[25,26] In this active configuration, the GTPases bridge the interaction between EGO and mTORC1 at the vacuolar surface through contacts with subunits Kog1 and Tco89.[25,27] A similar mechanism is seen in mammals with the pentameric lysosomal complex Ragulator, except that while in yeast mTORC1 typically remains associated with the vacuole regardless of amino acid availability, mammals have integrated mTORC1 recruitment to the lysosome as a secondary level of control.[28-30] Ragulator contains a scaffolding subunit in p18 (LAMTOR1), a heterodimer of p14 (LAMTOR2) and MP1 (LAMTOR3) that is functionally orthologous to Ego3, and a poorly understood, recently discovered heterodimer of C7orf59 (LAMTOR4) and HBXIP (LAMTOR5).[29,31-34] Ragulator also associates with 2 Rag family GTPases, RagA/B and RagC/D.[29] When amino acids are abundant, the vacuolar ATPase (v-ATPase) interacts with Ragulator to promote Ragulator's GEF activity toward the Rag GTPases, while the RagA/B and RagC/D GAP activities of the GATOR1 and the FLCN-FNIP1/2 complexes are diminished and activated, respectively.[34-37] These inputs result in a GTP-bound form of RagA/B and a GDP-bound RagC/D, which is the active signaling form of the Rag heterodimer complex.[38] The Rags then link mTORC1 to the lysosome through associations with Raptor (Kog1 ortholog), resulting in mTORC1 activation and downstream phosphorylation of mTORC1 substrates.[30]

mTORC1 activation by mitogen/growth factor signaling

Metazoans have evolved additional layers of mTORC1 regulation to allow coordination of an individual cell's metabolism

within the context of the entire organism. Specifically, mTORC1 is activated by extracellular signals relayed by mitogens and/or growth factors, such as insulin, to promote cell growth and proliferation. Typically, mitogen/growth factor signaling leads to the activation of the membrane-associated phosphoinositide-3-kinase (PI3K), which then triggers the production of phosphoinositide-3-phosphate (PtdIns[3,4,5]P_3) and, subsequently, activation of the Akt/PKB kinase.[39] Akt activation results in the phosphorylation of PRAS40, which relieves its inhibitory effect on mTORC1 signaling.[40] Work in *D. melanogaster* demonstrated that Akt/PKB also activates mTORC1 by phosphorylating components of the tuberous sclerosis (TSC) complex.[41] TSC is a heterodimer of the Tsc1 and Tsc2 proteins and functions as a GAP for the Rheb GTPase, a positive upstream regulator of mTORC1.[42,43] By promoting hydrolysis of Rheb-GTP to Rheb-GDP, TSC suppresses Rheb-dependent mTORC1 activation.[43] Conversely, Akt-mediated TSC phosphorylation inhibits the complex's GAP activity, ultimately resulting in elevated mTORC1 signaling. A negative feedback mechanism also exists by which the downstream mTORC1 effector ribosomal S6 kinase 1 (S6K1) can target the insulin receptor substrate 1 (IRS-1) to downregulate Akt/PKB signaling under conditions of persistent nutrient signaling.[44-46] Other key mTORC1 activators, including the ERK and S6K1 kinases, have also been reported to phosphorylate the TSC complex and alleviate its negative regulation of Rheb function, thus providing additional links between mTORC1 and other cell growth and proliferation pathways.[47,48]

The Nuclear Functions of mTORC1 Signaling

As mTORC1 is regulated by the environmental stimuli discussed above, it is uniquely qualified to determine if a cell's nutrient environment is conducive to cell growth and proliferation. Surprisingly, the majority of mTORC1 studies have focused predominantly on its cytoplasmic signaling activities, in particular its role in controlling translational responses.[1,2] This emphasis on mTORC1 cytoplasmic signaling mechanisms has been due in large part to publications purporting that the majority of mTORC1 components are cytoplasmic and localized predominantly to the vacuole/lysosomal compartment.[28-30,35,49] However, numerous laboratories have established that mTORC1 components also localize to the nuclear compartment.[49-53] These studies suggest mTORC1 may directly bridge nutrient signaling to nuclear functions, including gene transcription, mitotic regulation, and epigenetic control. From herein, we will focus specifically on these aspects of mTORC1 signaling, as well as how their deregulation may contribute to the development of disease.

Role of mTORC1 in RNA polymerase I- and III-dependent rDNA transcription

Under favorable nutrient and environmental conditions, mTORC1 promotes the transcription of metabolic genes involved in many aspects of cell growth and proliferation.[54] These include most, if not all, genes required for ribosome biogenesis, as well as genes key to both lipid formation and mitochondrial function. To date, the best-characterized direct transcriptional role for Tor kinases is in the transcription of rRNA genes in the

nucleolus. Nucleoli are the largest of the sub-nuclear structures, and they form around the tandem rDNA (rDNA) loci, which are repeated hundreds of times in eukaryotic cells. While the RNA polymerase III (RNAPIII) transcribed 5S rDNA and the RNA polymerase I (RNAPI) transcribed 47S rDNA (35S in yeast) are localized on different chromosomes in mammals, in yeast these genes are contiguous, divergently transcribed, and repeated in a head-to-tail configuration 150–200 times on chromosome 12 to form a single crescent-shaped nucleolus.[55-57] Because ribosome production is a key determinant of cellular biosynthetic capacity and accounts for nearly 60% of total cellular transcription in rapidly growing cells, significant coordination occurs between all 3 RNAPs to maintain ribosomal components in the appropriate stoichiometries.[58,59] This coordination is largely dependent on signaling through mTORC1, as decreased mTORC1 activity rapidly reduces ribosomal protein gene transcription (see below), rDNA transcription, and rRNA maturation in yeast and mammalian cells.[60-64] In both models, Tor kinases are known to be recruited to the 35S/47S and 5S rDNA in a nutrient-dependent fashion.[51,65-67] Although the specific functions of Tor at these promoters remains unclear, Tor kinase recruitment is critical for nutrient-dependent rDNA transcription, which suggests the possibility that Tor may phosphorylate components of the transcriptional machinery on the rDNA. Other subunits of the mammalian mTORC1, including Raptor, are known to localize to the nucleolus, thus suggesting that the entire complex may signal locally to control rDNA transcription and/or rRNA processing.[53]

In yeast, mTORC1 also controls 5S rRNA and tRNA expression by regulating the nuclear localization of the RNAPIII-negative regulator, Maf1. Specifically, mTORC1 activation of Sch9 results in Sch9-dependent Maf1 phosporylation and Maf1 nuclear exclusion, which permits RNAPIII-dependent transcription.[65,68-71] Upon decreased mTORC1 signaling, Maf1 is dephosphorylated, localizes to the nucleus, and actively represses 5S rRNA synthesis. mTORC1-dependent negative regulation of Maf1 has also been suggested to include a Sch9-independent pathway, thus providing mTORC1 with multiple means for controlling RNAPIII-dependent transcription.[71] In mammals, the mTor kinase is recruited to RNAPIII-transcribed genes, where it directly phosphorylates Maf1 to prevent Maf1-dependent transcriptional repression.[72] Thus, the regulation of Maf1 function through mTORC1 signaling is a common theme in regulating expression of RNAPIII-transcribed genes. mTORC1 may also indirectly regulate rDNA transcription through altered localization or function of rDNA-specific transcription factors or components of the basal transcriptional apparatus. For example, rapamycin treatment or nutrient starvation in yeast rapidly relocates RNAPI from the nucleolus to the nucleoplasm.[62] This may be due, in part, to effects on the essential and conserved RNAPI transcription factor Rrn3 (TIF-IA in mammals).[73-77] In yeast, inhibition of mTORC1 signaling results in rapid proteasomal degradation of Rrn3, leading to decreased RNAPI recruitment to the rDNA promoter and an overall downregulation of ribosome production.[78] Structural analyses of Rrn3 identified a "serine patch" that, when phosphorylated, prevented association with RNAPI and inhibited rDNA transcription, although the kinase

responsible for phosphorylating Rrn3 has not yet been identified.[79] Mammalian mTor also regulates rRNA transcription in part by directly phosphorylating mammalian TIF-IA.[80] TIF-IA is also phosphorylated by a variety of other kinases besides mTor, including JNK2, ERK, RSK, and AMPK.[80-83] Regulatory inputs from these distinct kinase pathways may serve to couple nutrient-dependent rRNA biogenesis mediated by mTor with other key environmental stimuli, such as mitogen/growth factor availability or the presence of energy stress.

Additional links between mTORC1 and RNAPI/III have recently been described. For example, Todaka and colleagues have shown that mTORC1 upstream amino acid-sensing complexes such as yeast EGO and mammalian Ragulator directly associate with these polymerases via interactions between the Rag-GTPases and the conserved RNAPI/III polymerase subunit Rpc19 (mammalian RPA16).[84] This association is dependent on the nucleotide loading status of the Gtr1/RagA GTPase, such that when in the active (GTP-bound) configuration, they associate to promote downstream RNAPI/III-regulated transcription. However, when they are in the inactive (GDP-bound) state, or cells are *gtr1Δ*, there are deficiencies in rRNA synthesis, RP mRNA production, and RNAPI/III activity. These results suggest these Rag GTPases may promote the association or stability of Rpc19/RPA16 with the core RNAPI/III complex, providing another potential bridge between the mTORC1 pathway and RNAPI/III transcriptional control.

mTORC1 also regulates ribosomal transcription via high mobility group (HMG) proteins, specifically Hmo1 (in yeast) and UBF1/2 (in mammals).[85-87] These HMGs are architectural proteins that bind DNA in a non-sequence specific, but chromatin context-dependent fashion, and bend the DNA to form enhanceosomes that promote ribosomal gene transcription.[88-92] Hmo1 has been suggested to associate with and organize the rDNA repeats in a manner that promotes their high-level transcription in a mTORC1-dependent fashion in lieu of traditional nucleosomes.[93] UBF is a direct target of S6K1 and maintains heterochromatin structure and rDNA stability while preventing extrachromosomal circle formation.[86,87] Like Hmo1, the UBF-dependent enhanceosome has been implicated in rDNA organization, but whether it replaces traditional nucleosomes at the rDNA promoter or binds the DNA crossover junction in existing nucleosomes remains controversial.[94-96] Although UBF is dispensable for rDNA transcription, as its depletion only modestly affects steady-state rRNA transcription, changes to the number of "open" rDNA repeats do occur in its absence, suggesting UBF may control the epigenetic state of rDNA chromatin.[97] These studies implicate specific HMG factors as key regulators of mTORC1-dependent transcriptional processes necessary for ribosome biogenesis.

