Signalling networks in focus

The TOR signalling network from yeast to man

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Abstract

The target of rapamycin, TOR, is an essential ser/thr protein kinase that functions in two distinct multiprotein complexes, TOR complex 1 and 2. The structure and functions of these complexes have been conserved from yeast to man. TOR complex 1 is inhibited by rapamycin and is thought to couple growth cues to cellular metabolism; TOR complex 2 is not inhibited by rapamycin and appears to regulate spatial aspects of growth such as cell polarity. Work done in a variety of model systems, continues to contribute to our current understanding of this TOR signalling network. Recent studies in flies and mammalian tissue culture cells have elucidated many signalling components upstream of TOR complex 1. These studies also suggest that aberrant mammalian TOR complex 1 signalling contributes to a number of pathologies ranging from metabolic diseases to hyperproliferative disorders and cancers. Consequently the efficacies of rapamycin and related compounds in treating such diseases are being evaluated in clinical trials.
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1. Signalling network facts

- The target of rapamycin (TOR) is a ser/thr protein kinase conserved in all eukaryotes.
- TOR functions in two distinct multi-protein complexes, TOR complex 1 (TORC1) and TORC2, which regulate distinct aspects of cell growth.
- Many upstream signalling factors that positively or negatively regulate mammalian TORC1 are known oncoproteins or tumour suppressors, respectively.

- The efficacy of TORC1 inhibitors in a variety of disease settings are currently being assessed in clinical trials.

2. Introduction

The antifungal action of the lipophilic macrolide rapamycin was exploited to identify the target of rapamycin (TOR) in the yeast *Saccharomyces cerevisiae*. TOR is a ser/thr protein kinase conserved in all eukaryotes that associates with and is inhibited (via a still unknown mechanism) by the toxic complex formed between rapamycin and a conserved proline-isomerase (FKBP12). Genetic studies in yeast demonstrated that TOR performs at least two major functions, one sensitive to rapamycin and one insensitive to rapamycin. Interestingly, TOR resides in two distinct multi-protein

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complexes: TORC1 and TORC2 (Inoki, Ouyang, Li, & Guan, 2005b). TORC1 couples growth cues, such as nutrient and environmental stress, and in metazoans signals from circulating growth factors and hormones, to metabolic flux and consequently mass accumulation. TOR in TORC1 is bound and inhibited by rapamycin-FKBP12. TORC2 appears to govern cell polarity, but upstream regulators of TORC2 are not known. Curiously, TOR in TORC2 is not bound by rapamycin-FKBP12 and therefore is insensitive to rapamycin. As elaborated below, these complexes reside in separate signalling pathways and regulate distinct aspects of cell growth. Together these pathways define the TOR signalling network.

3. Functions

The downstream effectors of TORC1 and TORC2 have been extensively characterized in yeast. Due to the availability rapamycin, TORC1 effectors are generally better characterized than TORC2 effectors. Although both complexes regulate a wide array of growth-related readouts, only a few direct substrates of these kinases are actually known.

3.1. Rapamycin-sensitive TORC1 regulates various aspects of cellular metabolism

In general, rapamycin treatment causes a dramatic down-regulation of cellular anabolic processes, and correspondingly, an up-regulation of catabolic and growth-inhibitory processes (Fig. 1). Consequently, most cells treated with rapamycin abruptly arrest growth and enter a G_0-like state.

