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REVIEW

Cell growth control: little eukaryotes make big contributions

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The story of rapamycin is a pharmaceutical fairytale. Discovered as an antifungal activity in a soil sample collected on Easter Island, this macrocyclic lactone and its derivatives are now billion dollar drugs, used in, and being evaluated for, a number of clinical applications. Taking advantage of its antifungal property, the molecular *T*arget *O*f *R*apamycin, *TOR*, was first described in the budding yeast *Saccharomyces cerevisiae*. *TOR*s encode large, Ser/Thr protein kinases that reside in two distinct, structurally and functionally conserved, multi-protein complexes. In yeast, these complexes coordinate many different aspects of cell growth. TOR complex 1, TORC1, promotes protein synthesis and other anabolic processes, while inhibiting macroautophagy and other catabolic and stress-response processes. TORC2 primarily regulates cell polarity, although additional readouts of this complex are beginning to be characterized. TORC1 appears to be activated by nutrient cues and inhibited by stresses and rapamycin; however, detailed mechanisms are not known. In contrast, TORC2 is insensitive to rapamycin and physiological regulators of this complex have yet to be defined. Given the unsurpassed resources available to yeast researchers, this simple eukaryote continues to contribute to our understanding of eukaryotic cell growth in general and TOR function in particular.
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Discovery of TOR

Rapamycin was initially characterized in 1975 as the potent antifungal principle produced by a strain of *Streptomyces hygroscopicus*, which was isolated from a soil sample collected on Easter Island, locally known as Rapa Nui, hence the name (Sehgal, 2003). By the end of the 1980s, rapamycin was known to also possess powerful antitumour and immunosuppressant activities, and interest regarding the mode of action of this intriguing macrocyclic lactone was growing. To address this, in 1991, Joe Heitman and his postdoctoral supervisor Michael Hall at the University of Basel Biozentrum, teamed up with Rao Movva, a staff scientist across the street in Sandoz Pharma Limited (now Novartis). Together, these researchers identified budding yeast (*Saccharomyces cerevisiae*) mutants that are completely resistant to the cytostatic affects of rapamycin and in the process genetically defined the molecular *T*arget *O*f *R*apamycin (*TOR*; Heitman *et al*., 1991).

These rapamycin-resistant mutants fall into three distinct classes. The most populous class of mutants displays recessive resistance to rapamycin resulting from disruption of *FPR1*. *FPR1* encodes a highly conserved peptidylprolyl isomerase known as the FK506-binding protein (FKBP12). Both rapamycin and FK506 (a structurally related immunosuppressant) bind to FKBP12 and inhibit its isomerase activity. However, *FPR1* is not an essential gene in yeast and therefore FKBP12 is not the cytostatic target of rapamycin; rather, it was determined to be a cofactor required for toxicity of rapamycin (Heitman *et al*., 1991; Koltin *et al*., 1991). Parenthetically, FKBP12 is also a cofactor required for FK506 to inhibit its target – calcineurin. The cytostatic targets of FKBP12-rapamycin are defined by the two other classes of rapamycin-resistant mutants. These classes were represented by much fewer isolates and exhibit dominant resistance to rapamycin resulting from a gain-of-function mutation in either of the two genes, *TOR1* or *TOR2*.

Cloning of *TOR1* and *TOR2* demonstrated that their encoded proteins are homologues (67% identical) and that they resemble phosphatidylinositol (PI) kinases (Cafferkey *et al*., 1993; Kunz *et al*., 1993; Helliwell *et al*., 1994). In subsequent studies, *TOR* homologues have been found in all eukaryotic genomes examined but, unlike yeast, which possess two *TOR* genes, nearly all other eukaryotic genomes possess only one *TOR* gene. Although TORs resemble lipid kinases, they actually belong to a family of Ser/Thr protein kinases known as phosphatidylinositol kinase-related kinases (PIKKs; Keith and Schreiber, 1995). Protein kinase activity is required for TOR function; the significance, if any, of the resemblance to lipid kinases is not understood.