Intriguingly, recent studies have suggested that the profound block in nascent rRNA synthesis that occurs upon mTORC1 inhibition by rapamycin treatment is actually independent of immediate effects on RNAPI.[78,98] Instead, it was argued that the rapid and robust suppression of RP translation occurring during mTORC1 inhibition prevents rRNA processing events and leads to the sequestration of a subset of ribosomal proteins and

ribosomal biogenesis factors in the nucleolus, resulting in a consequent downregulation of RNAPI-dependent rDNA transcription.[98] Given the direct role of Tor kinase-dependent regulation of RNAPI/III transcription discussed above, further studies exploring the nature of mTORC1-regulated RNAPI/III transcription, and whether reduced mTORC1 signaling affects these processes directly or indirectly through RP translation, seem both necessary and appropriate to fully understand the underlying mechanisms.

mTORC1 and RNA polymerase II-dependent ribosomal protein gene expression

mTORC1 signaling regulates RP gene transcription in all eukaryotes, yet the mechanisms underlying this process have been best characterized in yeast. The Hall and Shore laboratories have identified one particular pathway, which includes the stress-responsive forkhead-like transcription factor Fhl1, its transcriptional co-activator Ifh1, and its co-repressor Crf1.[99-101] During nutrient starvation, the active Yak1 kinase phosphorylates Crf1, resulting in Crf1 accumulation in the nucleus, where it associates with constitutively RP promoter bound Fhl1. The Crf1–Fhl1 interaction outcompetes binding of the transcriptional co-activator Ifh1, resulting in decreased RP gene transcription. Upon increased nutrient availability, mTORC1 is activated and signals to downstream effector kinases, possibly Sch9 or PKA, to inhibit Yak1. Yak1 inhibition prevents nuclear transport of Crf1, thus allowing Ifh1 to interact with Fhl1. In conjunction with the Rap1 transcription factor, RP transcription is then activated.[99,100,102-104] Simultaneously, mTORC1 also promotes the nuclear localization of another RP transcriptional regulator, the split finger protein Sfp1.[105-107] Sfp1 has functional homology to the mammalian c-Myc transcription factor and is known to be a direct kinase target of mTORC1.[108] Importantly, Sfp1 promotes the nuclear localization of Ifh1, which further facilitates mTORC1-dependent RP gene transcription.[106] Interestingly, there also exists a negative feedback mechanism by which Sfp1-dependent, Fhl1-Ifh1 RP gene transcription opposes mTORC1 activity.[108] Besides nutrient availability, Sfp1 activity is also markedly sensitive to environmental stressors and chemical exposure, thus further reinforcing the link between environmental stimuli, mTORC1 signaling, and the regulation of cellular biosynthetic activity through ribosome biogenesis.[109]

Another regulator of RP gene transcription is the previously discussed HMG factor Hmo1, which is bound to the majority of RP gene promoters and contributes to their transcription by recruiting both Fhl1 and Ifh1.[92] Notably, Hmo1 expression is regulated by mTORC1 signaling, thus providing cells with a means to affect transcription of the rDNA and the RP genes simultaneously in response to nutrient stress.[110] mTORC1 also regulates the expression of the ribosome biogenesis (Ribi) genes, which code for proteins that, while required for ribosome production, are not components of the ribosome themselves. Under unfavorable nutrient conditions, the transcriptional repressors Stb3, Dot6, and Tod6 recruit the histone deacetylase complex RPD3L to Ribi promoters, resulting in histone deacetylation and decreased transcription.[12] Upon nutrient stimulation, mTORC1 activates Sch9, which then phosphorylates these transcriptional

repressors and ultimately promotes Ribi gene transcription.[12,111] Given that mTORC1 affects RNA polymerase II-dependent RP gene transcription in part through direct phosphorylation of select transcriptional regulators, determining the consensus phosphorylation sequence for the Tor kinases will thus be critical for identifying the complete spectrum of mTORC1-regulated transcription factors.

mTORC1 as a regulator of cell cycle progression

In addition to controlling transcriptional and translational processes, mTORC1 also contributes to the regulation of the G_1–S and G_2–M cell cycle transitions. The role of mTORC1 is best understood as it relates to G_1- and S-phase control, since anabolic processes are most active during these periods.[2,112-114] Intriguingly, progression through the START phase of the cell cycle in yeast depends on ribosome biogenesis mediated by mTORC1 as a means by which cells ensure sufficient anabolic potential prior to cell cycle commitment. However, the mechanisms underlying this regulation remain largely unknown.[112,113] In this section, we will focus specifically on the role of mTORC1 in mitotic regulation, as the G_2/M transition is only now being recognized as a significant target of the mTORC1 signaling pathway.

Initial studies from Nakashima and colleagues determined that dampened mTORC1 signaling in yeast, either through rapamycin treatment or by the generation of a conditional *kog1* mutant, reduced the rate of progression through G_2/M of the cell cycle.[115] This defect was attributed to reduced activity of the Polo-like kinase Cdc5, as its overexpression rescued the G_2/M-phase defects, and when isolated from cells exhibiting reduced mTORC1 signaling, Cdc5 catalytic activity was impaired. As PP2A regulates the function and localization of Cdc5, part of the effect reduced mTORC1 signaling had on Cdc5 activity was attributed to disruption of the normal mTORC1–PP2A signaling axis. Recent findings from the Tatchell laboratory have further reinforced the link between mTORC1 signaling and mitotic regulation. A genetic screen for yeast mutants that could suppress a temperature-sensitive mutation within the essential mitotic Aurora kinase, Ipl1, identified *tco89Δ*.[116] Loss of Tco89 resulted in reduced nuclear accumulation of the PP1 phosphatase, Glc7, which opposes Ipl1-mediated substrate phosphorylation during mitosis. These studies provide support for a previously unappreciated link between nutrient signaling and the maintenance of genome stability through the control of mitotic chromosome segregation and suggest that mTORC1 may play a greater role in mitosis than is currently appreciated.

Other connections between the mTORC1 pathway and mitosis have been examined in mammalian cells within the last few years as well. For example, specific phosphorylated forms of the mTor kinase are known to co-localize along the spindle mid-zone, suggesting mTor may play a direct role in regulating aspects of mitotic progression and/or cytokinesis.[117] Additionally, recent studies reported that rapamycin treatment promotes phosphorylation and 14-3-3-dependent cytoplasmic sequestration of the CDC25B phosphatase, a critical regulator of the G_2/M-phase checkpoint.[118,119] Dissociation of the CDC25B:14-3-3 complex requires Cdk2 activity and promotes entry into mitosis.[119]

Interestingly, 14-3-3 proteins also bind both phosphorylated histone H3 serine 10 and 28, which are well-characterized marks of mitotic chromatin.[120] In yeast, histone H3S28 mutants that prevent phosphorylation exhibit significant rapamycin sensitivity, suggesting that phosphorylation of this residue may be functionally linked to mTORC1 signaling during mitosis.[121] A recent study from Smith and colleagues in mammalian cells has determined that the Cdk1–cyclin B complex, another key regulator of mitotic progression, mediates protein synthesis during mitosis by controlling the activity of the eukaryotic elongation factor kinase eEF2K.[122] Cdk1–cyclin B activity was decreased by amino acid starvation and activated by deletion of Tsc2, suggesting that mTORC1 is a direct regulator of Cdk1–cyclin B activity. Whether mTORC1-dependent Cdk1–cyclin B regulation contributes to other aspects of mitotic progression attributed to the Cdk1–cyclin B complex remains unknown. Interestingly, the mTORC1 subunit Raptor is a known Cdk1–cyclin B substrate, which further strengthens the link between mTORC1 and mitotic regulation.[123] mTORC1 also controls Cdk1/cyclin B activity in part by regulating cyclin B mRNA stability in yeast.[124] Specifically, the Dbf2 kinase phosphorylates the arginine methyltransferase, Hmt1, which leads to Hmt1-dependent methylation of mRNA binding proteins that specifically stabilize *CLB2* (cyclin B) mRNA. Upon rapamycin treatment or nutrient starvation, the PP2A phosphatase, Pph22, is activated and dephosphorylates Hmt1. As a result, there is destabilization of *CLB2* mRNA transcripts and significantly delayed accumulation of protein, ultimately slowing transit through anaphase and the completion of mitosis. Taken together, these studies suggest that mammalian mTORC1 has an important, yet poorly understood, role in mitosis that involves interactions with the both the CDC25B and Cdk1–cyclin B signaling cascades.