Yeast TORC1 (yTORC1) positively controls the anabolic process of protein synthesis at multiple levels, specifically by regulating (i) ribosome biogenesis via control of rRNA, ribosomal protein, and tRNA gene expression, and rRNA processing, (ii) mRNA stability, (iii) translation initiation factor activities, and (iv) high-affinity amino acid permeases that serve to provide amino acids for immediate use by the translation machin-
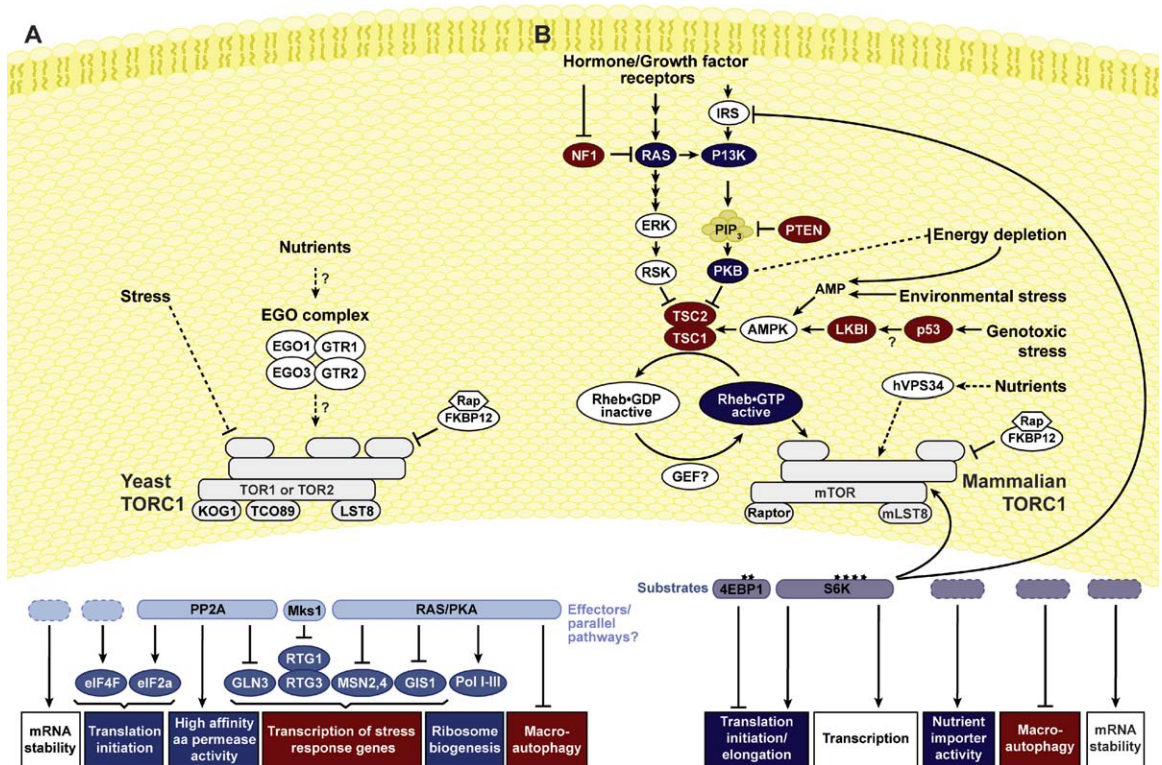

Fig. 1. Rapamycin-sensitive yeast TORC1 (yTORC1, A) and mammalian TORC1 (mTORC1, B) couple growth cues to cellular metabolism. Growth factors, nutrients, stresses and/or energy are sensed by signalling pathways that impinge upon TORC1 as described in the text. Many factors upstream of mTORC1 are known proto-oncogenes (dark blue) or tumour suppressors (red). Bona fide TORC1 substrates are marked with asterisks. TORC1 regulated processes that promote growth are boxed in dark blue; TORC1 regulated processes that inhibit growth are boxed in dark red. Arrows and bars denote positive and negative interactions, respectively, and dashed arrows and bars refer to potential interactions.

ery (Fig. 1A; Loewith & Hall, 2004; Powers, 2004). In analogy to yTORC1, mammalian TORC1 (mTORC1) also controls protein synthesis at multiple levels (Inoki et al., 2005b).

yTORC1 also promotes growth by sequestering a number of nutrient and/or general stress-responsive transcription factors (e.g. GLN3, RTG3, and MSN2) in the cytoplasm (Fig. 1A). Accordingly, rapamycin treatment triggers nuclear entry of these transcription factors, which stimulate the expression of genes whose products are required for the uptake and assimilation of suboptimal nitrogen sources, the de novo synthesis of amino acids, the synthesis of nutrient storage reserves, and cell survival under various stress conditions (Loewith & Hall, 2004). Both yTORC1 and mTORC1 negatively control the catabolic process of macroautophagy, a membrane trafficking pathway that sequesters bulk cytoplasmic material into nascent autophagosomes for subsequent fusion with and degradation in the vacuole (Fig. 1; Inoki et al., 2005b). Nutrient starvation and/or TORC1 inhibition triggers macroautophagy, which serves to recycle surplus cytoplasmic mass and to turn over large structures and organelles such as ribosomes and mitochondria, thereby contributing simultaneously to both reduction of cellular energy consumption and cell growth arrest.

