Molecular Systems Biology 6; Article number 453; doi:10.1038/msb.2010.108
Citation: ***Molecular Systems Biology*** **6:453**

www.molecularsystemsbiology.com

molecular systems biology

REVIEW

A comprehensive map of the mTOR signaling network

Etienne Caron[1,2], Samik Ghosh[3], Yukiko Matsuoka[3,4], Dariel Ashton-Beaucage[1], Marc Therrien[1,5], Sébastien Lemieux[1,9], Claude Perreault[1,2], Philippe P Roux[1,5,*] and Hiroaki Kitano[3,6,7,8,*]

[1] Institute for Research in Immunology and Cancer, Université de Montréal, Montreal, Canada,
[2] Department of Medicine, Faculty of Medicine, Université de Montréal, Montreal, Quebec, Canada,
[3] The Systems Biology Institute, Minato-ku, Tokyo, Japan,
[4] JST ERATO KAWAOKA Infection-induced Host Responses Project, Tokyo, Japan,
[5] Department of Pathology and Cell biology, Faculty of Medicine, Université de Montréal, Montreal, Quebec, Canada,
[6] Sony Computer Science Laboratories, Shinagawa-ku, Tokyo, Japan,
[7] Division of Systems Biology, Cancer Institute, Tokyo, Japan and
[8] Open Biology Unit, Okinawa Institute of Science and Technology, Kunigami, Okinawa, Japan
[9] Department of Computer Science and Operations Research, Faculty of Arts and Sciences, Université de Montréal, Quebec, Canada
* Corresponding authors. PP Roux, Institute for Research in Immunology and Cancer, Université de Montréal, PO Box 6128 Station Centre-Ville, Montreal, Quebec, Canada H3C 3J7. Tel.: +1 514 343 6399; Fax: +1 514 343 5839; E-mail: philippe.roux@umontreal.ca or H Kitano, The Systems Biology Institute, Falcon Building 5F, 5-6-9 Shirokanedai, Minato, Tokyo 108-0071, Japan. Tel.: +81 3 5789 2181; Fax: +81 3 5789 2182; E-mail: kitano@sbi.jp

Received 13.7.10; accepted 12.11.10

The mammalian target of rapamycin (mTOR) is a central regulator of cell growth and proliferation. mTOR signaling is frequently dysregulated in oncogenic cells, and thus an attractive target for anticancer therapy. Using CellDesigner, a modeling support software for graphical notation, we present herein a comprehensive map of the mTOR signaling network, which includes 964 species connected by 777 reactions. The map complies with both the systems biology markup language (SBML) and graphical notation (SBGN) for computational analysis and graphical representation, respectively. As captured in the mTOR map, we review and discuss our current understanding of the mTOR signaling network and highlight the impact of mTOR feedback and crosstalk regulations on drug-based cancer therapy. This map is available on the *Payao* platform, a Web 2.0 based community-wide interactive process for creating more accurate and information-rich databases. Thus, this comprehensive map of the mTOR network will serve as a tool to facilitate systems-level study of up-to-date mTOR network components and signaling events toward the discovery of novel regulatory processes and therapeutic strategies for cancer.
Molecular Systems Biology **6**: 453; published online 21 December 2010; doi:10.1038/msb.2010.108
Subject Categories: metabolic and regulatory networks; signal transduction
Keywords: cancer; CellDesigner; graphical notation; mTOR; regulatory network

Introduction

The tale of the drug rapamycin and its functional target TOR (target of rapamycin) has attracted monumental scientific and clinical interest over the past few decades. Rapamycin was first identified as a potent antifungal metabolite produced by *Streptomyces hygroscopicus* (Vezina *et al*, 1975), a bacterial strain originally isolated from a soil sample collected on Easter Island in the South Pacific. Surprisingly, rapamycin was subsequently found to possess immunosuppressive and antitumorigenic properties (Chiang and Abraham, 2007; Guertin and Sabatini, 2007). The mode of action of rapamycin involves its interaction with the immunophilin FKbinding protein–12 (FKBP12; Harding *et al*, 1989). Studies in budding yeast determined that an FKBP12–rapamycin complex directly inhibits a ~290 kDa Ser/Thr kinase termed 'target of rapamycin' (TOR) (Heitman *et al*, 1991). Subsequently, the mammalian ortholog of TOR was identified and termed FKBP–rapamycin-associated protein (FRAP) and rapamycin and FKBP12 target (RAFT) (Brown *et al*, 1994; Sabatini *et al*, 1994), and is commonly referred to as mammalian TOR (mTOR). In the last two decades, scientists have used rapamycin to decipher mTOR's complex biological functions, which include the regulation of cell growth, proliferation and survival in response to nutrients, growth factors and hormones (Corradetti and Guan, 2006; Wullschleger *et al*, 2006; Foster and Fingar, 2010). mTOR has also attracted broad interest because of its involvement in many human diseases, including type II diabetes and several types of cancer (Efeyan and Sabatini, 2010). These observations have guided the development of additional mTOR inhibiting drugs (rapalogs and second-generation inhibitors), many of which are currently being evaluated for their therapeutic efficacy (Easton and Houghton, 2006).

Over the past few years, intense efforts have revealed many new mTOR regulatory proteins across a complex network of positive and negative regulatory mechanisms (Dunlop and Tee, 2009; Efeyan and Sabatini, 2010). This increased complexity impacts our ability to interpret and predict the regulation of the mTOR network, which is essential to better understand mTOR-related diseases. To unravel this complexity, computational approaches combined with mathematical modeling

techniques have emerged as a solution (Karlebach and Shamir, 2008). To this end, a crucial task involves the reconstruction of networks in a biologically meaningful manner by manual curation from literature or automated curation from pathway databases (Adriaens *et al*, 2008; Bauer-Mehren *et al*, 2009). Some of these databases represent pathways in computer-readable standard formats, such as biological pathway exchange (BioPAX; www.biopax.org) and systems biology markup language (SBML; Hucka *et al*, 2003), allowing exchange between different software platforms and further processing by network analysis, visualization and modeling tools. However, a recent evaluation conducted to determine the accuracy and completeness of current pathway databases concluded that manual intervention is still needed to obtain a comprehensive and accurate view of a particular signaling network (Bauer-Mehren *et al*, 2009). Furthermore, manual reconstruction of such networks has been shown to be crucial in analyzing and interpreting structural features and global properties of signaling pathways (Oda *et al*, 2005; Kitano and Oda, 2006; Oda and Kitano, 2006; Calzone *et al*, 2008).

On the basis of the current scientific and clinical interest in understanding the precise regulation and function of mTOR, we set out to communicate the mTOR network in both human- and computer-readable formats. Using CellDesigner (http://celldesigner.org), a modeling support software (Funahashi *et al*, 2007), we present a manually assembled map of the mTOR signaling network. This map complies with SBML and the systems biology graphical notation (SBGN) process diagram (Kitano *et al*, 2005; Le Novere *et al*, 2009) for machine readable and graphical representation, respectively. Despite its static nature, a comprehensive map of molecular interactions would serve as a valuable working model to gain a systems-level understanding of the mTOR network. The map would also serve as a useful reference, and greatly help research on mTOR signaling. In this regard, we have reviewed our current understanding of the mTOR signaling network and discuss its particular relevance to cancer therapy. In addition, we elaborate on future directions to ensure a community-based effort in updating the mTOR network accurately through concurrent interventions.

Graphical notations for network representation

Standardizing the visual representation is crucial for more efficient and accurate transmission of biological knowledge between different communities. Recently, a group of biochemists, modelers and computer scientists proposed the SBGN, a visual convention for graphical representation of biological networks (Le Novere *et al*, 2009; www.sbgn.org). The SBGN aims at standardizing a systematic and unambiguous graphical notation and enables software tool support for computational analysis. SBGN defines three complementary types of visual languages: (1) process diagram, (2) entity relationship diagram and (3) activity flow diagram. In order to stimulate implementation of SBGN support and the use of the notation, the symbols used to represent molecules and interactions related to the mTOR signaling network are based on the process diagram (Figures 2 and 3) and the activity flow diagram (Figure 4).

Process diagram

In Figure 1A, the process diagram is exemplified with the TSC1–TSC2 complex, a critical negative regulator of the rapamycin-sensitive mTOR complex-1 (mTORC1). In this diagram, the multiple phosphorylation states of TSC1–TSC2 are represented in a system of active or inactive transitive nodes. Using the unphosphorylated form as the initial node, we show that the TSC1–TSC2 complex is phosphorylated, altering its ability to act as a GAP toward Rheb *in vivo*. More precisely, the catalysis arrows show that the transition of TSC2 from an active to an inactive state is mediated by the protein kinases AKT, ERK1/2, RSK1/2, IKKβ and CDK1, whereas AMPK and GSK3 (glycogen synthase kinase-3) positively regulate TSC1–TSC2 function. In addition, the process diagram language used to illustrate the mTOR signaling network was created using the CellDesigner software, which enables users to store data for each molecule and reaction in the protein and reaction notes, respectively. Moreover, the CellDesigner software allows users to access references that are used for individual reaction using PubMed ID (Figure 1). For the purpose of this review, we used the process diagram language to draw the comprehensive mTOR network map (Figure 2) and to describe upstream regulators of mTORC1 signaling (Figure 3).

Activity flow diagram

The activity flow diagram language is essentially similar to traditional notation used by molecular biologists in the current literature. It depicts the flow of information between biochemical entities in a network and it omits information about the state transitions of entities. As exemplified in Figure 1B, activity flow diagrams show only influences such as 'activation' or 'inhibition' of the TSC1–TSC2 complex by different protein kinases. By ignoring biochemical details of processes in a network, the number of nodes in an activity flow diagram is greatly reduced compared with an equivalent process diagram. In Figure 4, the activity flow diagram has been used to represent a simplified version of the mTOR map based on key factors of the mTOR signaling network.