The original rapamycin resistance-conferring alleles, *TOR1-1* and *TOR2-1*, contain single missense mutations, Ser1972Arg and Ser1975Ile, respectively, which

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prevent association with FKBP12-rapamycin (Cafferkey *et al*., 1993; Stan *et al*., 1994). Binding of FKBP12-rapamycin to TOR is also conserved among many eukaryotes, with plants (i.e. *Arabidopsis thaliana*) and nematodes (i.e. *Caenorhabditis elegans*) being notable exceptions (Long *et al*., 2002; Robaglia *et al*., 2004). As rapamycin is produced by a soil bacterium, it is probable that, like *A. thaliana* and *C. elegans*, many eukaryotes that live in soil have developed natural resistance to rapamycin. In susceptible organisms, binding of FKBP12-rapamycin to TOR appears to inhibit the ability of TOR to phosphorylate its substrates. However, the specific biochemical mechanisms of this inhibition are not known.

Genetic dissection of TOR function in yeast

Loss of TOR1 function is tolerated by yeast, but mutant cells grow slowly and are hypersensitive to temperature extremes and osmotic stress (Cafferkey *et al*., 1993; Kunz *et al*., 1993; Helliwell *et al*., 1994; Crespo *et al*., 2001). In contrast, loss of TOR2 is lethal, causing a random cell-cycle arrest (Kunz *et al*., 1993). Loss of both TOR1 and TOR2, similar to rapamycin treatment, is also lethal, but under these conditions cells arrest in G_1 (actually G_0; Helliwell *et al*., 1998a; Kunz *et al*., 1993). These early genetic observations suggested that (i) TOR1 and TOR2 have a (largely) redundant or shared function that is required for transit through G_1, (ii) this redundant function is sensitive to rapamycin and (iii) TOR2 performs an additional, unique function that TOR1 is unable to perform. The fact that rapamycin-resistant alleles of *TOR1* support growth in the presence of rapamycin further suggested that (iv) the essential, unique function of TOR2 is insensitive to rapamycin (Kunz *et al*., 1993; Helliwell *et al*., 1994; Zheng *et al*., 1995; Loewith and Hall, 2004).

As described in detail below, we know now that the TOR-shared and TOR2-unique functions, defined genetically, correspond to two separate signalling branches that regulate numerous aspects of cell growth and metabolism. The signalling specificity and differential sensitivity of TOR to rapamycin in these two branches is explained by the observation that TOR operates in each branch as a component of a distinct multiprotein complex (Loewith *et al*., 2002). TOR-complexes, and the signalling branches they regulate, are conserved from yeast to man (Wullschleger *et al*., 2006). Building on this, the aim of this review is to further discuss the contributions yeast-based studies have made to our current understanding of TOR signalling in eukaryotic cells and how future studies in yeast will continue to refine this understanding.

Structures of TORC1 and TORC2

Very large (~280 kDa) modular proteins

The amino-terminal half of TOR contains $\geqslant$20 tandem HEAT repeats (Figures 1 and 2). HEAT repeats, identified as a motif common among *H*untingtin, *E*longation factor 3, the *A* subunit of PP2A and *T*OR, are composed of 40–50 amino acids and form anti-parallel α-helices that are thought to mediate protein–protein interactions (Hemmings *et al*., 1990; Andrade and Bork, 1995; Andrade *et al*., 2001). A divergent HEAT repeat known as the FAT domain follows the block of canonical HEAT repeats (Bosotti *et al*., 2000; Perry and Kleckner, 2003). This putative scaffolding domain is found in all PIKKs and is always accompanied by a small domain (FATC) located at the extreme carboxyl terminus. Overexpression of the FAT domain is toxic to yeast; and, although the significance of this is not yet appreciated, this toxicity is suppressed by overexpression of phospholipase C (Alarcon *et al*., 1999). The FATC domain is essential for TOR function and NMR structural data suggest that a disulphide bridge in this domain may function to couple intracellular redox potential to TOR stability/function (Dames *et al*., 2005). Indeed, mammalian TOR (mTOR) has been suggested to respond to redox potential (Sarbassov and Sabatini, 2005). Between the FAT and FATC domains is the FKBP12-rapamycin-binding domain (FRB) followed by the kinase domain.