Currently it is thought that yTORC1 controls many processes via the type 2A or type 2A-related protein phosphatases (and possibly some processes via an unknown component of the RAS/PKA pathway), yet a bona fide yTORC1 effector has yet to be identified. In contrast, mTORC1 substrates are known and include a negative regulator of translation initiation, i.e. 4E-BP1, which is inhibited by mTORC1 phosphorylation, and the AGC ser/thr protein kinase S6K1, which is activated by mTORC1 and is thought to positively regulate translation initiation (Inoki et al., 2005b). Many S6K1 substrates are known including IRS1 (a signalling component upstream of mTORC1) and, possibly as part of a feedback control mechanism, mTOR itself. Identifying additional TORC1 substrates will be required to fully appreciate how TORC1 regulates cellular metabolism.

3.2. Rapamycin-insensitive TORC2 regulates cell polarity

Genetic studies in yeast demonstrated that yTORC2 is required for polarization of the actin cytoskeleton, which serves to define the spatial orientation of growth, via control of the cell integrity pathway (Fig. 2A; Inoki et al., 2005b; Loewith & Hall, 2004). This pathway consists of a guanine-nucleotide exchange factor (GEF),

ROM2, which activates the RHO1 GTPase. GTP loaded RHO1 triggers the activation of the yeast protein kinase C (PKC1) and its associated MAP kinase module (consisting of BCK1, MKK1/2 and MPK1), which ultimately controls polarization of the actin cytoskeleton. Yeast TORC2 substrates include SLM1 and SLM2, a homologous pair of PH domain containing proteins, and the AGC kinase YPK2 (Levin, 2005). SLM1/2 and YPK2 are all required for polarization of the actin cytoskeleton but the mechanisms by which these yTORC2 substrates impinge upon ROM2 have not yet been elucidated. mTORC2 also influences actin polymerization and cell polarity by processes that appear to involve the AGC kinase PKB/Akt, RHO GTPases, and PKC (Fig. 2B; Inoki et al., 2005b). Recently, TORC2 in *Dictyostelium discoideum* has been characterized and also shown to regulate cell polarity (Lee et al., 2005).

4. Cascades

While it is not known how, or even if, y/mTORC2 is regulated, y/mTORC1 is—via yet poorly understood mechanisms—regulated by nutrient quality and/or abundance. In addition, mTORC1 activity is influenced by growth factors, energy, and cellular stress, and recent studies in fly and mammalian tissue culture systems have elucidated many of the signalling components that communicate these cellular cues to mTORC1.