General characteristics of the mTOR map

In Figure 2, we present a comprehensive map of the mTOR signaling network that was manually assembled based on the published literature. To facilitate map exploration, it was organized in different functional modules, including regulation of mTORC1 and mTORC2, hypoxia, energy content, growth factor, nutrient, Wnt and TNF signaling, ribosome and rRNA biogenesis, cap-dependent translation, autophagy, protein folding, mitochondrial metabolism, cytoskeleton dynamics, transcription and cell cycle. The map was created using CellDesigner 4.0.1 (http://celldesigner.org/), a modeling software tool that enable users to describe molecular interactions using the process diagram language, as described above. Also, the map complies with the SBML, a standard

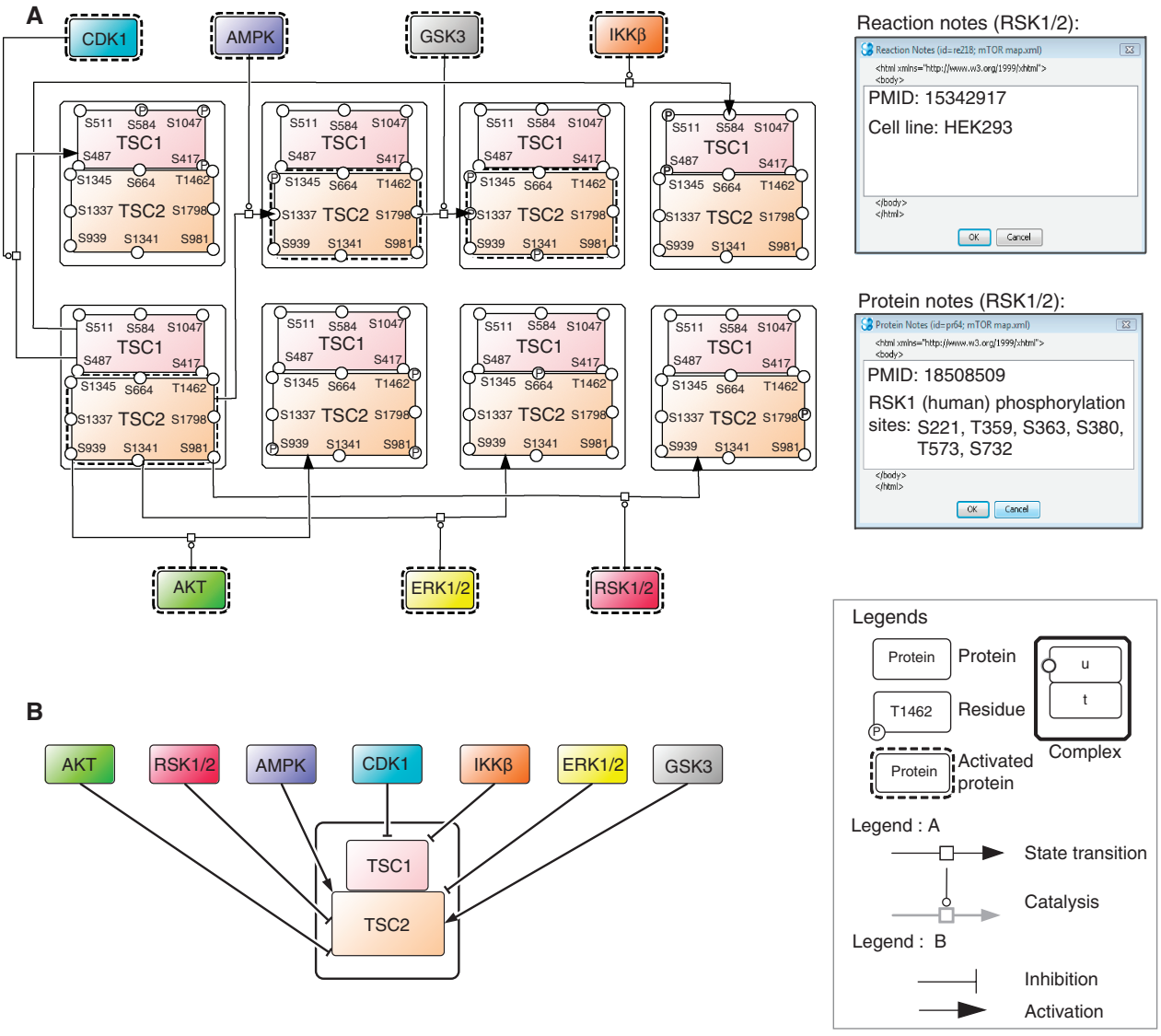


Figure 1 Graphical notations adopted by CellDesigner to illustrate the mTOR signaling network. (**A**) Process diagrams, explicitly displaying different phosphorylated forms of the TSC1–TSC2 complex, as well as the processes of phosphorylation on different serine and threonine residues by AKT, ERK1/2, RSK1/2, CDK1, AMPK, GSK3 and IKKβ. The active state of the molecule is indicated by a dashed line surrounding the molecule. Phosphorylation state of the component is represented with target residues and positions. For individual proteins and reactions, specific notes such as PubMed ID (PMID) were added, enabling a direct link to the relevant references. (**B**) Activity flow diagrams depicting the activation and inhibition of the TSC1–TSC2 complex by the enzymes illustrated in A. In this diagram language, the biochemical details are omitted.

machine-readable format for computational analysis (Hucka *et al*, 2003).

The map comprises 964 species and 777 reactions. 'Species' are defined in SBML as 'an entity that takes part in reactions' and it is used to distinguish the different states that are caused by enzymatic modification, association, dissociation and translocation. In the comprehensive mTOR map, species were categorized as follows: 380 proteins (241 unique proteins), 319 complexes, 20 simple molecules, 80 genes, 87 RNA, 2 ions, 24 degraded products, 6 unknown molecules and 46 phenotypes. Among all species, 602 were located in the cytoplasm and at the plasma membrane, 298 in the nucleus, 22 in endomembranes, 17 in the mitochondria, 12 in Rab7 vesicular structure, 6 in the extracellular environment, 4 at the centrosome and 3 in the ER–golgi network. The reactions were categorized as follows: 210 heterodimer associations, 251 state transitions, 224 known transitions omitted, 43 dissociations, 32 transports and 17 unknown transitions. The criteria for inclusion into the map are similar to those for the previously described maps of the epidermal growth factor receptor (EGFR) and Toll-like receptor (TLR; Oda *et al*, 2005; Oda and Kitano, 2006). The map was manually constructed based on 522 publications available in the Supplementary information section. This approach provides better quality in terms of coverage over major pathway and protein–protein interaction (PPI) databases (Supplementary Table S2; Bauer-Mehren *et al*, 2009). As illustrated in Figure 1, users can directly access references for individual reaction represented in the map.

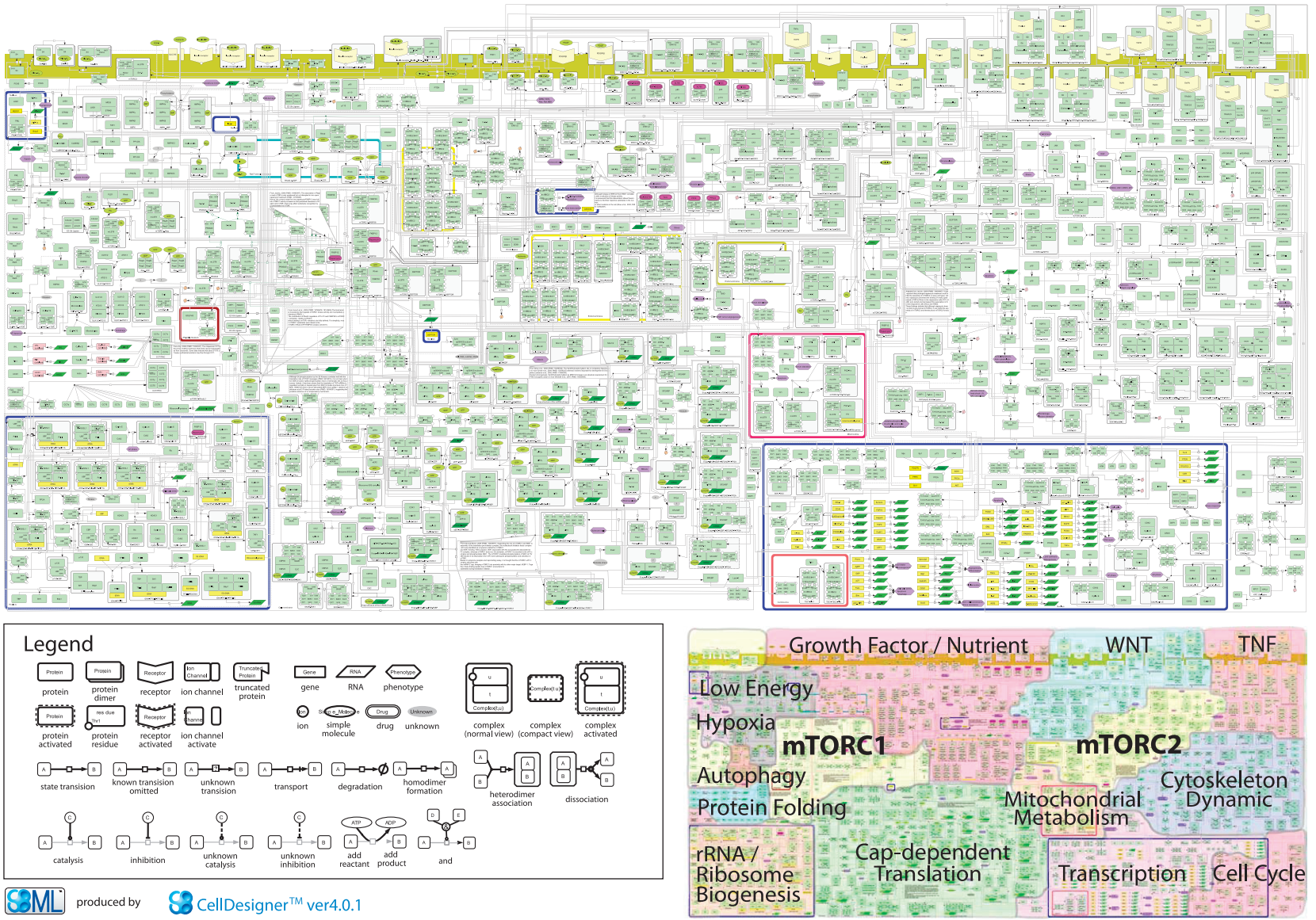


Figure 2 A comprehensive map of the mTOR signaling network. This map was created with CellDesigner version 4.0.1. A total number of 777 reactions and 964 species were included. The SBML and PDF files of the mTOR map are available from the Supplementary information. The map can be best viewed in the PDF format (see Supplementary Figure S3). All of the species, proteins, reactions and cellular compartments included in the map are listed in the SBML file when opened by CellDesigner (http://celldesigner.org/). All of the unique proteins were listed in Supplementary Table S1. A cartoon view of the map (bottom right) depicting different functional modules was drawn to facilitate the exploration of the map. Symbols adopted to build the map are illustrated in the legend. This image is also available as a high resolution pdf.