Recent studies have established that a key balance exists between mTORC1 signaling, the progression of the cell cycle, and cellular aging.[125,126] For example, when mammalian cells are serum starved or lack sufficient oxygen tension (hypoxia), mTORC1 signaling is downregulated, while autophagy is activated. Under these conditions, growth and proliferation ceases, and cells enter a reversible quiescent state which can be exited when environmental conditions improve. Intriguingly, cells arrested in the cell cycle, either through expression of the p21 cyclin-dependent kinase inhibitor or treatment with the DNA damaging agent doxorubicin, undergo an irreversible cell cycle arrest (senescence) when hypertrophic mTORC1 signaling is maintained, a process which is defined as gerogenic conversion (geroconversion).[126] Under these same conditions, downregulation of mTORC1 signaling through exposure to hypoxia or rapamycin suppresses the senescence response and instead causes cells to enter into a reversible quiescent state.[126,127] These studies have led to the concept that cellular aging can ultimately be explained as a consequence of hypertrophic signaling mediated by mTORC1, which may also explain why physiological or pharmacological suppressors of mTORC1 activity promote longevity in organisms ranging from yeast to mammals.[125,127,128] Whether the localization of mTORC1 (i.e., cytoplasmic or nuclear) impacts this process remains to be seen. Defining the role of these differentially

localized mTORC1 complexes will be an important issue to address, however, as they may have different biological functions (see below). Taken together, the findings discussed above outline multiple and diverse mechanisms by which mTORC1 signaling can impact cell cycle regulation and the determination of cell fate upon arrest of the cell cycle. Furthermore, they highlight the intriguing link between mTORC1 signaling, cell proliferation and the aging process. While still poorly understood, these processes will be essential to define going forward. Emerging links between mTORC1 and cell cycle control, in particular during mitosis, could suggest that the mTORC1 deregulation detected in most cancers may promote tumorigenesis in part by impacting the fidelity of chromosome segregation.

mTORC1 signaling and the epigenome

Besides the direct effects on RNA polymerases and transcription factor activity/localization described above, recent studies also suggest mTORC1 signaling has an emerging role in regulating the chromatin fiber.[121,129-132] DNA is packaged with the highly basic histone proteins, H2A, H2B, H3, and H4 to form chromatin. Specifically, heterodimers of H3/H4 and H2A/H2B assemble into an octameric complex, around which approximately 147 base pairs of DNA are wrapped, known collectively as the nucleosome.[133] Each histone in the octamer consists of an α-helical globular core that binds DNA, as well as N- and C-terminal extensions (tails) that protrude from this core structure. Both the tail and globular domains can be modified by a diverse set of chemical post-translational modifications that modulate chromatin structure to affect DNA-dependent processes, including gene transcription.[134] Studies of the yeast metabolic cycle from the Tu laboratory recently demonstrated that levels of intracellular acetyl-CoA, the universal donor for all protein acetylation reactions, correlate with nutrient availability and promote histone H3 and H4 acetylation.[129] Importantly, these marked increases in acetylation were detected predominantly at growth-promoting genes, including those coding for ribosomal components and the Cln3 cyclin.[129,130] This provides direct evidence linking environmental nutrient status and expression of pro-growth genes via histone post-translational modifications, although the authors did not specifically address whether nutrient signaling through mTORC1 was involved. These findings fit well with a previous study in yeast from the Cardenas lab that demonstrated the balance between the Esa1 histone acetyltransferase and the Rpd3 histone deacetylase at RP genes is in fact regulated by mTORC1 signaling, thus directly linking mTORC1-dependent chromatin regulation to the control of anabolic growth processes.[131]

A recently completed rapamycin-based chemical genomics screen against a yeast library of histone H3 and H4 mutants performed by our laboratory has provided further support that the mTORC1 pathway exhibits functional interactions with the histone H3/H4 epigenome.[121] In this study, a defined set of H3/H4 amino acid mutants were identified that altered the sensitivity of cells to sub-inhibitory doses of rapamycin in a manner suggesting post-translational modifications at these positions are either regulated by mTORC1 or are required for mTORC1-dependent cell growth and proliferation. Indeed, a subset of the mutants on histone H3 were shown to affect expression of the 5S and 35S rRNA

genes, as well as a model RP gene. Furthermore, this study identified a mutation at histone H3 lysine 37 (H3K37) to be invariably lethal in the context of even modestly impaired mTORC1 signaling, a phenomenon found to be due to the induction of necrosis. The observed necrosis in the H3K37 mutant was linked to the disruption in chromatin association of a subset of HMG factors, suggesting the intriguing possibility that mTORC1 regulates aspects of chromatin structure that promote HMG binding. HMG proteins constitute the largest class of chromatin-associated proteins outside of histones, and many of these factors are key regulators of gene transcription and chromatin function. They do so through their ability to bend DNA as architectural factors, as well as their incorporation into multimeric chromatin regulatory complexes, including the FACT histone chaperone and the INO80 ATP-dependent chromatin remodeling complexes.[88,96,121,135,136] Thus, mTORC1 modulation of HMG chromatin binding could have profound implications on both the 3-dimensional architecture of the genome and the transcriptional profile of the cell. How necrosis is selectively induced under these conditions, and if this involves a direct signaling role for the dislodged factors, or is caused by transcriptional changes associated with disrupted chromatin binding, is currently unclear.

Recently, mTORC1 has also been investigated as an effector of the sirtuin family of histone deacetylases. mTORC1 regulates the rDNA association of the yeast sirtuin Sir2.[137] Under conditions of diminished mTORC1 signaling, Sir2 binds the rDNA, possibly as a component of the RENT complex, leading to a more deacetylated and "closed" rDNA chromatin architecture.[137] In mammals, the sirtuin histone deacetylases SIRT1 and SIRT4 functionally interact with mTORC1, although they do so through different mechanisms. SIRT4 transcriptional regulation is downstream of mTORC1 signaling, and this pathway is a key regulator of glutamine metabolism.[138] Specifically, under nutrient-replete conditions mTORC1 represses transcription of SIRT4 by promoting protesomal degradation of its transcriptional regulator, CREB2. Decreased SIRT4 leads to activation of the glutamine dehydrogenase promoter and conversion of glutamate to α-ketoglutarate. α-ketoglutarate is then fed into the TCA cycle to fuel proliferation. Intriguingly, the expression of SIRT4 is downregulated in many cancers, suggesting that corruption of mTORC1 signaling may enable cancer cell metabolism by altering chromatin. Conversely, SIRT1 functions as a negative effector of mTORC1 signaling through association with the TSC complex, specifically Tsc2.[139] SIRT1 has also been suggested to affect rRNA transcription in response to environmental changes as a member of the energy-dependent nucleolar silencing complex (eNoSC).[140] Since both mTORC1 and sirtuins are known to regulate cellular aging, a further understanding of their functional interrelationships will not only illuminate their connection to cell growth control and oncogenesis, but their contributions to the aging process as well.

All of the previously described links between mTORC1 and histone post-translational modifications would suggest the possibility that mTORC1 signaling via these epigenetic marks may in fact alter gene expression through changes in chromatin structure. This idea is supported by findings from our laboratory that

histone H3 lysine 56 acetylation (H3K56ac), a key regulator of chromatin assembly/disassembly reactions mediated by the histone chaperone Asf1, is regulated by mTORC1 signaling.[132] mTORC1-dependent H3K56ac was demonstrated to directly regulate RNAPI-dependent transcription, as disruption of this modification reduced RNAPI binding across the 35S rDNA. H3K56ac mutants were also found to have increased levels of nascent, non-processed pre-rRNA, which was explained by the reduced rDNA binding of the SSU processome complex and Hmo1, both of which are crucial for RNAPI transcription and rRNA cleavage.[1,85,92,93] Interestingly, deletion of either the Hst3 or Hst4 sirtuin deacetylases rescued the H3K56Ac defect in a mTORC1 mutant background, suggesting mTORC1 may suppress sirtuin function to regulate H3K56ac.[132] Although H3K56ac is conserved in mammalian cells, the role of mTORC1 signaling in its regulation has yet to be determined. Interestingly, decreased mTORC1 activity in yeast has also been shown to promote rapid relocation of RNAPI from the nucleus to the nucleoplasm, as well as increased levels of Rpd3 on the rDNA.[62,141] Deacetylation of the histone H4 residues H4K5 and H4K12 under these conditions enables the condensin complex to bind to the rDNA and promote the chromatin compaction that reduces nucleolar volume and maintains genome stability.[62,142] Whether the RNAPI delocalization is a consequence of transcriptional repression due to Rpd3-dependent histone deacetylation, or is caused by a secondary Rpd3-independent mechanism, remains controversial.[62,143] In contrast to findings in yeast, work in HeLa cells found no gross morphological changes to the nucleolus upon rapamycin treatment; however, decreased mTORC1 signaling does lead to the nucleolar exclusion of the mTORC1 components mTor and Raptor.[61] These studies demonstrating differential mTor nuclear localization due to altered nutrient signaling are particularly interesting, since previous reports utilizing normal and transformed cell lines suggest that transformed cells display a shift toward increased nuclear-localized mTor kinase, as well as delocalization of some mTor substrates, regardless of their nutritional status.[144]

Beyond histone modifications, chromatin structure is also modified through the actions of ATP-dependent chromatin remodeling complexes, the incorporation of histone variants, and the assembly/disassembly of nucleosomes by histone chaperones.[145,146] Many of these chromatin processes are responsive to the cellular metabolic state and, as a consequence, are candidate factors downstream of nutrient and/or growth factor signaling pathways such as mTORC1.[147] Sekiguchi and colleagues reported that loss of EGO subunits is synthetically lethal in settings where the catalytic subunit of the ATP-dependent chromatin remodeling complex INO80 has been mutated, thus implicating the INO80 in mTORC1-dependent chromatin regulation.[148] Additionally, Laxman and Tu identified a number of yeast factors that interact with the mTORC1 subunit Kog1 via mass spectrometry. In this study, they specifically identified the Caf1 subunit of the Ccr4–Not complex, suggesting a functional link between mTORC1 and Ccr4-Not, although the relevance of these interactions were not explored.[149] Intriguingly, early rapamycin-based chemical genomic screening of the systematic yeast deletion collection identified Ccr4–Not mutants to have increased sensitivity to rapamycin, thus further implicating Ccr4-Not in mTORC1 signaling.[150] While still poorly understood, Ccr4-Not is known to regulate every aspect of the gene expression pathway, including gene transcription, epigenetic processes, mRNA export, and cytoplasmic mRNA decay.[151] As such, mTORC1-dependent Ccr4–Not regulation could have significant ramifications on a number of transcriptional and post-transcriptional processes.