The presence of multiple putative protein–protein interaction domains suggested that TOR may stably interact with other proteins and, consistently, gel filtration experiments indicated that both TOR1 and TOR2 migrate with an apparent molecular mass of ~2 MDa. Purification of TOR1 and TOR2 led to the identification of several proteins that associate with TOR1 and/or TOR2. These TOR-associated proteins were subsequently found to define two distinct multi-protein TOR complexes (Loewith *et al*., 2002).

TOR complex 1 (TORC1)

TORC1 contains LST8, KOG1, TCO89 and either TOR1 or TOR2 (Figure 1 and Table 1; Loewith *et al*., 2002; Chen and Kaiser, 2003; Wedaman *et al*., 2003; Reinke *et al*., 2004). TORC1 is likely dimeric, built on a TOR–TOR dimer; but, unlike the case for a related PIKK, ATM where dimer dissociation is a critical step in activation, dimerization, or loss thereof, does not appear to be correlated with TORC1 function (Bakkenist and Kastan, 2003; Wullschleger *et al*., 2005 and unpublished data). All TORC1 components can be co-precipitated with FKBP12 but only in the presence of rapamycin (Loewith *et al*., 2002; and unpublished data). This observation indicates that the integrity of TORC1 is unaffected by FKBP12-rapamycin and, importantly, implies that FKBP12-rapamycin does not inhibit TOR signalling via disruption of TORC1. Similarly, FKBP12-rapamycin inhibits mammalian TORC1 (mTORC1) activity; and, although initially thought otherwise (Kim *et al*., 2003; Oshiro *et al*., 2004), FKBP12-rapamycin does not affect mTORC1 stability (Sarbassov *et al*., 2004; McMahon *et al*., 2005).

TORC1 localization

Several studies have investigated the localization of TORC1 using a variety of techniques including sub-