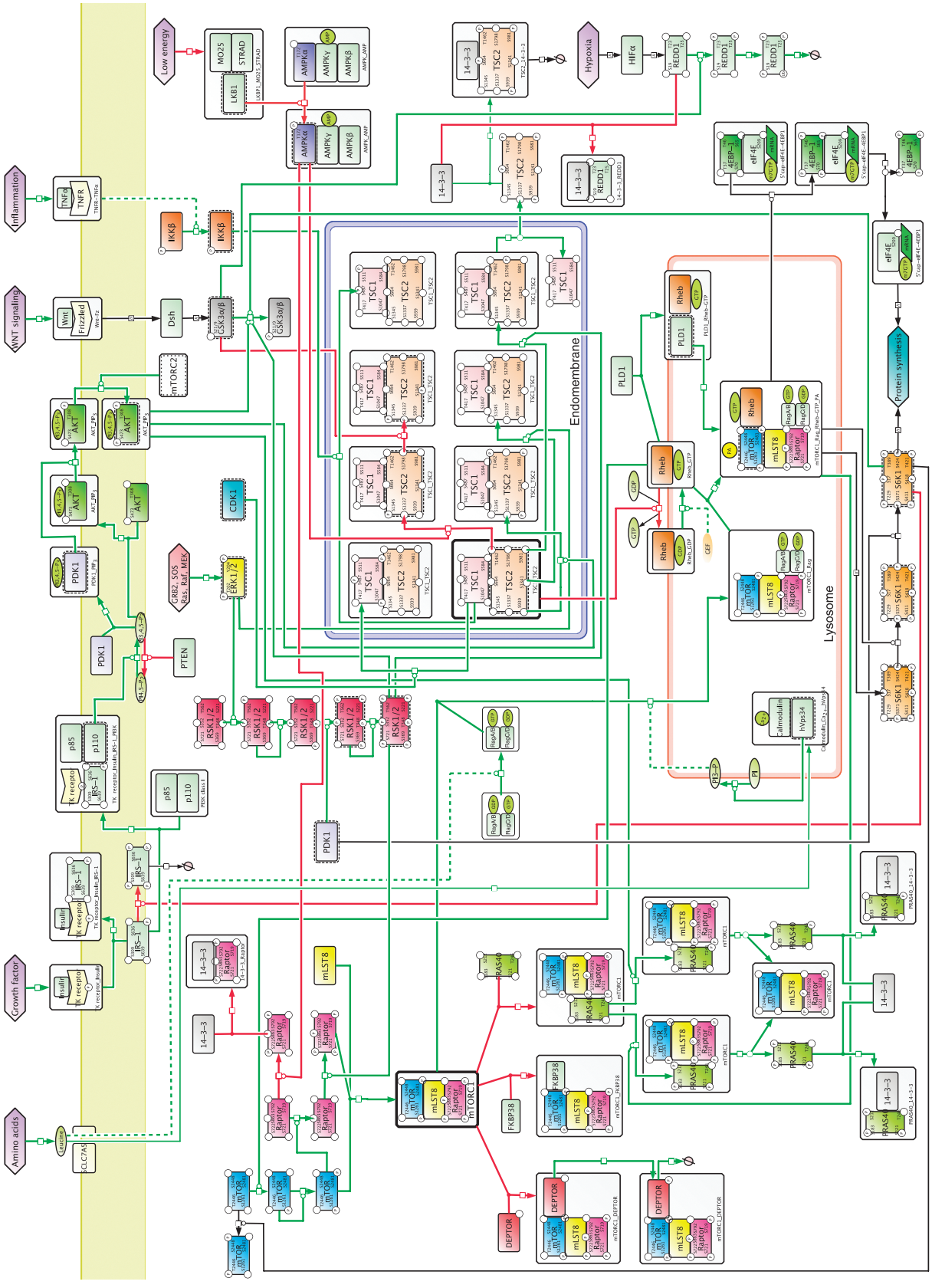


Figure 3 Upstream regulators of mTORC1 signaling. Species, proteins, reactions and cellular compartments involved in mTORC1 signaling were extracted from the comprehensive mTOR map and illustrated using the process diagram language. Green and red reactions indicate activation and inhibition of mTORC1, respectively. Size and color of each component are configurable. Symbols are similar to those used in the legend of the Figure 2. The SBML and PDF files (see Supplementary Figure S4) of mTORC1 signaling are available from the Supplementary information.

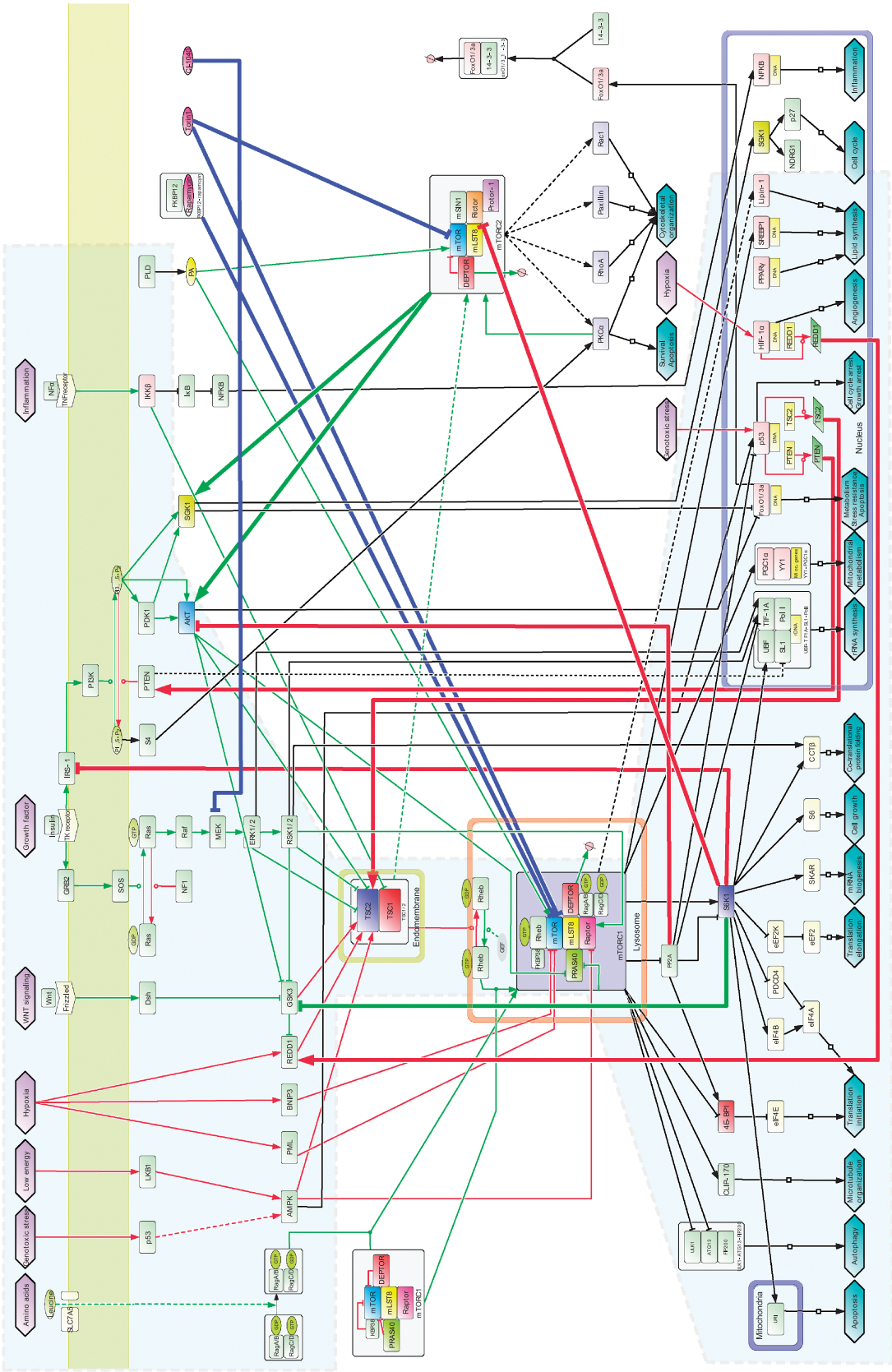


Figure 4 Activity flow of the mTOR signaling network. Central components and reactions were extracted from the comprehensive mTOR map and illustrated using the activity flow diagram language. This reduced map focuses on the flow of activation and inhibition and on the architecture of mTORC1 signaling. Components and signaling events related to mTORC1 consist of a possible bow-tie network (pale blue area). In this network architecture, input signals converge to TSC1–TSC2/Rheb/mTORC1, which forms the bow-tie core of the network. Activities of the core components have important roles in controlling diverse downstream responses. Positive and negative feedback loops in the network are represented by the bold lines. Green and red lines indicate activation and inhibition of mTORC1/2, respectively. The inhibition of mTORC1 by rapamycin, the inhibition mTORC1/2 by the ATP-competitive inhibitor Torin1, and the inhibition of MEK by CI-1040 are represented by the bold blue lines. Symbols are similar to those used in the legend of Figures 1 and 2. The SBML and PDF files (see Supplementary Figure S5) of the reduced mTOR map are available from the Supplementary information.

In order to achieve a better coverage of all proteins involved in the mTOR signaling network and to identify potential modulators, we constructed an mTOR protein interaction network (PIN) from a set of 85 central mTOR pathway proteins and physical interaction data from multiple protein interaction databases using APID2NET (Hernandez-Toro *et al*, 2007; Supplementary Figure S1). In the PIN, we identified 317 additional proteins not present in the current version of the mTOR map. Thus, integration of proteins and reactions from the curated mTOR signaling network and the constructed PIN from public databases offers opportunities to formulate and test new hypotheses. These are essential if we wish to expand the current mTOR map and continuously benefit to the community.