Overall, these studies reinforce the concept that mTORC1 signaling has a significant role in integrating environmental nutrient information with downstream effects on epigenetic, transcriptional, and perhaps post-transcriptional gene expression pathways controlling cell growth and proliferation. Given that mTORC1 activity is aberrantly regulated in many diseases, the resulting dysregulation of these downstream processes almost assuredly plays a significant role in promoting or maintaining the disease state. As such, the question of how mTORC1-dependent changes to these epigenetic and transcriptional pathways contribute to disease should remain a major focus of the field moving forward.

Concluding Remarks and Future Endeavors

To date, our understanding of the cellular processes regulated by mTORC1 has focused predominantly on its cytoplasmic signaling function as an overseer of translation. However, it appears now that this representation of mTORC1 signaling is an oversimplification of the pathway's complexity. The emerging data discussed above highlights a significant role for mTORC1 in the nucleus. Thus, the field may only now begin to see the proverbial tip of the iceberg in terms of the full extent of mTORC1's regulatory roles within the cell. Therefore, a concerted effort should be made to define mTORC1's nuclear functions, determine how they control cell growth and proliferation and delineate how their deregulation contributes to diseases such as cancer, cardiovascular disease, and diabetes. To facilitate this goal, we have outlined a few basic concept areas we believe will be important to address in the near future.

The nuclear localization of Tor kinases suggests the possibility that Tor-dependent phosphorylation of a set of nuclear substrates regulates both transcription and mitotic cell cycle regulation. Outside of Maf1, however, the extent of mTor nuclear substrates has been largely unexplored. Because Tor kinases individually, or within the context of the mTORC1 complex, are recruited to specific target genes, components of the RNA polymerase I, II, and III complexes could be significant downstream targets whose phosphorylation would directly couple nutrient signaling to gene expression. Transcriptional co-activators and/or co-repressors, as well as chromatin remodeling complexes, may also be targets for mTORC1-mediated phosphorylation. Furthermore, while current studies implicate mTORC1 in epigenetic regulation, how much of this is direct (i.e., mTORC1-dependent phosphorylation of histone proteins) vs. indirect via the regulation of histone-modifying enzymes, remains unknown. Determining which components of the mitotic machinery are phosphorylated by mTORC1 will also elaborate significantly on its role in cell cycle control and may identify possible mechanisms by which mTORC1 dysregulation contributes to genomic instability and

tumorigenesis. Lastly, it will be important to examine whether nuclear localization of Tor kinase activity is a regulated process that controls mTORC1 cytoplasmic signaling. A recent study has hinted at this possibility, since disrupting mammalian mTor nuclear import was determined to impair downstream S6K1 activation.[52] These data suggest that nuclear compartmentalization of the mTor kinase will likely be a significant regulatory mechanism in the control of mTORC1 cytoplasmic signaling.

Another major area of interest should be to examine how nutrient excess, such as that which occurs in obesity, alters mTORC1 signaling to affect those nuclear processes discussed above. Numerous studies have identified nutrient excess as a risk factor for many diseases, including cancer.[152] Yet it remains unclear how increased nutrient availability promotes all the necessary transcriptional and epigenetic changes needed promote the cancer phenotype. One possibility could be that elevated mTORC1 signaling caused by nutrient excess results in epigenetic and transcriptional changes that induce or propogate the disease state. Furthermore, given the link between mTORC1 and mitotic regulation, it seems that nutrient excess could hyperactivate mTORC1 and perhaps alter chromosome segregation fidelity during mitosis. This situation could induce or perpetuate genomic instability phenotypes that contribute to cancer development. Such a scenario might provide a partial explanation for why most cancers exhibit elevated mTORC1 activity. Aberrant mTORC1 activity would also deregulate ribosome production and elevate anabolic processes, which may facilitate tumorigenesis given the increased metabolic demand of tumors.[153] Since one of the rate-limiting steps in controlling commitment to cell cycle entry is ribosome production, increased ribosome biogenesis also has the potential to promote and/or enhance tumorigenesis by changing cell cycle kinetics.[112]

Finally, there is the hurdle of eventually translating the concepts discussed aboved into clinically relevant therapeutic approaches. To date, the mTORC1 inhibitor rapamycin, and the derivative rapalog compounds, have not lived up to their promise as anticancer agents due to their significant side effects.[9] Additionally, transformed cells treated with the rapalogs stop growing and dividing, but ultimately remain viable as their mechanism of action is generally cytostatic rather than cytotoxic.[9] Recent studies have demonstrated the clinical relevance of targeting key epigenetic and/or transcriptional processes with small-molecule inhibitors in the treatment of diseases ranging from cancer to heart disease.[154,155] If deregulated mTORC1 signaling is in fact a driver of cellular transformation, then pairing pharmacological agents targeting a subset of downstream mTORC1-regulated nuclear processes, such as those reviewed above, with mTORC1 inhibitors may lower the effective dose of mTORC1 inhibitor while simultaneously increasing its therapeutic benefits. Given the vast array of conditions that display aberrant mTORC1 function, this type of combinatorial therapy may lend itself to treating a number of diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell 2012; 149:274-93; PMID:22500797; http://dx.doi.org/10.1016/j.cell.2012.03.017

2. Loewith R, Hall MN. Target of rapamycin (TOR) in nutrient signaling and growth control. Genetics 2011; 189:1177-201; PMID:22174183; http://dx.doi.org/10.1534/genetics.111.133363

3. Vézina C, Kudelski A, Sehgal SN. Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. J Antibiot (Tokyo) 1975; 28:721-6; PMID:1102508; http://dx.doi.org/10.7164/antibiotics.28.721

4. Metcalfe SM, Richards FM. Cyclosporine, FK506, and rapamycin. Some effects on early activation events in serum-free, mitogen-stimulated mouse spleen cells. Transplantation 1990; 49:798-802; PMID:1691537; http://dx.doi.org/10.1097/00007890-199004000-00028

5. Barbet NC, Schneider U, Helliwell SB, Stansfield I, Tuite MF, Hall MN. TOR controls translation initiation and early G1 progression in yeast. Mol Biol Cell 1996; 7:25-42; PMID:8741837; http://dx.doi.org/10.1091/mbc.7.1.25

6. Heitman J, Movva NR, Hall MN. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. Science 1991; 253:905-9; PMID:1715094; http://dx.doi.org/10.1126/science.1715094

7. Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH. RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. Cell 1994; 78:35-43; PMID:7518356; http://dx.doi.org/10.1016/0092-8674(94)90570-3

8. Chiu MI, Katz H, Berlin V. RAPT1, a mammalian homolog of yeast Tor, interacts with the FKBP12/rapamycin complex. Proc Natl Acad Sci U S A 1994; 91:12574-8; PMID:7809080; http://dx.doi.org/10.1073/pnas.91.26.12574

9. Benjamin D, Colombi M, Moroni C, Hall MN. Rapamycin passes the torch: a new generation of mTOR inhibitors. Nat Rev Drug Discov 2011; 10:868-80; PMID:22037041; http://dx.doi.org/10.1038/nrd3531

10. Lovejoy CA, Cortez D. Common mechanisms of PIKK regulation. DNA Repair (Amst) 2009; 8:1004-8; PMID:19464237; http://dx.doi.org/10.1016/j.dnarep.2009.04.006

11. Urban J, Soulard A, Huber A, Lippman S, Mukhopadhyay D, Deloche O, Wanke V, Anrather D, Ammerer G, Riezman H, et al. Sch9 is a major target of TORC1 in Saccharomyces cerevisiae. Mol Cell 2007; 26:663-74; PMID:17560372; http://dx.doi.org/10.1016/j.molcel.2007.04.020

12. Huber A, French SL, Tekotte H, Yerlikaya S, Stahl M, Perepelkina MP, Tyers M, Rougemont J, Beyer AL, Loewith R. Sch9 regulates ribosome biogenesis via Stb3, Dot6 and Tod6 and the histone deacetylase complex RPD3L. EMBO J 2011; 30:3052-64; PMID:21730963; http://dx.doi.org/10.1038/emboj.2011.221

13. Wei Y, Zheng XF. Sch9 partially mediates TORC1 signaling to control ribosomal RNA synthesis. Cell Cycle 2009; 8:4085-90; PMID:19823048; http://dx.doi.org/10.4161/cc.8.24.10170

14. Huber A, Bodenmiller B, Uotila A, Stahl M, Wanka S, Gerrits B, Aebersold R, Loewith R. Characterization of the rapamycin-sensitive phosphoproteome reveals that Sch9 is a central coordinator of protein synthesis. Genes Dev 2009; 23:1929-43; PMID:19684113; http://dx.doi.org/10.1101/gad.532109

15. Di Como CJ, Arndt KT. Nutrients, via the Tor proteins, stimulate the association of Tap42 with type 2A phosphatases. Genes Dev 1996; 10:1904-16; PMID:8756348; http://dx.doi.org/10.1101/gad.10.15.1904

16. Jiang Y, Broach JR. Tor proteins and protein phosphatase 2A reciprocally regulate Tap42 in controlling cell growth in yeast. EMBO J 1999; 18:2782-92; PMID:10329624; http://dx.doi.org/10.1093/emboj/18.10.2782

17. Chung J, Kuo CJ, Crabtree GR, Blenis J. Rapamycin-FKBP specifically blocks growth-dependent activation of and signaling by the 70 kd S6 protein kinases. Cell 1992; 69:1227-36; PMID:1377606; http://dx.doi.org/10.1016/0092-8674(92)90643-Q

18. Dowling RJ, Topisirovic I, Alain T, Bidinosti M, Fonseca BD, Petroulakis E, Wang X, Larsson O, Selvaraj A, Liu Y, et al. mTORC1-mediated cell proliferation, but not cell growth, controlled by the 4E-BPs. Science 2010; 328:1172-6; PMID:20508131; http://dx.doi.org/10.1126/science.1187532