4.1. Regulation of mTORC1 by hormones and growth factors

Ligation of several hormone and growth factor receptors activates mTORC1 (Fig. 1B; Harris & Lawrence, 2003; Inoki et al., 2005b). Ligation of these receptors results in activation of phosphatidylinositol 3-kinases (PI3K), which produce the lipid second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP$_3$) that in turn recruits and thus activates the protein kinase PKB/Akt (the lipid phosphatase PTEN antagonizes this recruitment step). An important PKB/Akt substrate is TSC2 (tuberin), a GTPase activating protein (GAP) that forms a heterodimeric complex with TSC1 (hamartin). PKB/Akt-mediated phosphorylation antagonizes TSC2 function. PKB/Akt may also indirectly antagonize TSC2 function by virtue of its ability to maintain cellular energy reserves that prevent AMPK activation (see below; Hahn-Windgassen et al., 2005). TSC2 stimulates the GTPase activity of the small GTP-binding protein Rheb, which directly interacts with and positively regulates mTORC1 (Long, Lin, Ortiz-Vega, Yonezawa, & Avruch, 2005). Ligation of hormone and growth factor
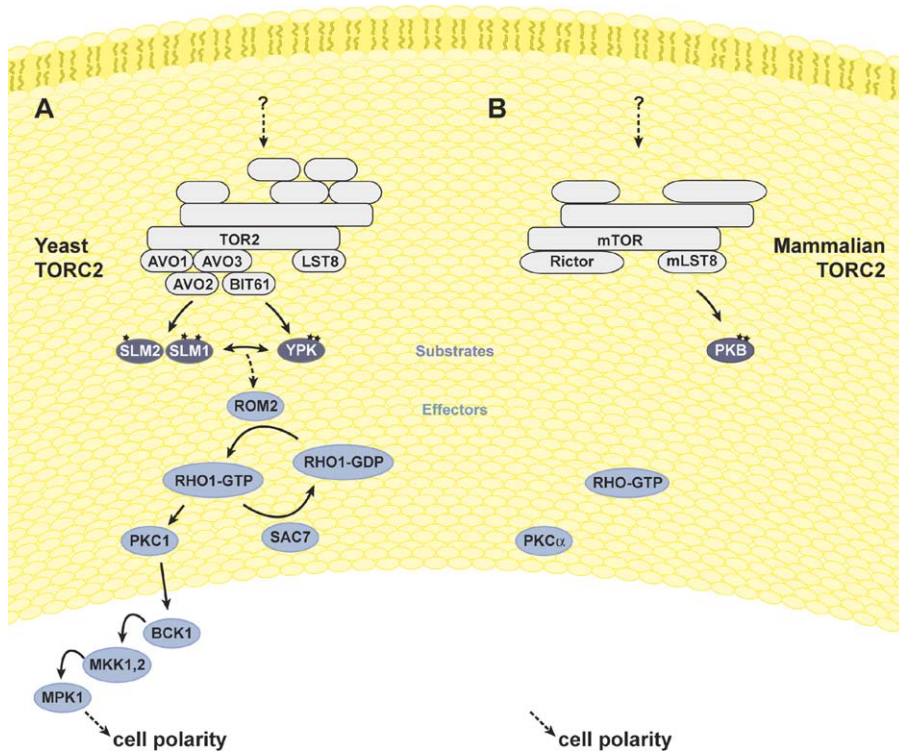
Fig. 2. Rapamycin-insensitive TORC2 regulates spatial aspects of cell growth. (A) yTORC2 regulates actin organization via the RHO1-PKC1-BCK1-MKK1,2-MPK1 cell integrity pathway. (B) mTORC2 also regulates actin organization by a process that may involve the AGC kinase PKB/Akt, RHO GTPases, and PKC (see text and legend of Fig. 1 for further details).

receptors also lead to increased activity of RasGEFs and decreased activity of RasGAPs (e.g. NF1) and subsequently activation of the small GTP-binding protein Ras (Coleman, Marshall, & Olson, 2004). GTP-loaded Ras binds and activates PI3K and/or activates the Raf-MEK-ERK-RSK1 kinase cascade. Importantly, RSK1 directly phosphorylates and inhibits TSC2 indicating that Ras-, and growth factor- and hormone-transduced signals may regulate mTORC1 through at least two separate routes (Fig. 1B; Tee & Blenis, 2005).

4.2. Regulation of mTORC1 by cellular energy status and stress

Reduction of cellular energy decreases mTORC1 activity via the 5'AMP-activated protein kinase (AMPK). Accordingly, energy depletion enhances both the level of cellular AMP and AMP-bound AMPK. The LKB1 protein kinase preferably phosphorylates and thereby activates AMP-bound AMPK, which in turn indirectly reduces mTORC1 activity by phosphorylation and activation of the Rheb-GAP TSC2 (Inoki et al., 2005b). Notably, a number of cellular stresses (e.g.

heat shock, oxidative and osmotic stress, hypoxia, or low glucose levels) increase AMP levels (due to ATP depletion) and thus activate AMPK, resulting in a reduction of mTORC1 activity (Hardie, 2005). Finally, genotoxic stress also activates mTORC1 in an AMPK-dependent fashion, which may involve association of LKB1 with p53 (Feng, Zhang, Levine, & Jin, 2005).