Description of the mTOR signaling network

In the following section, we review and highlight our current knowledge of the mechanisms regulating the mTOR signaling network, as captured in the comprehensive mTOR map (Figure 2). As mentioned earlier, the comprehensive map was organized in functional modules and includes annotations for the specific reactions and molecular species (PubMed ID of the relevant literature, outlinks to gene and protein databases, etc) to facilitate navigation by researchers.

Upstream regulators of mTORC1 signaling

mTORC1 is a rapamycin-sensitive multi-protein complex that contains mTOR, Raptor and mLST8 (also known as GβL; mTORC1 module in Figure 2) (Wullschleger *et al*, 2006; Bhaskar and Hay, 2007). Other proteins have been shown to be part of mTORC1, including FKBP38, PRAS40 and Deptor (Laplante and Sabatini, 2009). Most recent studies also suggest that Raptor binds to PRAS40 (Sancak *et al*, 2007; Yip *et al*, 2010). Raptor positively regulates mTOR activity and is thought to function as a scaffold that recruits mTORC1 substrates (Hara *et al*, 2002; Kim *et al*, 2002), whereas PRAS40, Deptor and FKBP38 negatively regulate mTOR activity (Bai *et al*, 2007; Sancak *et al*, 2007; Vander Haar *et al*, 2007; Peterson *et al*, 2009). The role of mLST8 in mTORC1 function is currently unclear, as the chronic loss of this protein does not affect mTORC1 activity *in vivo* (Guertin *et al*, 2006b). mTORC1 senses and integrates various environmental cues and intracellular signals to regulate cellular processes involved in the promotion of cell growth (Jacinto, 2008). To facilitate interpretation of the major signaling inputs to mTORC1 described below, and to encourage the use of the SBGN notation, we have drawn from the mTOR map a small-scale scheme of mTORC1 signaling events using the SBGN process diagram language (Figure 3). This simplified mTORC1 map comprises 110 species, 77 reactions and 4 cellular compartments, and highlights positive and negative regulatory signals to mTORC1.

mTORC1 signaling is positively regulated by growth factors through the PI3K–AKT pathway (Growth Factor/Nutrient module in Figures 2 and 3). The binding of insulin to its cell surface receptor leads to the recruitment and phosphorylation of IRS-1, which promotes the recruitment and activation of PI3K at the cell surface membrane. Active PI3K converts phosphatidylinositol-4,5-phosphate to phosphatidylinositol-3,4,5-phosphate (PIP3), a process antagonized by the lipid phosphatase PTEN. When produced at the plasma membrane, PIP3 recruits both PDK1 and AKT, resulting in the phosphorylation and partial activation of AKT. Whereas PDK1 phosphorylates AKT at Thr308, additional phosphorylation at Ser473 by mTORC2 (see below) is necessary for optimal activation of AKT *in vitro* (Sarbassov *et al*, 2005). mTORC1 is thought to be activated in part by AKT through the tuberous sclerosis complex proteins, TSC1 and TSC2. The TSC1–TSC2 complex is a critical negative regulator of mTORC1 (Huang and Manning, 2008b). Because of its central role in regulating mTORC1, 34 species depicting extensive details about the TSC1–TSC2 complex (post-translation modifications, interactors, cellular locations) were represented in the comprehensive mTOR map. In response to growth factors, TSC2 is phosphorylated and functionally inactivated by AKT (Inoki *et al*, 2002; Manning *et al*, 2002). ERK1/2 and RSK1/2 were also shown to phosphorylate and inactivate TSC2 in response to growth factors (Roux *et al*, 2004; Ballif *et al*, 2005; Ma *et al*, 2005), suggesting that PI3K and Ras/MAPK pathways collaborate to inhibit TSC1–TSC2 function in response to growth factors. Whereas TSC2 functions as a GAP toward the small Ras-related GTPase Rheb, TSC1 is required to stabilize TSC2 and prevent its proteasomal degradation (Huang and Manning, 2008b). While the active GTP-bound form of Rheb was shown to directly interact with mTOR to stimulate its catalytic activity (Long *et al*, 2005), Rheb may also promote substrate recognition by mTORC1 (Sancak *et al*, 2007; Sato *et al*, 2009).

Nutrients, such as amino acids, regulate mTORC1 signaling via different mechanisms. Amino acid availability regulates mTORC1 in a TSC2-independent but Rheb-dependent manner (Smith *et al*, 2005; Gulati and Thomas, 2007), but the exact mechanism remains poorly understood (Growth Factor/Nutrient module in Figures 2 and 3). Two complementary studies have provided compelling evidence that the Rag family of small GTPases is both necessary and sufficient to transmit a positive signal from amino acids to mTOR (Kim *et al*, 2008; Sancak *et al*, 2008). The current model proposes that amino acids induce the movement of mTORC1 to lysosomal membranes, where Rag proteins reside. More precisely, a complex encoded by the *MAPKSP1*, *ROBLD3* and *c11orf59* genes, interacts with the Rag GTPases, recruits them to lysosomes, and was shown to be essential for mTORC1 activation (Sancak *et al*, 2010).

mTORC1 activity is sensitive to oxygen deprivation, and one pathway by which this occurs involves activation of the TSC1–TSC2 complex by REDD1, a hypoxia-inducible protein (Hypoxia module in Figures 2 and 3l; Brugarolas *et al*, 2004). Newly synthesized REDD1 was found to interact with 14–3–3 and relieve TSC2 from 14–3–3-dependent repression (DeYoung *et al*, 2008). mTORC1 also senses insufficient cellular energy levels through AMPK, a protein kinase activated in response to a low ATP/AMP ratio (Inoki *et al*, 2003) and by LKB1-mediated phosphorylation (Low Energy module in Figures 2 and 3; Lizcano *et al*, 2004; Shaw *et al*, 2004). Upon energy depletion, AMPK phosphorylates and activates TSC2, resulting in the inhibition of mTORC1 (Inoki *et al*, 2003). The GSK3 may also

be involved in the activation of TSC1–TSC2, as it was shown to phosphorylate TSC2 at Ser1337 and Ser1341 in response to AMPK-mediated TSC2 phosphorylation (Wnt module in Figures 2 and 3; Inoki *et al*, 2006). Site-specific phosphorylation of Raptor also has an important role in the activation of mTORC1 in response to energy depletion. Indeed, low energy status promotes Raptor phosphorylation at Ser722 and Ser792 by AMPK, thereby providing binding sites for 14–3–3, which attenuates mTORC1 signaling (mTORC1 module in Figures 2 and 3; Gwinn *et al*, 2008). In contrast, insulin- and Rheb-stimulated mTOR phosphorylates Raptor on Ser863 to promote mTORC1 signaling (Wang *et al*, 2009; Foster *et al*, 2010). The Ras/MAPK pathway activated protein kinase RSK was also shown to directly phosphorylate Raptor on Ser719, Ser721 and Ser722, thereby promoting mTORC1 signaling (Carriere *et al*, 2008a, b). Four mTOR phosphorylation sites have been identified so far: Ser1261, Ser2448, Ser2481 (an autophosphorylation site) and Thr2446 (Foster and Fingar, 2010). While Ser1261 is the only site demonstrated to affect mTOR activity (Acosta-Jaquez *et al*, 2009), phosphorylation of Ser2481 was shown to correlate with the activation status of mTOR (Soliman *et al*, 2010). Phosphorylation of Ser2448 was originally found to be regulated by Akt (Nave *et al*, 1999), but more recent studies demonstrated that S6K1 phosphorylates this site as part of a feedback loop with unknown functions (Chiang and Abraham, 2005; Holz and Blenis, 2005). The phosphorylation status of PRAS40 and Deptor was also found to affect mTORC1 signaling. Indeed, phosphorylation of PRAS40 by both AKT (on Thr246) and mTORC1 (on Ser183, Ser212, Ser221) and subsequent association of PRAS40 with 14–3–3 is critical for the activation of mTORC1 (Fonseca *et al*, 2007; Oshiro *et al*, 2007; Vander Haar *et al*, 2007; Wang *et al*, 2008). Upon activation, mTORC1 also directly phosphorylates Deptor to promote its degradation, which further activates mTORC1 signaling (Peterson *et al*, 2009). Although 13 specific phosphorylation sites have been identified on Deptor (see protein notes from the SBML-format file of the comprehensive mTOR map), functional role for individual phosphorylated residues have yet to be characterized. Collectively, these reports suggest that multiple phosphorylation events on mTORC1 components cooperate to regulate mTORC1 signaling in response to a wide variety of upstream signals.

Downstream targets of mTORC1 signaling

As described above, mTORC1 responds to diverse external and intracellular signals to promote anabolic and inhibit catabolic cellular processes (Foster and Fingar, 2010). Under favorable conditions, mTORC1 promotes protein synthesis, cell growth and proliferation (Ma and Blenis, 2009). Conversely, various cellular stresses inhibit mTORC1 signaling to reduce biosynthetic rates and allow initiation of macroautophagy. To review major output responses from mTORC1, we have drawn a reduced version of the mTOR map based on central mTOR network components using the SBGN activity flow diagram language (Figure 4). This simplified map also highlights the architecture of mTORC1 signaling and underscores the flow of positive and negative regulatory signals, which are briefly discussed in the last section.

mTORC1 regulates protein synthesis via phosphorylation of both S6K1 and 4E-BP1 (Cap-dependent translation module in Figures 2–4). Upon growth factor or nutrient stimulations, mTORC1-mediated phosphorylation of 4E-BP1 induces its dissociation from eIF4E, which enables assembly of the eIF4F complex for competent translation initiation (Ma and Blenis, 2009). When activated by mTORC1, S6K1 phosphorylates both PDCD4 and eIF4B, resulting in activation of the eIF4A RNA helicase, which unwinds secondary structures in the 5′ untranslated region of mRNA to facilitate scanning of the ribosome (Ma and Blenis, 2009). S6K1 also controls translation elongation by regulating the activity of eEF2K. SKAR-mediated recruitment of activated S6K1 also increases the translation efficiency of spliced mRNA during the pioneer round of translation (Ma and Blenis, 2009). While both 4E-BP1 and S6K1 regulate protein synthesis, 4E-BP1 appears to be mainly involved in cell proliferation, while S6K1 regulates cell size (Dowling *et al*, 2010a). Recently, S6K1 and RSK were found to phosphorylate the β-subunit of CCT (Abe *et al*, 2009). CCT is part of a chaperone network linked to protein synthesis and has been shown to facilitate folding of newly translated proteins *in vivo* (Protein Folding module in Figures 2 and 4; Camasses *et al*, 2003; Albanese *et al*, 2006). Although the functional role of S6K1-mediated phosphorylation of CCTβ remains elusive, these findings suggest that mTORC1 may have roles in optimizing folding of newly synthesized proteins during or shortly after translation of polypeptides.