19. Yokogami K, Wakisaka S, Avruch J, Reeves SA. Serine phosphorylation and maximal activation of STAT3 during CNTF signaling is mediated by the rapamycin target mTOR. Curr Biol 2000; 10:47-50; PMID:10660304; http://dx.doi.org/10.1016/S0960-9822(99)00268-7

20. Loewith R, Jacinto E, Wullschleger S, Lorberg A, Crespo JL, Bonenfant D, Oppliger W, Jenoe P, Hall MN. Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Mol Cell 2002; 10:457-68; PMID:12408816; http://dx.doi.org/10.1016/S1097-2765(02)00636-6

21. Cybulski N, Hall MN. TOR complex 2: a signaling pathway of its own. Trends Biochem Sci 2009; 34:620-7; PMID:19875293; http://dx.doi.org/10.1016/j.tibs.2009.09.004

22. Lloyd AC. The regulation of cell size. Cell 2013; 154:1194-205; PMID:24034244; http://dx.doi.org/10.1016/j.cell.2013.08.053

23. Dubouloz F, Deloche O, Wanke V, Cameroni E, De Virgilio C. The TOR and EGO protein complexes orchestrate microautophagy in yeast. Mol Cell 2005; 19:15-26; PMID:15989961; http://dx.doi.org/10.1016/j.molcel.2005.05.020

24. Zhang T, Péli-Gulli MP, Yang H, De Virgilio C, Ding J. Ego3 functions as a homodimer to mediate the interaction between Gtr1-Gtr2 and Ego1 in the ego complex to activate TORC1. Structure 2012; 20:2151-60; PMID:23123112; http://dx.doi.org/10.1016/j.str.2012.09.019

25. Binda M, Péli-Gulli MP, Bonfils G, Panchaud N, Urban J, Sturgill TW, Loewith R, De Virgilio C. The Vam6 GEF controls TORC1 by activating the EGO complex. Mol Cell 2009; 35:563-73; PMID:19748353; http://dx.doi.org/10.1016/j.molcel.2009.06.033

26. Panchaud N, Péli-Gulli MP, De Virgilio C. Amino acid deprivation inhibits TORC1 through a GTPase-activating protein complex for the Rag family GTPase Gtr1. Sci Signal 2013; 6:ra42; PMID:23716719; http://dx.doi.org/10.1126/scisignal.2004112

27. Gong R, Li L, Liu Y, Wang P, Yang H, Wang L, Cheng J, Guan KL, Xu Y. Crystal structure of the Gtr1p-Gtr2p complex reveals new insights into the amino acid-induced TORC1 activation. Genes Dev 2011; 25:1668-73; PMID:21816923; http://dx.doi.org/10.1101/gad.16968011

28. Sturgill TW, Cohen A, Diefenbacher M, Trautwein M, Martin DE, Hall MN. TOR1 and TOR2 have distinct locations in live cells. Eukaryot Cell 2008; 7:1819-30; PMID:18723607; http://dx.doi.org/10.1128/EC.00088-08

29. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell 2010; 141:290-303; PMID:20381137; http://dx.doi.org/10.1016/j.cell.2010.02.024

30. Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, Sabatini DM. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science 2008; 320:1496-501; PMID:18497260; http://dx.doi.org/10.1126/science.1157535

31. Nada S, Hondo A, Kasai A, Koike M, Saito K, Uchiyama Y, Okada M. The novel lipid raft adaptor p18 controls endosome dynamics by anchoring the MEK-ERK pathway to late endosomes. EMBO J 2009; 28:477-89; PMID:19177150; http://dx.doi.org/10.1038/emboj.2008.308

32. Wunderlich W, Fialka I, Teis D, Alpi A, Pfeifer A, Parton RG, Lottspeich F, Huber LA. A novel 14-kilodalton protein interacts with the mitogen-activated protein kinase scaffold mp1 on a late endosomal/lysosomal compartment. J Cell Biol 2001; 152:765-76; PMID:11266467; http://dx.doi.org/10.1083/jcb.152.4.765

33. Schaeffer HJ, Catling AD, Eblen ST, Collier LS, Krauss A, Weber MJ. MP1: a MEK binding partner that enhances enzymatic activation of the MAP kinase cascade. Science 1998; 281:1668-71; PMID:9733512; http://dx.doi.org/10.1126/science.281.5383.1668

34. Bar-Peled L, Schweitzer LD, Zoncu R, Sabatini DM. Ragulator is a GEF for the rag GTPases that signal amino acid levels to mTORC1. Cell 2012; 150:1196-208; PMID:22980980; http://dx.doi.org/10.1016/j.cell.2012.07.032

35. Zoncu R, Bar-Peled L, Efeyan A, Wang S, Sancak Y, Sabatini DM. mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H(+)-ATPase. Science 2011; 334:678-83; PMID:22053050; http://dx.doi.org/10.1126/science.1207056

36. Bar-Peled L, Chantranupong L, Cherniack AD, Chen WW, Ottina KA, Grabiner BC, Spear ED, Carter SL, Meyerson M, Sabatini DM. A Tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. Science 2013; 340:1100-6; PMID:23723238; http://dx.doi.org/10.1126/science.1232044

37. Tsun ZY, Bar-Peled L, Chantranupong L, Zoncu R, Wang T, Kim C, Spooner E, Sabatini DM. The folliculin tumor suppressor is a GAP for the RagC/D GTPases that signal amino acid levels to mTORC1. Mol Cell 2013; 52:495-505; PMID:24095279; http://dx.doi.org/10.1016/j.molcel.2013.09.016

38. Jeong JH, Lee KH, Kim YM, Kim DH, Oh BH, Kim YG. Crystal structure of the Gtr1p(GTP)-Gtr2p(GDP) protein complex reveals large structural rearrangements triggered by GTP-to-GDP conversion. J Biol Chem 2012; 287:29648-53; PMID:22807443; http://dx.doi.org/10.1074/jbc.C112.384420

39. Himpe E, Kooijman R. Insulin-like growth factor-I receptor signal transduction and the Janus Kinase/Signal Transducer and Activator of Transcription (JAK-STAT) pathway. Biofactors 2009; 35:76-81; PMID:19319849; http://dx.doi.org/10.1002/biof.20

40. Wang H, Zhang Q, Wen Q, Zheng Y, Lazarovici P, Jiang H, Lin J, Zheng W. Proline-rich Akt substrate of 40kDa (PRAS40): a novel downstream target of PI3k/Akt signaling pathway. Cell Signal 2012; 24:17-24; PMID:21906675; http://dx.doi.org/10.1016/j.cellsig.2011.08.010

41. Potter CJ, Pedraza LG, Xu T. Akt regulates growth by directly phosphorylating Tsc2. Nat Cell Biol 2002; 4:658-65; PMID:12172554; http://dx.doi.org/10.1038/ncb840

42. Inoki K, Li Y, Xu T, Guan KL. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. Genes Dev 2003; 17:1829-34; PMID:12869586; http://dx.doi.org/10.1101/gad.1110003

43. Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J. Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. Curr Biol 2003; 13:1259-68; PMID:12906785; http://dx.doi.org/10.1016/S0960-9822(03)00506-2

44. Takano A, Usui I, Haruta T, Kawahara J, Uno T, Iwata M, Kobayashi M. Mammalian target of rapamycin pathway regulates insulin signaling via subcellular redistribution of insulin receptor substrate 1 and integrates nutritional signals and metabolic signals of insulin. Mol Cell Biol 2001; 21:5050-62; PMID:11438661; http://dx.doi.org/10.1128/MCB.21.15.5050-5062.2001

45. Haruta T, Uno T, Kawahara J, Takano A, Egawa K, Sharma PM, Olefsky JM, Kobayashi M. A rapamycin-sensitive pathway down-regulates insulin signaling via phosphorylation and proteasomal degradation of insulin receptor substrate-1. Mol Endocrinol 2000; 14:783-94; PMID:10847581; http://dx.doi.org/10.1210/mend.14.6.0446

46. Tremblay F, Marette A. Amino acid and insulin signaling via the mTOR/p70 S6 kinase pathway. A negative feedback mechanism leading to insulin resistance in skeletal muscle cells. J Biol Chem 2001; 276:38052-60; PMID:11498541

47. Roux PP, Ballif BA, Anjum R, Gygi SP, Blenis J. Tumor-promoting phorbol esters and activated Ras inactivate the tuberous sclerosis tumor suppressor complex via p90 ribosomal S6 kinase. Proc Natl Acad Sci U S A 2004; 101:13489-94; PMID:15342917; http://dx.doi.org/10.1073/pnas.0405659101

48. Ma L, Chen Z, Erdjument-Bromage H, Tempst P, Pandolfi PP. Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. Cell 2005; 121:179-93; PMID:15851026; http://dx.doi.org/10.1016/j.cell.2005.02.031

49. Betz C, Hall MN. Where is mTOR and what is it doing there? J Cell Biol 2013; 203:563-74; PMID:24385483; http://dx.doi.org/10.1083/jcb.201306041

50. Kim JE, Chen J. Cytoplasmic-nuclear shuttling of FKBP12-rapamycin-associated protein is involved in rapamycin-sensitive signaling and translation initiation. Proc Natl Acad Sci U S A 2000; 97:14340-5; PMID:11114166; http://dx.doi.org/10.1073/pnas.011511898

51. Li H, Tsang CK, Watkins M, Bertram PG, Zheng XF. Nutrient regulates Tor1 nuclear localization and association with rDNA promoter. Nature 2006; 442:1058-61; PMID:16900101; http://dx.doi.org/10.1038/nature05020

52. Bachmann RA, Kim JH, Wu AL, Park IH, Chen J. A nuclear transport signal of rapamycin is critical for its cytoplasmic signaling to S6 kinase 1. J Biol Chem 2006; 281:7357-63; PMID:16407298; http://dx.doi.org/10.1074/jbc.M512218200