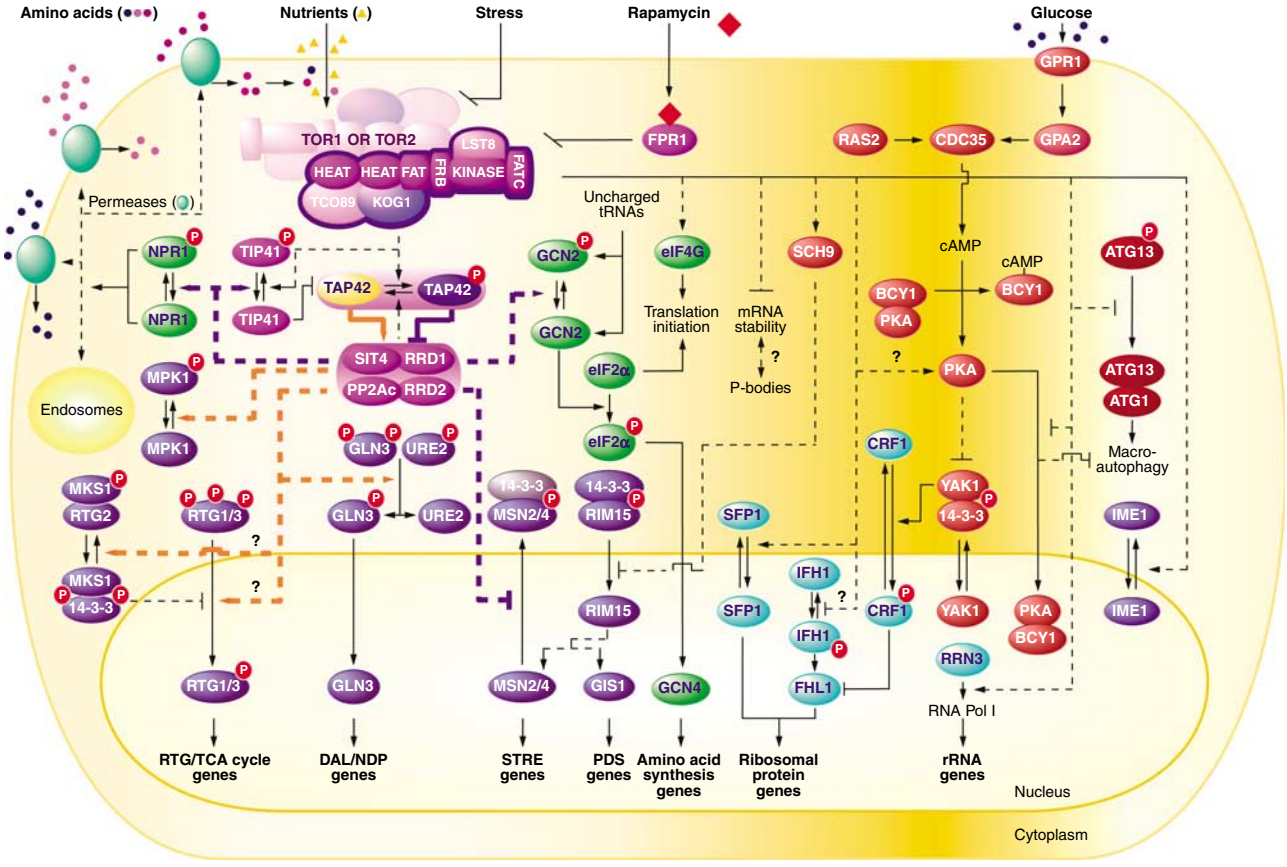

Figure 1 Diagram of the TORC1 signalling network in budding yeast. TORC1, pictured as a dimer, is found at the upper-left corner. The domains found in TOR are indicated, as are the other TORC1 subunits. TORC1 promotes cell growth by stimulating anabolic processes such as translation initiation and permease activity (green proteins), and by stimulating expression of the translation machinery (via turquoise proteins). TORC1 inhibits catabolic process such as autophagy (dark red proteins), and blocks transcriptional stress responses mediated by RTG1/3, GLN3, MSN2/4 and IME1 (violet proteins). Some TORC1 readouts are also influenced by the protein kinase A (PKA) signalling pathway and SCH9 (red proteins). GPR1 is a plasma membrane G protein-coupled receptor (GPCR) that interacts with the heterotrimeric G protein α-subunit, GPA2. GPR1, GPA2 and RAS2 sense nutritional signals and correspondingly regulate adenylyl cyclase (CDC35) activity and consequently cAMP synthesis. TORC1 controls other readouts via type 2A (PP2A) and/or the PP2A-like protein phosphatase SIT4 (magenta proteins). TORC1 controls nuclear import and/or nuclear export of SFP1 and IME1. Arrows and bars denote positive and negative interactions, respectively. Solid arrows and bars refer to direct interactions, dashed arrows and bars refer to indirect and/or potential interactions. Red circles containing the letter P denote phosphorylated amino-acid residues. STRE, stress-responsive element; PDS, post-diauxic shift element; DAL, degradation of urea and allantoin; NDP, nitrogen discrimination pathway; RTG, retrograde regulation; TCA, tricarboxylic acid; P bodies, processing bodies (i.e. discrete cytoplasmic foci to which mRNA is routed for degradation/storage). See text for further details.

cellular fractionation, immunogold electron microscopy, and direct and indirect immunofluorescence microscopy (Cardenas and Heitman, 1995; Kunz *et al*., 2000; Chen and Kaiser, 2003; Wedaman *et al*., 2003; Reinke *et al*., 2004; Araki *et al*., 2005 see also at http://yeastgfp.ucsf.edu). These studies all agree that TORC1 associates with membranes – most likely the plasma membrane, the vacuolar (lysosome) membrane and perhaps endosomal membranes – but the functional significance of association with (multiple) membranes is not yet appreciated. The association with the vacuolar membrane is intriguing, given that TORC1 is widely believed to be regulated by nutrient cues (see below) and the vacuole is a major nutrient store in yeast (De Virgilio and Loewith, 2006; Wullschleger *et al*., 2006). There is currently no indication that TORC1 localization is influenced by either rapamycin treatment or nutrient cues (Kunz *et al*., 2000; Chen and Kaiser, 2003). Localization of TOR in mammalian cells is also ambiguous with reports suggesting that mTOR can be found in intracellular polyglutamine-containing protein aggregates, associated with mitochondrial, endoplasmic reticulum and Golgi apparatus membranes, and shuttling in and out of the nucleus (Desai *et al*., 2002; Drenan *et al*., 2004; Ravikumar *et al*., 2004; Bachmann *et al*., 2006).