While rapamycin treatment has been shown many years ago to promote macroautophagy in yeast (Noda and Ohsumi, 1998), mTORC1 was recently demonstrated to regulate this process through a protein complex composed of ULK1, ATG13 and FIP200 (Jung *et al*, 2009). Under nutrient-rich conditions, mTORC1 inhibits autophagy through phosphorylation and inactivation of ULK1/2 and ATG13 (Autophagy module in Figures 2 and 4). Upon nutrient starvation or following rapamycin treatment, inactivation of mTORC1 results in the activation of ULK1/2, which promotes phosphorylation of FIP200 and induces macroautophagy (Jung *et al*, 2009). Intriguingly, FIP200 was also shown to interact with the TSC1–TSC2 complex in response to nutrients, resulting in increased S6K1 activity and cell growth (Growth Factor/Nutrient module in Figure 2; Gan *et al*, 2005). Taken together, these observations raise the possibility that FIP200 binds distinct protein complexes both upstream and downstream of mTORC1 to regulate macroautophagy in response to nutrient status.

Gene expression profiling experiments have shown that ~5% of the transcriptome was differentially expressed in response to rapamycin-mediated mTOR inhibition (Guertin *et al*, 2006a), suggesting a role for mTORC1 in regulating gene expression. Additional studies have demonstrated that mTORC1 controls lipid biosynthesis by activating PPAR-γ and SREBP-1, two transcription factors that control expression of many lipogenic and adipogenic genes (Transcription module in Figures 2 and 4) (Laplante and Sabatini, 2009). mTORC1 also targets Lipin1, which was shown to have a key role in adipogenesis by promoting triglyceride synthesis and serving as a coactivator of PPAR-γ (Laplante and Sabatini, 2009). A recent study also identified mTORC1 as an important regulator

of mitochondrial gene expression by altering the physical interaction of YY1 and PGC1-α (Cunningham *et al*, 2007), two transcription factors that have key roles in mitochondrial biogenesis (Mitochondrial Metabolism module in Figures 2 and 4). In the transcription module of the comprehensive mTOR map, we have drawn several mTOR network-related genes that were shown to be differentially expressed in response to various stimulations. Although most regulatory mechanisms involved in mTOR-dependent gene expression remain to be determined, these studies highlight the important roles of mTORC1 in regulating the transcriptome.

Regulation of mTORC2 signaling

The second mTOR complex, mTORC2, controls cell survival, metabolism and proliferation in part by phosphorylating both AKT and SGK1 (mTORC2 module in Figures 2 and 4; Jacinto *et al*, 2006; Guertin *et al*, 2006b; Garcia-Martinez and Alessi, 2008). Some studies have also suggested a role for mTORC2 in the control of cytoskeleton organization by promoting PKCα and paxillin phosphorylation (Cytoskeleton Dynamic module in Figures 2 and 4; Jacinto *et al*, 2004; Sarbassov *et al*, 2004). mTORC2 is comprised of mTOR, Rictor, mSin1, mLST8, and is often accompanied with PROTOR-1/PPR5 and Deptor (Wullschleger *et al*, 2006; Bhaskar and Hay, 2007). Similar to its role in mTORC1 signaling, Deptor negatively regulates mTORC2 activity (Peterson *et al*, 2009). Deletion or knockdown of the mTORC2 components mTOR, Rictor, mSin1 and mLST8 has a dramatic effect on mTORC2 assembly and activation of AKT and SGK1 (Jacinto *et al*, 2006; Guertin *et al*, 2006b). To be fully activated and stabilized, AKT requires phosphorylation of both Ser308 and Ser473 by PDK1 and mTORC2, respectively (Bhaskar and Hay, 2007; Oh *et al*, 2010). While phosphorylation of both AKT sites is required for full AKT activity *in vitro*, Ser473 phosphorylation is dispensable for phosphorylation of most AKT substrates *in vivo* with the exception of FOXO3 and PKCα (Guertin *et al*, 2006b). The mechanisms leading to PDK1-mediated phosphorylation of AKT are well described, but the functional interaction between mTORC2 and AKT is incompletely understood (Jacinto, 2008). One possibility hinges on the presence of a PH-like domain in mSIN1, which would promote the translocation of mTORC2 to the plasma membrane much like what was found for PDK1 (Schroder *et al*, 2007).

In contrast to mTORC1, recent work revealed that the TSC1–TSC2 complex promotes mTORC2 activity (Huang *et al*, 2008a, 2009). Interestingly, a physical interaction between these two complexes was identified, but the exact role for this new interaction is not currently known. mTORC2 is also generally described as being rapamycin insensitive, but it is now becoming apparent that while short-term rapamycin treatment does not inhibit mTORC2, longer treatments suppress the assembly and function of mTORC2 through a mechanism that may involve dephosphorylation and delocalization of Rictor and mSin1 (Sarbassov *et al*, 2006; Rosner and Hengstschlager, 2008). Although we still know very little about the function and regulation of mTORC2, the availability of competitive ATP antagonists against mTOR will likely help deciphering mTORC2-mediated cellular processes.

Inhibition of mTOR in drug-based cancer therapy

Inappropriate amplification of the mTOR signaling pathway, as a result of diverse genetic lesions, is implicated in a variety of human cancers (Shaw and Cantley, 2006). Consequently, mTOR has emerged as a key target for the treatment of cancer. Rapamycin has initially been shown to possess strong cytostatic activities against a wide range of tumor cells *in vitro*, and was found to be effective at suppressing growth of cancer cells *in vivo* (Gibbons *et al*, 2009). A number of clinical trials using rapalogs as anticancer drugs have been performed, and has led to FDA approval in the case of renal cell carcinoma. However, the overall efficacy of rapamycin analogs as single agents for cancer therapy did not meet expectations (Guertin and Sabatini, 2009; Dowling *et al*, 2009). As highlighted in Figure 4, one notable architectural feature of mTORC1 signaling is the possible existence of a bow-tie structure. Typically, bow-tie networks are composed of a highly conserved core part of the network connected by diverse and redundant input and output subnetworks with various feedback control loops (Csete and Doyle, 2004; Oda *et al*, 2005; Oda and Kitano, 2006). In this section, we briefly review key mTOR regulatory mechanisms within the architecture of the mTOR network and discuss their implications in cancer therapy.

Impact of mTOR feedback and crosstalk regulations in rapamycin-based therapy

In recent years, mTORC1 was shown to be involved in the initiation of several negative feedback regulatory mechanisms (Efeyan and Sabatini, 2010; Figure 4). Many of the described feedback and crosstalk reactions have not been confirmed *in vivo* and may be cell and stimuli dependent. Nonetheless, upon growth factor stimulation, mTORC1 activates S6K1, which in turn phosphorylates IRS-1 at the plasma membrane, and ultimately suppresses PI3K-mediated activation of AKT (Manning, 2004; Harrington *et al*, 2005). Rapamycin-mediated mTORC1 inhibition results in the attenuation of this negative feedback loop, leading to increased AKT activity and activation of prosurvival signals, which would be a possible explanation for the relative inefficacy of rapamycin in cancer treatment (Manning, 2004; Harrington *et al*, 2005). Activation of elements in collateral pathways has been observed in the bow-tie architecture of the TLR signaling network (Oda and Kitano, 2006). As illustrated in Figure 4, mTORC1-mediated S6K1 activation might engage mTORC2 in regulating collateral signals that modulate upstream components of the main bow-tie network. Indeed, two independent studies have recently found a new crosstalk between the two mTOR complexes that could also promote cell survival signaling in rapamycin-based cancer therapy. Thus, mTORC2-dependent AKT activation in the upper wing of the bow-tie was found to be negatively regulated through S6K1-mediated phosphorylation of Rictor on Thr1135 (Dibble *et al*, 2009; Julien *et al*, 2010). In addition, inhibition of mTORC1 was found to stimulate Ras-dependent activation of the MAPK pathway, a signaling cascade frequently activated in human cancers (Carracedo *et al*, 2008). While the exact molecular mechanisms underlying this

negative feedback loop remain elusive, these results demonstrate the potential benefit of using combinatorial mTOR and MEK inhibitors for the treatment of certain cancer types.

Recent studies have also shown that rapamycin, previously thought to completely inhibit mTORC1 activity, does not equally suppress mTORC1 signaling to its substrates (Choo *et al*, 2008; Choo and Blenis, 2009; Thoreen *et al*, 2009). While rapamycin treatment completely and sustainably inhibits S6K1 activation, it only partly and variably inhibits 4E-BP1 phosphorylation (Choo *et al*, 2008). Because 4E-BP1 is critically involved in cap-dependent translation via regulation of eIF4E, rapamycin-resistant mTORC1 signaling toward 4E-BP1 is a plausible explanation for failed rapamycin-based cancer therapies.

The mTOR signaling map outlined in this review provides a picture of the intricate maze of regulatory interactions and feedback control loops mediated by mTOR, both within a bow-tie architecture and between components of different pathways.