53. Vazquez-Martin A, Cufí S, Oliveras-Ferraros C, Menendez JA. Raptor, a positive regulatory subunit of mTOR complex 1, is a novel phosphoprotein of the rDNA transcription machinery in nucleoli and chromosomal nucleolus organizer regions (NORs). Cell Cycle 2011; 10:3140-52; PMID:21900751; http://dx.doi.org/10.4161/cc.10.18.17376

54. Smets B, Ghillebert R, De Snijder P, Binda M, Swinnen E, De Virgilio C, Winderickx J. Life in the midst of scarcity: adaptations to nutrient availability in Saccharomyces cerevisiae. Curr Genet 2010; 56:1-32; PMID:20054690; http://dx.doi.org/10.1007/s00294-009-0287-1

55. Henderson AS, Warburton D, Atwood KC. Location of ribosomal DNA in the human chromosome complement. Proc Natl Acad Sci U S A 1972; 69:3394-8; PMID:4508329; http://dx.doi.org/10.1073/pnas.69.11.3394

56. Petes TD. Yeast ribosomal DNA genes are located on chromosome XII. Proc Natl Acad Sci U S A 1979; 76:410-4; PMID:370829; http://dx.doi.org/10.1073/pnas.76.1.410

57. Mélèse T, Xue Z. The nucleolus: an organelle formed by the act of building a ribosome. Curr Opin Cell Biol 1995; 7:319-24; PMID:7662360; http://dx.doi.org/10.1016/0955-0674(95)80085-9

58. Warner JR. The economics of ribosome biosynthesis in yeast. Trends Biochem Sci 1999; 24:437-40; PMID:10542411; http://dx.doi.org/10.1016/S0968-0004(99)01460-7

59. Mayer C, Grummt I. Ribosome biogenesis and cell growth: mTOR coordinates transcription by all three classes of nuclear RNA polymerases. Oncogene 2006; 25:6384-91; PMID:17041624; http://dx.doi.org/10.1038/sj.onc.1209883

60. Mahajan PB. Modulation of transcription of rRNA genes by rapamycin. Int J Immunopharmacol 1994; 16:711-21; PMID:7528736; http://dx.doi.org/10.1016/0192-0561(94)90091-4

61. Iadevaia V, Zhang Z, Jan E, Proud CG. mTOR signaling regulates the processing of pre-rRNA in human cells. Nucleic Acids Res 2012; 40:2527-39; PMID:22121221; http://dx.doi.org/10.1093/nar/gkr1040

62. Tsang CK, Bertram PG, Ai W, Drenan R, Zheng XF. Chromatin-mediated regulation of nucleolar structure and RNA Pol I localization by TOR. EMBO J 2003; 22:6045-56; PMID:14609951; http://dx.doi.org/10.1093/emboj/cdg578

63. Powers T, Walter P. Regulation of ribosome biogenesis by the rapamycin-sensitive TOR-signaling pathway in Saccharomyces cerevisiae. Mol Biol Cell 1999; 10:987-1000; PMID:10198052; http://dx.doi.org/10.1091/mbc.10.4.987

64. Zaragoza D, Ghavidel A, Heitman J, Schultz MC. Rapamycin induces the G0 program of transcriptional repression in yeast by interfering with the TOR signaling pathway. Mol Cell Biol 1998; 18:4463-70; PMID:9671456

65. Wei Y, Tsang CK, Zheng XF. Mechanisms of regulation of RNA polymerase III-dependent transcription by TORC1. EMBO J 2009; 28:2220-30; PMID:19574957; http://dx.doi.org/10.1038/emboj.2009.179

66. Tsang CK, Liu H, Zheng XF. mTOR binds to the promoters of RNA polymerase I- and III-transcribed genes. Cell Cycle 2010; 9:953-7; PMID:20038818; http://dx.doi.org/10.4161/cc.9.5.10876

67. Kantidakis T, Ramsbottom BA, Birch JL, Dowding SN, White RJ. mTOR associates with TFIIIC, is found at tRNA and 5S rRNA genes, and targets their repressor Maf1. Proc Natl Acad Sci U S A 2010; 107:11823-8; PMID:20543138; http://dx.doi.org/10.1073/pnas.1005188107

68. Vannini A, Ringel R, Kusser AG, Berninghausen O, Kassavetis GA, Cramer P. Molecular basis of RNA polymerase III transcription repression by Maf1. Cell 2010; 143:59-70; PMID:20887893; http://dx.doi.org/10.1016/j.cell.2010.09.002

69. Michels AA. MAF1: a new target of mTORC1. Biochem Soc Trans 2011; 39:487-91; PMID:21428925; http://dx.doi.org/10.1042/BST0390487

70. Roberts DN, Wilson B, Huff JT, Stewart AJ, Cairns BR. Dephosphorylation and genome-wide association of Maf1 with Pol III-transcribed genes during repression. Mol Cell 2006; 22:633-44; PMID:16762836; http://dx.doi.org/10.1016/j.molcel.2006.04.009

71. Lee J, Moir RD, Willis IM. Regulation of RNA polymerase III transcription involves SCH9-dependent and SCH9-independent branches of the target of rapamycin (TOR) pathway. J Biol Chem 2009; 284:12604-8; PMID:19299514; http://dx.doi.org/10.1074/jbc.C900020200

72. Michels AA, Robitaille AM, Buczynski-Ruchonnet D, Hodroj W, Reina JH, Hall MN, Hernandez N. mTORC1 directly phosphorylates and regulates human MAF1. Mol Cell Biol 2010; 30:3749-57; PMID:20516213; http://dx.doi.org/10.1128/MCB.00319-10

73. Yamamoto RT, Nogi Y, Dodd JA, Nomura M. RRN3 gene of Saccharomyces cerevisiae encodes an essential RNA polymerase I transcription factor which interacts with the polymerase independently of DNA template. EMBO J 1996; 15:3964-73; PMID:8670901

74. Peyroche G, Milkereit P, Bischler N, Tschochner H, Schultz P, Sentenac A, Carles C, Riva M. The recruitment of RNA polymerase I on rDNA is mediated by the interaction of the A43 subunit with Rrn3. EMBO J 2000; 19:5473-82; PMID:11032814; http://dx.doi.org/10.1093/emboj/19.20.5473

75. Buttgereit D, Pflugfelder G, Grummt I. Growth-dependent regulation of rRNA synthesis is mediated by a transcription initiation factor (TIF-IA). Nucleic Acids Res 1985; 13:8165-80; PMID:4070001; http://dx.doi.org/10.1093/nar/13.22.8165

76. Schnapp A, Pfleiderer C, Rosenbauer H, Grummt I. A growth-dependent transcription initiation factor (TIF-IA) interacting with RNA polymerase I regulates mouse ribosomal RNA synthesis. EMBO J 1990; 9:2857-63; PMID:2390974

77. Stepanchick A, Zhi H, Cavanaugh AH, Rothblum K, Schneider DA, Rothblum LI. DNA binding by the ribosomal DNA transcription factor rrn3 is essential for ribosomal DNA transcription. J Biol Chem 2013; 288:9135-44; PMID:23393135; http://dx.doi.org/10.1074/jbc.M112.444265

78. Philippi A, Steinbauer R, Reiter A, Fath S, Leger-Silvestre I, Milkereit P, Griesenbeck J, Tschochner H. TOR-dependent reduction in the expression level of Rrn3p lowers the activity of the yeast RNA Pol I machinery, but does not account for the strong inhibition of rRNA production. Nucleic Acids Res 2010; 38:5315-26; PMID:20421203; http://dx.doi.org/10.1093/nar/gkq264

79. Blattner C, Jennebach S, Herzog F, Mayer A, Cheung AC, Witte G, Lorenzen K, Hopfner KP, Heck AJ, Aebersold R, et al. Molecular basis of Rrn3-regulated RNA polymerase I initiation and cell growth. Genes Dev 2011; 25:2093-105; PMID:21940764; http://dx.doi.org/10.1101/gad.17363311

80. Mayer C, Zhao J, Yuan X, Grummt I. mTOR-dependent activation of the transcription factor TIF-IA links rRNA synthesis to nutrient availability. Genes Dev 2004; 18:423-34; PMID:15004009; http://dx.doi.org/10.1101/gad.285504

81. Mayer C, Bierhoff H, Grummt I. The nucleolus as a stress sensor: JNK2 inactivates the transcription factor TIF-IA and down-regulates rRNA synthesis. Genes Dev 2005; 19:933-41; PMID:15805466; http://dx.doi.org/10.1101/gad.333205

82. Zhao J, Yuan X, Frödin M, Grummt I. ERK-dependent phosphorylation of the transcription initiation factor TIF-IA is required for RNA polymerase I transcription and cell growth. Mol Cell 2003; 11:405-13; PMID:12620228; http://dx.doi.org/10.1016/S1097-2765(03)00036-4

83. Hoppe S, Bierhoff H, Cado I, Weber A, Tiebe M, Grummt I, Voit R. AMP-activated protein kinase adapts rRNA synthesis to cellular energy supply. Proc Natl Acad Sci U S A 2009; 106:17781-6; PMID:19815529; http://dx.doi.org/10.1073/pnas.0909873106

84. Todaka Y, Wang Y, Tashiro K, Nakashima N, Nishimoto T, Sekiguchi T. Association of the GTP-binding protein Gtr1p with Rpc19p, a shared subunit of RNA polymerase I and III in yeast Saccharomyces cerevisiae. Genetics 2005; 170:1515-24; PMID:15937128; http://dx.doi.org/10.1534/genetics.105.042366

85. Berger AB, Decourty L, Badis G, Nehrbass U, Jacquier A, Gadal O. Hmo1 is required for TOR-dependent regulation of ribosomal protein gene transcription. Mol Cell Biol 2007; 27:8015-26; PMID:17875934; http://dx.doi.org/10.1128/MCB.01102-07

86. Sanij E, Hannan RD. The role of UBF in regulating the structure and dynamics of transcriptionally active rDNA chromatin. Epigenetics 2009; 4:374-82; PMID:19717978; http://dx.doi.org/10.4161/epi.4.6.9449

