A comprehensive map of the mTOR signaling network

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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.

Keywords: cancer; CellDesigner; graphical notation; mTOR; regulatory network

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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; Guerlin 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 (glucogen 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 enables users to describe molecular interactions using the process diagram language, as described above. Also, the map complies with the SBML, a standard
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, 20 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.
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 of 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 API2DNET (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**

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Upstream regulators of mTORC1 signaling

mTORC1 is a rapamycin-sensitive multi-protein complex that contains mTOR, Raptor and mLST8 (also known as GmTORC1 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 proteosomal 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; Gualti 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, whereas 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 3; 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 promoting 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). 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 has 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 PPARγ and SREBP-1, two transcription factors that control expression of many lipidogenic 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 PPARγ (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 knock-down 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 regimens 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.payaoogle.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).

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Conflict of interest
The authors declare that they have no conflict of interest.

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