Combination therapies and second generation of mTOR inhibitors

The implication of mTOR signaling in oncogenesis and the interaction of mTOR with other pathway components suggests the potential benefit of combination therapy (Dancey, 2010). Indeed, properly designed multicomponent therapies are the first step in controlling robustness to achieve clinical efficacy (Kitano, 2007). Efforts to combine the antitumor effects of therapeutic agents targeting different molecular components have been studied by several groups (Nelander *et al*, 2008; Lehar *et al*, 2008a, b). With respect to mTOR, synergisms between rapalogs and conventional therapeutics have already been explored (reviewed in Abraham and Eng, 2008). The ability of mTOR inhibitors to downregulate HIF1 and VEGF has made them interesting combination partners with VEGFR inhibitors, such as Sorafenib and Sunitinib (Hudson *et al*, 2002; El-Hashemite *et al*, 2003). The feedback interaction between MAPK and mTOR pathways also makes them potential combination partners. MEK inhibitors, such as CI-1040, in combination with rapalogs exhibit dose-dependent synergism in human lung cancer cell lines (Legrier *et al*, 2007). Targeting mTOR/AKT and MEK/ERK pathways have shown synergistic antigrowth phenotypes in androgen-dependent prostate tumors in the mouse (Kinkade *et al*, 2008). Combination therapies with IGF-1 or MAPK2 inhibitors and rapalogs are currently in various stages of clinical evaluation (Dancey, 2010). Combination therapies with an mTOR inhibitor provide a promising avenue in eliciting synergistic efficacy by exploiting regulatory interaction between different pathways, which might not be possible in monotherapy. However, combination of therapeutic agents can also lead to synergistic toxicity. Thus, strategies for evaluation of toxicity and side effects need to be systematically explored, and as signaling pathway interactions are context-specific, different dosage regimen need to be evaluated based on cancer subtypes or patient subgrouping.

While combination therapies with rapamycin or a rapalog may turn out to be efficacious in controlling deregulated mTOR signaling, several groups have developed small-molecule active-site inhibitors of mTOR (reviewed in Guertin and Sabatini, 2009; Dowling *et al*, 2010b). This research has been bolstered by the fact that acute rapamycin treatment does not inhibit mTORC2 and incompletely inhibits mTORC1 signaling (Choo *et al*, 2008; Thoreen *et al*, 2009). Several ATP-competitive mTOR inhibitors have been described (Torin1, WYE-125132, PP242, KU-0063794) that inhibit mTOR irrespective of whether it is within mTORC1 or mTORC2 (Growth Factor/Nutrient module in Figures 2 and 4; Feldman *et al*, 2009; Garcia-Martinez *et al*, 2009; Thoreen *et al*, 2009; Yu *et al*, 2009; Janes *et al*, 2010). Generally, these inhibitors are more potent suppressors of protein synthesis and 4E-BP1 phosphorylation, and strongly promote autophagy. Some of these have already been tested for their antitumor activities in various cancer subtypes (breast cancer, glioma, lung and renal tumors), which generally appear to be more potent than what was found for rapamycin (Mayer *et al*, 2004; Yu *et al*, 2009).

The comprehensive machine-readable interaction map presented in this review will likely facilitate computational modeling and systems-level study of mTOR pathway components and interacting partners toward the discovery of novel targets and therapeutic intervention strategies for cancer.

Perspectives

We foresee the comprehensive mTOR network to be a guidance map in the study of mTOR signaling and its regulation. Akin to a geographical map that facilitates the navigation of new territories by explorers, the mTOR signaling map will provide researchers with a tool to quickly and efficiently maneuver through the complexity of mTOR interactions in their quest for novel interactions and potential pharmacological targets. Further, the availability of the map in standardized formats (SBML and SBGN) renders the network amenable to computational analyses based on various SBML compliant tools (http://sbml.org/SBML_Software_Guide/SBML_Software_Summary).

Our knowledge of the mTOR signaling network evolves rapidly. Thus, a key feature to the construction of large-scale biological networks is the ability to enrich the product with up-to-date information curated from the latest scientific literature. Further, it is of paramount importance to allow this knowledge to be available for community-wide curation and collaboration—allowing researchers to not only access the information, but also to curate the reactions and share comments on possible scientific implications, hypotheses, in a community-wide collaborative manner (Web 2.0). In this direction, we have developed a platform for community-based curation and enrichment of biological pathways called *Payao* (Matsuoka *et al*, 2010). *Payao* is a community-based, collaborative web service platform for gene regulatory and biochemical pathway model curation, based on SBML and uses CellDesigner for rendering the network. The *Payao* system (www.payaologue.org) enables a community to work on the same models simultaneously, insert tags as pop-up balloon to the parts of the model, exchange comments, record the discussions and eventually update the models accurately and concurrently. The current mTOR network elucidated in this review as well as the maps in Figures 3 and 4 have been published on the *Payao*

platform. From the *Payao* site (http://sblab.celldesigner.org/Payao10/bin/), search for mTOR in the 'Search Models' field to access the models (a snapshot of the map on *Payao* is available in Supplementary Figure S2).

We envision that the comprehensive mTOR map presented in this review will provide researchers with access to up-to-date and annotated mTOR knowledge base, allow for the sharing of information through comment tags that will enrich the network in a continuous cycle of open-flow community-collaborative framework.

Supplementary information

Supplementary information is available at the *Molecular Systems Biology* website (www.nature.com/msb).

Acknowledgements

We thank Valeria de Azcoitia and Martin Giroux for critically reading this paper and for helpful discussions. EC is supported by training grants from the Japan Society for the Promotion of Science and the Canadian Institutes of Health Research. PPR holds a Canada Research Chair in Signal Transduction and Proteomics, and a Career Development Award from the Human Frontier Science Program. Research in PPR laboratory is funded by a Terry Fox Foundation grant provided through the Canadian Cancer Society Research Institute. CP holds a Canada Research Chair in Immunobiology. MT holds a Canada Research Chair in Intracellular Signalling.

Author contributions: EC, CP, PR and HK conceived the idea of the mTOR map. EC assembled the map and coordinated the project. SG, YM and SL provided guidance for analyses. HK, YM and SG developed the *Payao* platform. DAB and MT provided the PPI network. EC, SG and PR contributed to the writing of, and all authors approved, the paper.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Abe Y, Yoon SO, Kubota K, Mendoza MC, Gygi SP, Blenis J (2009) p90 ribosomal S6 kinase and p70 ribosomal S6 kinase link phosphorylation of the eukaryotic chaperonin containing TCP-1 to growth factor, insulin, and nutrient signalling. *J Biol Chem* **284:** 14939–14948

Abraham RT, Eng CH (2008) Mammalian target of rapamycin as a therapeutic target in oncology. *Expert Opin Ther Targets* **12:** 209–222

Acosta-Jaquez HA, Keller JA, Foster KG, Ekim B, Soliman GA, Feener EP, Ballif BA, Fingar DC (2009) Site-specific mTOR phosphorylation promotes mTORC1-mediated signalling and cell growth. *Mol Cell Biol* **29:** 4308–4324

Adriaens ME, Jaillard M, Waagmeester A, Coort SL, Pico AR, Evelo CT (2008) The public road to high-quality curated biological pathways. *Drug Discov Today* **13:** 856–862

Albanese V, Yam AY, Baughman J, Parnot C, Frydman J (2006) Systems analyses reveal two chaperone networks with distinct functions in eukaryotic cells. *Cell* **124:** 75–88

Bai X, Ma D, Liu A, Shen X, Wang QJ, Liu Y, Jiang Y (2007) Rheb activates mTOR by antagonizing its endogenous inhibitor, FKBP38. *Science* **318:** 977–980

Ballif BA, Roux PP, Gerber SA, MacKeigan JP, Blenis J, Gygi SP (2005) Quantitative phosphorylation profiling of the ERK/p90 ribosomal S6 kinase-signalling cassette and its targets, the tuberous sclerosis tumor suppressors. *Proc Natl Acad Sci USA* **102:** 667–672

Bauer-Mehren A, Furlong LI, Sanz F (2009) Pathway databases and tools for their exploitation: benefits, current limitations and challenges. *Mol Syst Biol* **5:** 290

Bhaskar PT, Hay N (2007) The two TORCs and Akt. *Dev Cell* **12:** 487–502

Brown EJ, Albers MW, Shin TB, Ichikawa K, Keith CT, Lane WS, Schreiber SL (1994) A mammalian protein targeted by G1-arresting rapamycin-receptor complex. *Nature* **369:** 756–758

Brugarolas J, Lei K, Hurley RL, Manning BD, Reiling JH, Hafen E, Witters LA, Ellisen LW, Kaelin Jr WG (2004) Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes Dev* **18:** 2893–2904

Calzone L, Gelay A, Zinovyev A, Radvanyi F, Barillot E (2008) A comprehensive modular map of molecular interactions in RB/E2F pathway. *Mol Syst Biol* **4:** 173

Camasses A, Bogdanova A, Shevchenko A, Zachariae W (2003) The CCT chaperonin promotes activation of the anaphase-promoting complex through the generation of functional Cdc20. *Mol Cell* **12:** 87–100

Carracedo A, Ma L, Teruya-Feldstein J, Rojo F, Salmena L, Alimonti A, Egia A, Sasaki AT, Thomas G, Kozma SC, Papa A, Nardella C, Cantley LC, Baselga J, Pandolfi PP (2008) Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. *J Clin Invest* **118:** 3065–3074

Carriere A, Cargnello M, Julien LA, Gao H, Bonneil E, Thibault P, Roux PP (2008a) Oncogenic MAPK signalling stimulates mTORC1 activity by promoting RSK-mediated raptor phosphorylation. *Curr Biol* **18:** 1269–1277

Carriere A, Ray H, Blenis J, Roux PP (2008b) The RSK factors of activating the Ras/MAPK signalling cascade. *Front Biosci* **13:** 4258–4275

Chiang GG, Abraham RT (2005) Phosphorylation of mammalian target of rapamycin (mTOR) at Ser-2448 is mediated by p70S6 kinase. *J Biol Chem* **280:** 25485–25490

Chiang GG, Abraham RT (2007) Targeting the mTOR signalling network in cancer. *Trends Mol Med* **13:** 433–442

Choo AY, Blenis J (2009) Not all substrates are treated equally: implications for mTOR, rapamycin-resistance and cancer therapy. *Cell Cycle* **8:** 567–572

Choo AY, Yoon SO, Kim SG, Roux PP, Blenis J (2008) Rapamycin differentially inhibits S6Ks and 4E-BP1 to mediate cell-type-specific repression of mRNA translation. *Proc Natl Acad Sci USA* **105:** 17414–17419

Corradetti MN, Guan KL (2006) Upstream of the mammalian target of rapamycin: do all roads pass through mTOR? *Oncogene* **25:** 6347–6360

Csete M, Doyle J (2004) Bow ties, metabolism and disease. *Trends Biotechnol* **22:** 446–450

Cunningham JT, Rodgers JT, Arlow DH, Vazquez F, Mootha VK, Puigserver P (2007) mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. *Nature* **450:** 736–740