87. Hannan KM, Brandenburger Y, Jenkins A, Sharkey K, Cavanaugh A, Rothblum L, Moss T, Poortinga G, McArthur GA, Pearson RB, et al. mTOR-dependent regulation of ribosomal gene transcription requires S6K1 and is mediated by phosphorylation of the carboxy-terminal activation domain of the nucleolar transcription factor UBF. Mol Cell Biol 2003; 23:8862-77; PMID:14612424; http://dx.doi.org/10.1128/MCB.23.23.8862-8877.2003

88. Putnam CD, Copenhaver GP, Denton ML, Pikaard CS. The RNA polymerase I transactivator upstream binding factor requires its dimerization domain and high-mobility-group (HMG) box 1 to bend, wrap, and positively supercoil enhancer DNA. Mol Cell Biol 1994; 14:6476-88; PMID:7935371

89. Lu J, Kobayashi R, Brill SJ. Characterization of a high mobility group 1/2 homolog in yeast. J Biol Chem 1996; 271:33678-85; PMID:8969238; http://dx.doi.org/10.1074/jbc.271.52.33678

90. Hock R, Furusawa T, Ueda T, Bustin M. HMG chromosomal proteins in development and disease. Trends Cell Biol 2007; 17:72-9; PMID:17169561; http://dx.doi.org/10.1016/j.tcb.2006.12.001

91. Albert B, Colleran C, Léger-Silvestre I, Berger AB, Dez C, Normand C, Perez-Fernandez J, McStay B, Gadal O. Structure-function analysis of Hmo1 unveils an ancestral organization of HMG-Box factors involved in ribosomal DNA transcription from yeast to human. Nucleic Acids Res 2013; 41:10135-49; PMID:24021628; http://dx.doi.org/10.1093/nar/gkt770

92. Hall DB, Wade JT, Struhl K. An HMG protein, Hmo1, associates with promoters of many ribosomal protein genes and throughout the rRNA gene locus in Saccharomyces cerevisiae. Mol Cell Biol 2006; 26:3672-9; PMID:16612005; http://dx.doi.org/10.1128/MCB.26.9.3672-3679.2006

93. Merz K, Hondele M, Goetze H, Gmelch K, Stoeckl U, Griesenbeck J. Actively transcribed rRNA genes in S. cerevisiae are organized in a specialized chromatin associated with the high-mobility group protein Hmo1 and are largely devoid of histone molecules. Genes Dev 2008; 22:1190-204; PMID:18451108; http://dx.doi.org/10.1101/gad.466908

94. Bazett-Jones DP, Leblanc B, Herfort M, Moss T. Short-range DNA looping by the Xenopus HMG-box transcription factor, xUBF. Science 1994; 264:1134-7; PMID:8178172; http://dx.doi.org/10.1126/science.8178172

95. Stefanovsky VY, Pelletier G, Bazett-Jones DP, Crane-Robinson C, Moss T. DNA looping in the RNA polymerase I enhancesome is the result of non-cooperative in-phase bending by two UBF molecules. Nucleic Acids Res 2001; 29:3241-7; PMID:11470882; http://dx.doi.org/10.1093/nar/29.15.3241

96. Hu CH, McStay B, Jeong SW, Reeder RH. xUBF, an RNA polymerase I transcription factor, binds cross-over DNA with low sequence specificity. Mol Cell Biol 1994; 14:2871-82; PMID:8164649

97. Sanij E, Poortinga G, Sharkey K, Hung S, Holloway TP, Quin J, Robb E, Wong LH, Thomas WG, Stefanovsky V, et al. UBF levels determine the number of active ribosomal RNA genes in mammals. J Cell Biol 2008; 183:1259-74; PMID:19103806; http://dx.doi.org/10.1083/jcb.200805146

98. Reiter A, Steinbauer R, Philippi A, Gerber J, Tschochner H, Milkereit P, Griesenbeck J. Reduction in ribosomal protein synthesis is sufficient to explain major effects on ribosome production after short-term TOR inactivation in Saccharomyces cerevisiae. Mol Cell Biol 2011; 31:803-17; PMID:21149576; http://dx.doi.org/10.1128/MCB.01227-10

99. Schawalder SB, Kabani M, Howald I, Choudhury U, Werner M, Shore D. Growth-regulated recruitment of the essential yeast ribosomal protein gene activator Ifh1. Nature 2004; 432:1058-61; PMID:15616569; http://dx.doi.org/10.1038/nature03200

100. Martin DE, Soulard A, Hall MN. TOR regulates ribosomal protein gene expression via PKA and the Forkhead transcription factor FHL1. Cell 2004; 119:969-79; PMID:15620355; http://dx.doi.org/10.1016/j.cell.2004.11.047

101. Wade JT, Hall DB, Struhl K. The transcription factor Ifh1 is a key regulator of yeast ribosomal protein genes. Nature 2004; 432:1054-8; PMID:15616568; http://dx.doi.org/10.1038/nature03175

102. Rudra D, Mallick J, Zhao Y, Warner JR. Potential interface between ribosomal protein production and pre-rRNA processing. Mol Cell Biol 2007; 27:4815-24; PMID:17452446; http://dx.doi.org/10.1128/MCB.02062-06

103. Garbett KA, Tripathi MK, Cencki B, Layer JH, Weil PA. Yeast TFIID serves as a coactivator for Rap1p by direct protein-protein interaction. Mol Cell Biol 2007; 27:297-311; PMID:17074814; http://dx.doi.org/10.1128/MCB.01558-06

104. Planta RJ, Gonçalves PM, Mager WH. Global regulators of ribosome biosynthesis in yeast. Biochem Cell Biol 1995; 73:825-34; PMID:8721998; http://dx.doi.org/10.1139/o95-090

105. Fingerman I, Nagaraj V, Norris D, Vershon AK. Sfp1 plays a key role in yeast ribosome biogenesis. Eukaryot Cell 2003; 2:1061-8; PMID:14555489; http://dx.doi.org/10.1128/EC.2.5.1061-1068.2003

106. Jorgensen P, Rupes I, Sharom JR, Schneper L, Broach JR, Tyers M. A dynamic transcriptional network communicates growth potential to ribosome synthesis and critical cell size. Genes Dev 2004; 18:2491-505; PMID:15466158; http://dx.doi.org/10.1101/gad.1228804

107. Marion RM, Regev A, Segal E, Barash Y, Koller D, Friedman N, O'Shea EK. Sfp1 is a stress- and nutrient-sensitive regulator of ribosomal protein gene expression. Proc Natl Acad Sci U S A 2004; 101:14315-22; PMID:15353587; http://dx.doi.org/10.1073/pnas.0405353101

108. Lempiäinen H, Uotila A, Urban J, Dohnal I, Ammerer G, Loewith R, Shore D. Sfp1 interaction with TORC1 and Mrs6 reveals feedback regulation on TOR signaling. Mol Cell 2009; 33:704-16; PMID:19328065; http://dx.doi.org/10.1016/j.molcel.2009.01.034

109. Hosiner D, Lempiäinen H, Reiter W, Urban J, Loewith R, Ammerer G, Schweyen R, Shore D, Schüller C. Arsenic toxicity to Saccharomyces cerevisiae is a consequence of inhibition of the TORC1 kinase combined with a chronic stress response. Mol Biol Cell 2009; 20:1048-57; PMID:19073887; http://dx.doi.org/10.1091/mbc.E08-04-0438

110. Xiao L, Kamau E, Donze D, Grove A. Expression of yeast high mobility group protein HMO1 is regulated by TOR signaling. Gene 2011; 489:55-62; PMID:21924331; http://dx.doi.org/10.1016/j.gene.2011.08.017

111. Beck T, Hall MN. The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. Nature 1999; 402:689-92; PMID:10604478; http://dx.doi.org/10.1038/45287

112. Bernstein KA, Bleichert F, Bean JM, Cross FR, Baserga SJ. Ribosome biogenesis is sensed at the Start cell cycle checkpoint. Mol Biol Cell 2007; 18:953-64; PMID:17192414; http://dx.doi.org/10.1091/mbc.E06-06-0512

113. Wang X, Proud CG. Nutrient control of TORC1, a cell cycle regulator. Trends Cell Biol 2009; 19:260-7; PMID:19419870; http://dx.doi.org/10.1016/j.tcb.2009.03.005

114. Foster DA, Yellen P, Xu L, Saqcena M. Regulation of G1 Cell Cycle Progression: Distinguishing the Restriction Point from a Nutrient-Sensing Cell Growth Checkpoint(s). Genes Cancer 2010; 1:1124-31; PMID:21779436; http://dx.doi.org/10.1177/1947601910392989

115. Nakashima A, Maruki Y, Imamura Y, Kondo C, Kawamata T, Kawanishi I, Takata H, Matsuura A, Lee KS, Kikkawa U, et al. The yeast Tor signaling pathway is involved in G2/M transition via polo-kinase. PLoS One 2008; 3:e2223; PMID:18493323; http://dx.doi.org/10.1371/journal.pone.0002223

116. Tatchell K, Makrantoni V, Stark MJ, Robinson LC. Temperature-sensitive ipl1-2/Aurora B mutation is suppressed by mutations in TOR complex 1 via the Glc7/PP1 phosphatase. Proc Natl Acad Sci U S A 2011; 108:3994-9; PMID:21368139; http://dx.doi.org/10.1073/pnas.1014406108

117. Vazquez-Martin A, Sauri-Nadal T, Menendez OJ, Oliveras-Ferraros C, Cufí S, Corominas-Faja B, López-Bonet E, Menendez JA. Ser2481-autophosphorylated mTOR colocalizes with chromosomal passenger proteins during mammalian cell cytokinesis. Cell Cycle 2012; 11:4211-21; PMID:23095638; http://dx.doi.org/10.4161/cc.22551