4.3. Regulation of mTORC1 by nutrient abundance

Nitrogen starvation and rapamycin treatment elicit very similar responses in *S. cerevisiae*, suggesting that yTORC1 is regulated by the abundance and/or quality of the available nitrogen source. Similarly, starving mammalian cells of (especially branched-chain) amino acids, results in mTORC1 inhibition. However, it is not known how nutrient abundance/quality is sensed and how this information is transmitted to TORC1. While TSC2 does not appear to be involved in this process (notably yeast lack a TSC2 homolog), some groups have suggested that Rheb may be involved in communicating amino acid sufficiency to mTORC1 (Kwiatkowski & Manning, 2005). Yet, other groups have presented evidence that the class

III PI 3-kinase hVps34 communicates amino acid sufficiency to mTORC1 independently of Rheb (Byfield, Murray, & Backer, 2005; Nobukuni et al., 2005).

Another possible component of the nutrient signalling cascade upstream of TORC1 is the vacuolar membrane associated EGO protein complex. The vacuole constitutes the major nutrient reservoir in yeast; the EGO complex (EGOC) consists of EGO1, EGO3, GTR1 and GTR2 (Dubouloz, Deloche, Wanke, Cameroni, & De Virgilio, 2005; and unpublished observations). The function of EGO3 is not yet clear; EGO1 is myristoylated and likely recruits EGOC to the vacuolar membrane. GTR1 and GTR2 are small GTPases and the yeast orthologues of the mammalian RagA, -B, -C, and -D proteins. Overexpression of GTR2 or EGO3 increases rapamycin resistance (Dubouloz et al., 2005), and EGO3 is the target of a synthetic molecule (SMIR4) that promotes rapamycin resistance. Furthermore, transcript profiling suggests that *ego3Δ* mutants show considerable similarities to rapamycin treated cells (Huang et al., 2004). These observations strongly suggest that EGOC acts upstream of TORC1. As the GTPase components of EGOC are conserved in mammals it will be interesting to determine whether they also participate in the regulation mTORC1.

5. Key molecules

5.1. TORC1

y/mTORC1 contains KOG1/raptor, LST8/mLST8, TCO89/(?) and either TOR1 or TOR2/mTOR (Harris & Lawrence, 2003). Other than TOR, the specific functions of TORC1 components are speculative. Raptor has been proposed to bind and recruit substrates to TOR, and the stability of the raptor–mTOR interaction may be the target of upstream signals. LST8/mLST8 has been proposed both to receive upstream signals and to transmit downstream outputs from TORC1 (Butow & Avadhani, 2004).

5.2. TORC2

y/mTORC2 contains AVO1/(?), AVO2/(?), AVO3/ rictor, BIT61/(?), LST8/mLST8, and TOR2/mTOR. TORC2 also exists as a multimer, likely assembled on a TOR2-TOR2 dimer (Wullschleger, Loewith, Oppliger, & Hall, 2005). AVO1 and AVO3 play important roles in the structural integrity of TORC2, while AVO2 and perhaps BIT61 serve to recruit substrates to the complex. LST8 is required for full catalytic potential of TOR2 and to a lesser extent, for the structural stability of TORC2.

6. Associated pathologies and therapeutic implications

mTORC1 inhibitors are currently used to inhibit host rejection of transplanted organs and in-stent restenosis of cardiac arteries following angioplasty. The elucidation of the signalling pathways upstream of mTORC1 has indicated that aberrantly high mTORC1 activity is the underlying cause of both malignant disease and cancer-predisposition hypertrophic disorders (Guertin & Sabatini, 2005; Inoki, Corradetti, & Guan, 2005a; Tee & Blenis, 2005). This is highlighted by the fact that signals from many proto-oncoproteins (RAS, PI3K, PKB/Akt, and Rheb) activate mTORC1, while several tumour suppressor proteins (NF1, PTEN, TSC1/2, LKB1 and p53) dampen mTORC1 activity (Fig. 1B). Mutations in most of the tumour suppressor genes connected to mTORC1 signalling result in hamartoma (benign tumour) syndromes, and are associated with an increased risk of developing malignant disease. Consequently, the efficacy of mTORC1 inhibitors as anti-cancer agents are currently being assessed in clinical trials (Bjornsti & Houghton, 2004). Lastly, mTORC1 signalling has been implicated in cardiac hypertrophy and, via the negative regulation of IRS1 by S6K1, insulin resistance. Thus, mTORC1 inhibitors may also turn out to be effective tools in the treatment of heart disease and/or metabolic disorders such as type 2 diabetes.

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