Dancey J (2010) mTOR signalling and drug development in cancer. *Nat Rev Clin Oncol* **7:** 209–219

DeYoung MP, Horak P, Sofer A, Sgroi D, Ellisen LW (2008) Hypoxia regulates TSC1/2-mTOR signalling and tumor suppression through REDD1-mediated 14-3-3 shuttling. *Genes Dev* **22:** 239–251

Dibble CC, Asara JM, Manning BD (2009) Characterization of Rictor phosphorylation sites reveals direct regulation of mTOR complex 2 by S6K1. *Mol Cell Biol* **29:** 5657–5670

Dowling RJ, Pollak M, Sonenberg N (2009) Current status and challenges associated with targeting mTOR for cancer therapy. *BioDrugs* **23:** 77–91

Dowling RJ, Topisirovic I, Alain T, Bidinosti M, Fonseca BD, Petroulakis E, Wang X, Larsson O, Selvaraj A, Liu Y, Kozma SC, Thomas G, Sonenberg N (2010a) mTORC1-mediated cell proliferation, but not cell growth, controlled by the 4E-BPs. *Science* **328:** 1172–1176

Dowling RJ, Topisirovic I, Fonseca BD, Sonenberg N (2010b) Dissecting the role of mTOR: lessons from mTOR inhibitors. *Biochim Biophys Acta* **1804:** 433–439

Dunlop EA, Tee AR (2009) Mammalian target of rapamycin complex 1: signalling inputs, substrates and feedback mechanisms. *Cell Signal* **21:** 827–835

Easton JB, Houghton PJ (2006) mTOR and cancer therapy. *Oncogene* **25:** 6436–6446

Efeyan A, Sabatini DM (2010) mTOR and cancer: many loops in one pathway. *Curr Opin Cell Biol* **22:** 169–176

El-Hashemite N, Walker V, Zhang H, Kwiatkowski DJ (2003) Loss of Tsc1 or Tsc2 induces vascular endothelial growth factor production through mammalian target of rapamycin. *Cancer Res* **63:** 5173–5177

Feldman ME, Apsel B, Uotila A, Loewith R, Knight ZA, Ruggero D, Shokat KM (2009) Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. *PLoS Biol* **7:** e38

Fonseca BD, Smith EM, Lee VH, MacKintosh C, Proud CG (2007) PRAS40 is a target for mammalian target of rapamycin complex 1 and is required for signalling downstream of this complex. *J Biol Chem* **282:** 24514–24524

Foster KG, Acosta-Jaquez HA, Romeo Y, Ekim B, Soliman GA, Carriere A, Roux PP, Ballif BA, Fingar DC (2010) Regulation of mTOR complex 1 (mTORC1) by raptor Ser863 and multisite phosphorylation. *J Biol Chem* **285:** 80–94

Foster KG, Fingar DC (2010) Mammalian target of rapamycin (mTOR): conducting the cellular signalling symphony. *J Biol Chem* **285:** 14071–14077

Funahashi A, Jouraku A, Matsuoka Y, Kitano H (2007) Integration of CellDesigner and SABIO-RK. *In Silico Biol* **7:** S81–S90

Gan B, Melkoumian ZK, Wu X, Guan KL, Guan JL (2005) Identification of FIP200 interaction with the TSC1-TSC2 complex and its role in regulation of cell size control. *J Cell Biol* **170:** 379–389

Garcia-Martinez JM, Alessi DR (2008) mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). *Biochem J* **416:** 375–385

Garcia-Martinez JM, Moran J, Clarke RG, Gray A, Cosulich SC, Chresta CM, Alessi DR (2009) Ku-0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR). *Biochem J* **421:** 29–42

Gibbons JJ, Abraham RT, Yu K (2009) Mammalian target of rapamycin: discovery of rapamycin reveals a signalling pathway important for normal and cancer cell growth. *Semin Oncol* **36**(Suppl 3)**:** S3–S17

Guertin DA, Guntur KV, Bell GW, Thoreen CC, Sabatini DM (2006a) Functional genomics identifies TOR-regulated genes that control growth and division. *Curr Biol* **16:** 958–970

Guertin DA, Sabatini DM (2007) Defining the role of mTOR in cancer. *Cancer Cell* **12:** 9–22

Guertin DA, Sabatini DM (2009) The pharmacology of mTOR inhibition. *Sci Signal* **2:** pe24

Guertin DA, Stevens DM, Thoreen CC, Burds AA, Kalaany NY, Moffat J, Brown M, Fitzgerald KJ, Sabatini DM (2006b) Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signalling to Akt-FOXO and PKCalpha, but not S6K1. *Dev Cell* **11:** 859–871

Gulati P, Thomas G (2007) Nutrient sensing in the mTOR/S6K1 signalling pathway. *Biochem Soc Trans* **35:** 236–238

Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, Turk BE, Shaw RJ (2008) AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell* **30:** 214–226

Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S, Tokunaga C, Avruch J, Yonezawa K (2002) Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell* **110:** 177–189

Harding MW, Galat A, Uehling DE, Schreiber SL (1989) A receptor for the immunosuppressant FK506 is a cis-trans peptidyl-prolyl isomerase. *Nature* **341:** 758–760

Harrington LS, Findlay GM, Lamb RF (2005) Restraining PI3K: mTOR signalling goes back to the membrane. *Trends Biochem Sci* **30:** 35–42

Heitman J, Movva NR, Hall MN (1991) Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* **253:** 905–909

Hernandez-Toro J, Prieto C, De las Rivas J (2007) APID2NET: unified interactome graphic analyzer. *Bioinformatics* **23:** 2495–2497

Holz MK, Blenis J (2005) Identification of S6 kinase 1 as a novel mammalian target of rapamycin (mTOR)-phosphorylating kinase. *J Biol Chem* **280:** 26089–26093

Huang J, Dibble CC, Matsuzaki M, Manning BD (2008a) The TSC1-TSC2 complex is required for proper activation of mTOR complex 2. *Mol Cell Biol* **28:** 4104–4115

Huang J, Manning BD (2008b) The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. *Biochem J* **412:** 179–190

Huang J, Wu S, Wu CL, Manning BD (2009) Signalling events downstream of mammalian target of rapamycin complex 2 are attenuated in cells and tumors deficient for the tuberous sclerosis complex tumor suppressors. *Cancer Res* **69:** 6107–6114

Hucka M, Finney A, Sauro HM, Bolouri H, Doyle JC, Kitano H, Arkin AP, Bornstein BJ, Bray D, Cornish-Bowden A, Cuellar AA, Dronov S, Gilles ED, Ginkel M, Gor V, Goryanin II, Hedley WJ, Hodgman TC, Hofmeyr JH, Hunter PJ *et al* (2003) The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. *Bioinformatics* **19:** 524–531

Hudson CC, Liu M, Chiang GG, Otterness DM, Loomis DC, Kaper F, Giaccia AJ, Abraham RT (2002) Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. *Mol Cell Biol* **22:** 7004–7014

Inoki K, Li Y, Zhu T, Wu J, Guan KL (2002) TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol* **4:** 648–657

Inoki K, Ouyang H, Zhu T, Lindvall C, Wang Y, Zhang X, Yang Q, Bennett C, Harada Y, Stankunas K, Wang CY, He X, MacDougald OA, You M, Williams BO, Guan KL (2006) TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. *Cell* **126:** 955–968

Inoki K, Zhu T, Guan KL (2003) TSC2 mediates cellular energy response to control cell growth and survival. *Cell* **115:** 577–590

Jacinto E (2008) What controls TOR? *IUBMB Life* **60:** 483–496

Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, Jung SY, Huang Q, Qin J, Su B (2006) SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. *Cell* **127:** 125–137

Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A, Hall MN (2004) Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol* **6:** 1122–1128

Janes MR, Limon JJ, So L, Chen J, Lim RJ, Chavez MA, Vu C, Lilly MB, Mallya S, Ong ST, Konopleva M, Martin MB, Ren P, Liu Y, Rommel C, Fruman DA (2010) Effective and selective targeting of leukemia cells using a TORC1/2 kinase inhibitor. *Nat Med* **16:** 205–213

Julien LA, Carriere A, Moreau J, Roux PP (2010) mTORC1-Activated S6K1 Phosphorylates Rictor on Threonine 1135 and Regulates mTORC2 Signalling. *Mol Cell Biol* **30:** 908–921

Jung CH, Jun CB, Ro SH, Kim YM, Otto NM, Cao J, Kundu M, Kim DH (2009) ULK-Atg13-FIP200 complexes mediate mTOR signalling to the autophagy machinery. *Mol Biol Cell* **20:** 1992–2003

Karlebach G, Shamir R (2008) Modelling and analysis of gene regulatory networks. *Nat Rev Mol Cell Biol* **9:** 770–780

Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM (2002) mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* **110:** 163–175

Kim E, Goraksha-Hicks P, Li L, Neufeld TP, Guan KL (2008) Regulation of TORC1 by Rag GTPases in nutrient response. *Nat Cell Biol* **10:** 935–945

Kinkade CW, Castillo-Martin M, Puzio-Kuter A, Yan J, Foster TH, Gao H, Sun Y, Ouyang X, Gerald WL, Cordon-Cardo C, Abate-Shen C (2008) Targeting AKT/mTOR and ERK MAPK signalling inhibits hormone-refractory prostate cancer in a preclinical mouse model. *J Clin Invest* **118:** 3051–3064

Kitano H (2007) A robustness-based approach to systems-oriented drug design. *Nat Rev Drug Discov* **6:** 202–210

Kitano H, Funahashi A, Matsuoka Y, Oda K (2005) Using process diagrams for the graphical representation of biological networks. *Nat Biotechnol* **23:** 961–966

Kitano H, Oda K (2006) Robustness trade-offs and host-microbial symbiosis in the immune system. *Mol Syst Biol* **2**, 2006 0022

Laplante M, Sabatini DM (2009) An emerging role of mTOR in lipid biosynthesis. *Curr Biol* **19:** R1046–R1052

Le Novere N, Hucka M, Mi H, Moodie S, Schreiber F, Sorokin A, Demir E, Wegner K, Aladjem MI, Wimalaratne SM, Bergman FT, Gauges R, Ghazal P, Kawaji H, Li L, Matsuoka Y, Villeger A, Boyd SE, Calzone L, Courtot M *et al* (2009) The systems biology graphical notation. *Nat Biotechnol* **27:** 735–741