118. Chen RQ, Yang QK, Lu BW, Yi W, Cantin G, Chen YL, Fearns C, Yates JR 3rd, Lee JD. CDC25B mediates rapamycin-induced oncogenic responses in cancer cells. Cancer Res 2009; 69:2663-8; PMID:19276368; http://dx.doi.org/10.1158/0008-5472.CAN-08-3222

119. Margolis SS, Walsh S, Weiser DC, Yoshida M, Shenolikar S, Kornbluth S. PP1 control of M phase entry exerted through 14-3-3-regulated Cdc25 dephosphorylation. EMBO J 2003; 22:5734-45; PMID:14592972; http://dx.doi.org/10.1093/emboj/cdg545

120. Wang F, Higgins JM. Histone modifications and mitosis: countermarks, landmarks, and bookmarks. Trends Cell Biol 2013; 23:175-84; PMID:23246430; http://dx.doi.org/10.1016/j.tcb.2012.11.005

121. Chen H, Workman JJ, Tenga A, Laribee RN. Target of rapamycin signaling regulates high mobility group protein association to chromatin, which functions to suppress necrotic cell death. Epigenetics Chromatin 2013; 6:29; PMID:24044743; http://dx.doi.org/10.1186/1756-8935-6-29

122. Smith EM, Proud CG. cdc2-cyclin B regulates eEF2 kinase activity in a cell cycle- and amino acid-dependent manner. EMBO J 2008; 27:1005-16; PMID:18337751; http://dx.doi.org/10.1038/emboj.2008.39

123. Gwinn DM, Asara JM, Shaw RJ. Raptor is phosphorylated by cdc2 during mitosis. PLoS One 2010; 5:e9197; PMID:20169205; http://dx.doi.org/10.1371/journal.pone.0009197

124. Messier V, Zenklusen D, Michnick SW. A nutrient-responsive pathway that determines M phase timing through control of B-cyclin mRNA stability. Cell 2013; 153:1080-93; PMID:23706744; http://dx.doi.org/10.1016/j.cell.2013.04.035

125. Blagosklonny MV, Hall MN. Growth and aging: a common molecular mechanism. Aging (Albany NY) 2009; 1:357-62; PMID:20157523

126. Demidenko ZN, Blagosklonny MV. Growth stimulation leads to cellular senescence when the cell cycle is blocked. Cell Cycle 2008; 7:3355-61; PMID:18948731; http://dx.doi.org/10.4161/cc.7.21.6919

127. Blagosklonny MV. Hypoxia, MTOR and autophagy: converging on senescence or quiescence. Autophagy 2013; 9:260-2; PMID:23192222; http://dx.doi.org/10.4161/auto.22783

128. Demidenko ZN. Rapamycin for life: a step to immortality. Cell Cycle 2011; 10:4206; PMID:22107962; http://dx.doi.org/10.4161/cc.10.24.18562

129. Cai L, Sutter BM, Li B, Tu BP. Acetyl-CoA induces cell growth and proliferation by promoting the acetylation of histones at growth genes. Mol Cell 2011; 42:426-37; PMID:21596309; http://dx.doi.org/10.1016/j.molcel.2011.05.004

130. Shi L, Tu BP. Acetyl-CoA induces transcription of the key G1 cyclin CLN3 to promote entry into the cell division cycle in Saccharomyces cerevisiae. Proc Natl Acad Sci U S A 2013; 110:7318-23; PMID:23589851; http://dx.doi.org/10.1073/pnas.1302490110

131. Rohde JR, Cardenas ME. The tor pathway regulates gene expression by linking nutrient sensing to histone acetylation. Mol Cell Biol 2003; 23:629-35; PMID:12509460; http://dx.doi.org/10.1128/MCB.23.2.629-635.2003

132. Chen H, Fan M, Pfeffer LM, Laribee RN. The histone H3 lysine 56 acetylation pathway is regulated by target of rapamycin (TOR) signaling and functions directly in ribosomal RNA biogenesis. Nucleic Acids Res 2012; 40:6534-46; PMID:22553361; http://dx.doi.org/10.1093/nar/gks345

133. Luger K, Mäder AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature 1997; 389:251-60; PMID:9305837; http://dx.doi.org/10.1038/38444

134. Zentner GE, Henikoff S. Regulation of nucleosome dynamics by histone modifications. Nat Struct Mol Biol 2013; 20:259-66; PMID:23463310; http://dx.doi.org/10.1038/nsmb.2470

135. Conaway RC, Conaway JW. The INO80 chromatin remodeling complex in transcription, replication and repair. Trends Biochem Sci 2009; 34:71-7; PMID:19062292; http://dx.doi.org/10.1016/j.tibs.2008.10.010

136. Winkler DD, Luger K. The histone chaperone FACT: structural insights and mechanisms for nucleosome reorganization. J Biol Chem 2011; 286:18369-74; PMID:21454601; http://dx.doi.org/10.1074/jbc.R110.180778

137. Ha CW, Huh WK. Rapamycin increases rDNA stability by enhancing association of Sir2 with rDNA in Saccharomyces cerevisiae. Nucleic Acids Res 2011; 39:1336-50; PMID:20947565; http://dx.doi.org/10.1093/nar/gkq895

138. Csibi A, Fendt SM, Li C, Poulogiannis G, Choo AY, Chapski DJ, Jeong SM, Dempsey JM, Parkhitko A, Morrison T, et al. The mTORC1 pathway stimulates glutamine metabolism and cell proliferation by repressing SIRT4. Cell 2013; 153:840-54; PMID:23663782; http://dx.doi.org/10.1016/j.cell.2013.04.023

139. Ghosh HS, McBurney M, Robbins PD. SIRT1 negatively regulates the mammalian target of rapamycin. PLoS One 2010; 5:e9199; PMID:20169165; http://dx.doi.org/10.1371/journal.pone.0009199

140. Murayama A, Ohmori K, Fujimura A, Minami H, Yasuzawa-Tanaka K, Kuroda T, Oie S, Daitoku H, Okuwaki M, Nagata K, et al. Epigenetic control of rDNA loci in response to intracellular energy status. Cell 2008; 133:627-39; PMID:18485871; http://dx.doi.org/10.1016/j.cell.2008.03.030

141. Humphrey EL, Shamji AF, Bernstein BE, Schreiber SL. Rpd3p relocation mediates a transcriptional response to rapamycin in yeast. Chem Biol 2004; 11:295-9; PMID:15123258; http://dx.doi.org/10.1016/j.chembiol.2004.03.001

142. Tsang CK, Li H, Zheng XS. Nutrient starvation promotes condensin loading to maintain rDNA stability. EMBO J 2007; 26:448-58; PMID:17203076; http://dx.doi.org/10.1038/sj.emboj.7601488

143. Sandmeier JJ, French S, Osheim Y, Cheung WL, Gallo CM, Beyer AL, Smith JS. RPD3 is required for the inactivation of yeast ribosomal DNA genes in stationary phase. EMBO J 2002; 21:4959-68; PMID:12234935; http://dx.doi.org/10.1093/emboj/cdf498

144. Zhang X, Shu L, Hosoi H, Murti KG, Houghton PJ. Predominant nuclear localization of mammalian target of rapamycin in normal and malignant cells in culture. J Biol Chem 2002; 277:28127-34; PMID:12000755; http://dx.doi.org/10.1074/jbc.M202625200

145. Hargreaves DC, Crabtree GR. ATP-dependent chromatin remodeling: genetics, genomics and mechanisms. Cell Res 2011; 21:396-420; PMID:21358755; http://dx.doi.org/10.1038/cr.2011.32

146. Das C, Tyler JK, Churchill ME. The histone shuffle: histone chaperones in an energetic dance. Trends Biochem Sci 2010; 35:476-89; PMID:20444609; http://dx.doi.org/10.1016/j.tibs.2010.04.001

147. Vaquero A, Reinberg D. Calorie restriction and the exercise of chromatin. Genes Dev 2009; 23:1849-69; PMID:19608767; http://dx.doi.org/10.1101/gad.1807009

148. Sekiguchi T, Hayashi N, Wang Y, Kobayashi H. Genetic evidence that Ras-like GTPases, Gtr1p, and Gtr2p, are involved in epigenetic control of gene expression in Saccharomyces cerevisiae. Biochem Biophys Res Commun 2008; 368:748-54; PMID:18258182; http://dx.doi.org/10.1016/j.bbrc.2008.01.133

149. Laxman S, Tu BP. Multiple TORC1-associated proteins regulate nitrogen starvation-dependent cellular differentiation in Saccharomyces cerevisiae. PLoS One 2011; 6:e26081; PMID:22043304; http://dx.doi.org/10.1371/journal.pone.0026081

150. Chan TF, Carvalho J, Riles L, Zheng XF. A chemical genomics approach toward understanding the global functions of the target of rapamycin protein (TOR). Proc Natl Acad Sci U S A 2000; 97:13227-32; PMID:11078525; http://dx.doi.org/10.1073/pnas.240444197

151. Miller JE, Reese JC. Ccr4-Not complex: the control freak of eukaryotic cells. Crit Rev Biochem Mol Biol 2012; 47:315-33; PMID:22416820; http://dx.doi.org/10.3109/10409238.2012.667214

152. Pischon T, Nöthlings U, Boeing H. Obesity and cancer. Proc Nutr Soc 2008; 67:128-45; PMID:18412987; http://dx.doi.org/10.1017/S0029665108006976

153. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144:646-74; PMID:21376230; http://dx.doi.org/10.1016/j.cell.2011.02.013

154. Dawson MA, Kouzarides T. Cancer epigenetics: from mechanism to therapy. Cell 2012; 150:12-27; PMID:22770212; http://dx.doi.org/10.1016/j.cell.2012.06.013

155. Flemming A. Cardiology: Bromodomain inhibition halts heart failure. Nat Rev Drug Discov 2013; 12:740; PMID:24080694; http://dx.doi.org/10.1038/nrd4134