Legrier ME, Yang CP, Yan HG, Lopez-Barcons L, Keller SM, Perez-Soler R, Horwitz SB, McDaid HM (2007) Targeting protein translation in human non small cell lung cancer via combined MEK and mammalian target of rapamycin suppression. *Cancer Res* **67:** 11300–11308

Lehar J, Krueger A, Zimmermann G, Borisy A (2008a) High-order combination effects and biological robustness. *Mol Syst Biol* **4:** 215

Lehar J, Stockwell BR, Giaever G, Nislow C (2008b) Combination chemical genetics. *Nat Chem Biol* **4:** 674–681

Lizcano JM, Goransson O, Toth R, Deak M, Morrice NA, Boudeau J, Hawley SA, Udd L, Makela TP, Hardie DG, Alessi DR (2004) LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. *EMBO J* **23:** 833–843

Long X, Lin Y, Ortiz-Vega S, Yonezawa K, Avruch J (2005) Rheb binds and regulates the mTOR kinase. *Curr Biol* **15:** 702–713

Ma L, Chen Z, Erdjument-Bromage H, Tempst P, Pandolfi PP (2005) Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. *Cell* **121:** 179–193

Ma XM, Blenis J (2009) Molecular mechanisms of mTOR-mediated translational control. *Nat Rev Mol Cell Biol* **10:** 307–318

Manning BD (2004) Balancing Akt with S6K: implications for both metabolic diseases and tumorigenesis. *J Cell Biol* **167:** 399–403

Manning BD, Tee AR, Logsdon MN, Blenis J, Cantley LC (2002) Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. *Mol Cell* **10:** 151–162

Matsuoka Y, Ghosh S, Kikuchi N, Kitano H (2010) Payao: a community platform for SBML pathway model curation. *Bioinformatics* **26:** 1381–1383

Mayer C, Zhao J, Yuan X, Grummt I (2004) mTOR-dependent activation of the transcription factor TIF-IA links rRNA synthesis to nutrient availability. *Genes Dev* **18:** 423–434

Nave BT, Ouwens M, Withers DJ, Alessi DR, Shepherd PR (1999) Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation. *Biochem J* **344** (Pt 2)**:** 427–431

Nelander S, Wang W, Nilsson B, She QB, Pratilas C, Rosen N, Gennemark P, Sander C (2008) Models from experiments: combinatorial drug perturbations of cancer cells. *Mol Syst Biol* **4:** 216

Noda T, Ohsumi Y (1998) Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast. *J Biol Chem* **273:** 3963–3966

Oda K, Kitano H (2006) A comprehensive map of the toll-like receptor signalling network. *Mol Syst Biol* **2**, 2006 0015

Oda K, Matsuoka Y, Funahashi A, Kitano H (2005) A comprehensive pathway map of epidermal growth factor receptor signalling. *Mol Syst Biol* **1**, 2005 0010

Oh WJ, Wu Cc, Kim SJ, Facchinetti V, Julien L-A, Finlan M, Roux PP, Su B, Jacinto E (2010) mTORC2 can associate with ribosomes to promote cotranslational phosphorylation and stability of nascent Akt polypeptide. *EMBO J* **29:** 3939–3951

Oshiro N, Takahashi R, Yoshino K, Tanimura K, Nakashima A, Eguchi S, Miyamoto T, Hara K, Takehana K, Avruch J, Kikkawa U, Yonezawa K (2007) The proline-rich Akt substrate of 40 kDa (PRAS40) is a physiological substrate of mammalian target of rapamycin complex 1. *J Biol Chem* **282:** 20329–20339

Peterson TR, Laplante M, Thoreen CC, Sancak Y, Kang SA, Kuehl WM, Gray NS, Sabatini DM (2009) DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. *Cell* **137:** 873–886

Rosner M, Hengstschlager M (2008) Cytoplasmic and nuclear distribution of the protein complexes mTORC1 and mTORC2: rapamycin triggers dephosphorylation and delocalization of the mTORC2 components rictor and sin1. *Hum Mol Genet* **17:** 2934–2948

Roux PP, Ballif BA, Anjum R, Gygi SP, Blenis J (2004) Tumor-promoting phorbol esters and activated Ras inactivate the tuberous sclerosis tumor suppressor complex via p90 ribosomal S6 kinase. *Proc Natl Acad Sci USA* **101:** 13489–13494

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

Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM (2010) Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell* **141:** 290–303

Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, Sabatini DM (2008) The Rag GTPases bind raptor and mediate amino acid signalling to mTORC1. *Science* **320:** 1496–1501

Sancak Y, Thoreen CC, Peterson TR, Lindquist RA, Kang SA, Spooner E, Carr SA, Sabatini DM (2007) PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. *Mol Cell* **25:** 903–915

Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM (2004) Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol* **14:** 1296–1302

Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, Markhard AL, Sabatini DM (2006) Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell* **22:** 159–168

Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* **307:** 1098–1101

Sato T, Nakashima A, Guo L, Tamanoi F (2009) Specific activation of mTORC1 by Rheb G-protein *in vitro* involves enhanced recruitment of its substrate protein. *J Biol Chem* **284:** 12783–12791

Schroder WA, Buck M, Cloonan N, Hancock JF, Suhrbier A, Sculley T, Bushell G (2007) Human Sin1 contains Ras-binding and pleckstrin homology domains and suppresses Ras signalling. *Cell Signal* **19:** 1279–1289

Shaw RJ, Cantley LC (2006) Ras, PI(3)K and mTOR signalling controls tumour cell growth. *Nature* **441:** 424–430

Shaw RJ, Kosmatka M, Bardeesy N, Hurley RL, Witters LA, DePinho RA, Cantley LC (2004) The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. *Proc Natl Acad Sci USA* **101:** 3329–3335

Smith EM, Finn SG, Tee AR, Browne GJ, Proud CG (2005) The tuberous sclerosis protein TSC2 is not required for the regulation of the mammalian target of rapamycin by amino acids and certain cellular stresses. *J Biol Chem* **280:** 18717–18727

Soliman GA, Acosta-Jaquez HA, Dunlop EA, Ekim B, Maj NE, Tee AR, Fingar DC (2010) mTOR Ser-2481 autophosphorylation monitors mTORC-specific catalytic activity and clarifies rapamycin mechanism of action. *J Biol Chem* **285:** 7866–7879

Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, Reichling LJ, Sim T, Sabatini DM, Gray NS (2009) An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. *J Biol Chem* **284:** 8023–8032

Vander Haar E, Lee SI, Bandhakavi S, Griffin TJ, Kim DH (2007) Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. *Nat Cell Biol* **9:** 316–323

Vezina C, Kudelski A, Sehgal SN (1975) Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *J Antibiot (Tokyo)* **28:** 721–726

Wang L, Harris TE, Lawrence Jr JC (2008) Regulation of proline-rich Akt substrate of 40 kDa (PRAS40) function by mammalian target of rapamycin complex 1 (mTORC1)-mediated phosphorylation. *J Biol Chem* **283:** 15619–15627

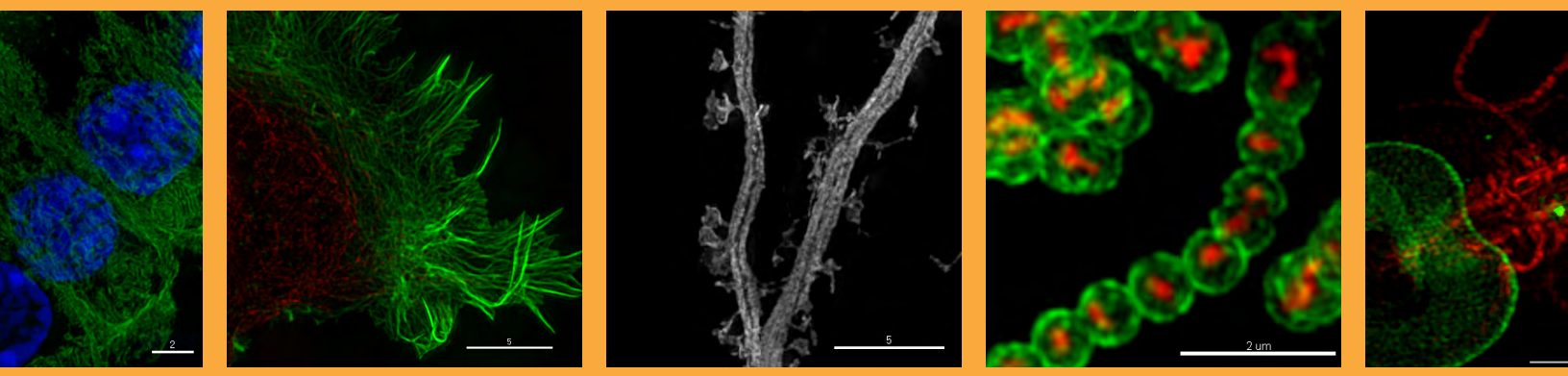
Wang L, Lawrence Jr JC, Sturgill TW, Harris TE (2009) Mammalian target of rapamycin complex 1 (mTORC1) activity is associated with phosphorylation of raptor by mTOR. *J Biol Chem* **284:** 14693–14697

Wullschleger S, Loewith R, Hall MN (2006) TOR signalling in growth and metabolism. *Cell* **124:** 471–484

Yip CK, Murata K, Walz T, Sabatini DM, Kang SA (2010) Structure of the human mTOR complex I and its implications for rapamycin inhibition. *Mol Cell* **38:** 768–774

Yu K, Toral-Barza L, Shi C, Zhang WG, Lucas J, Shor B, Kim J, Verheijen J, Curran K, Malwitz DJ, Cole DC, Ellingboe J, Ayral-Kaloustian S, Mansour TS, Gibbons JJ, Abraham RT, Nowak P, Zask A (2009) Biochemical, cellular, and *in vivo* activity of novel ATP-competitive and selective inhibitors of the mammalian target of rapamycin. *Cancer Res* **69:** 6232–6240

Real data.
Real installations.
Real super-resolution imaging.



Learn more about the DeltaVision OMX super-resolution imaging system at **www.superresolution.com**.

applied precision

1040 12th Ave NW | Issaquah, WA 98027