LST8

Like TOR2, but unlike TOR1 and other TOR-associated proteins, LST8 is found in both TORC1 and TORC2. LST8 is an essential, 34 kDa protein composed entirely of seven WD40 repeats and is conserved from yeast to man (mLST8 also known as G beta-like or

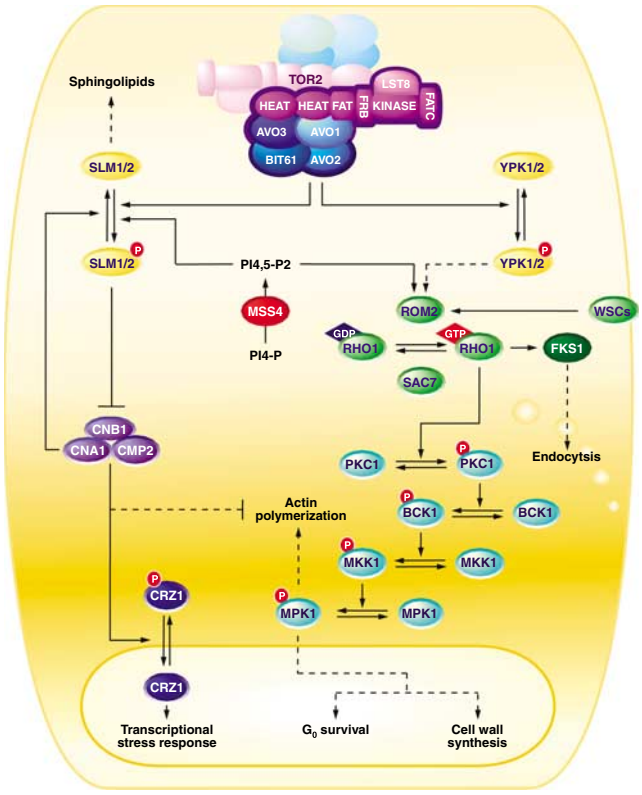

Figure 2 Diagram of the TORC2 signalling network in budding yeast. TORC2, pictured as a dimer, is found at the top-centre. The domains found in TOR2 are indicated, as are the other TORC2 subunits. Direct substrates of TORC2 (yellow) propagate TORC2 signals to the actin cytoskeleton. SLM1/2 bind to and inhibit the activity of calcineurin (violet), which promotes actin polarization. YPKs act upstream of the RHO1 GTPase switch (green). RHO1 couples TORC2 signals to the actin cytoskeleton and gene expression via a MAPK module (turquoise), and to the endocytosis machinery via a the glucan synthase FKS1 (dark green). TORC2 may also (indirectly) regulate the production of sphingolipids. TORC2 signals cross talk with phosphotidylinositides (PIs) generated by the PI4P-5 kinase MSS4 (red). See text and legend of Figure 1 for further details.

GβL; Table 1). After TOR, LST8 is the best-studied TORC component but its precise roles are far from clear. In TORC2, LST8 binds to the kinase domain of TOR2 and is required for TORC2 stability, *in vitro* kinase activity and *in vivo* function (Loewith *et al*., 2002; Wullschleger *et al*., 2005). LST8 also acts positively with TOR in TORC1 (Loewith *et al*., 2002). Several alleles of *LST8* have been generated and the differing effects of these alleles on various TORC1 readouts (detailed below) suggest that LST8 couples TORC1 to downstream effectors (Roberg *et al*., 1997; Liu *et al*., 2001; Chen and Kaiser, 2003). In contrast, mLST8 has been proposed to communicate upstream signals to mTORC1 (Kim *et al*., 2003).

KOG1

KOG1 is an essential, 176 kDa protein containing four HEAT repeats and seven C-terminal WD40 repeats. The presence of these putative protein–protein interaction domains is consistent with the proposal that the mammalian orthologue of KOG1, raptor, functions as a scaffold, coupling mTOR to substrates (Hara *et al*., 2002). Curiously, the N-terminus of KOG1 contains a caspase-like domain (Ginalski *et al*., 2004). Although the catalytic cys-his dyad is conserved, protease activity of KOG1/raptor has not been reported. KOG1 acts positively in TORC1 (Loewith *et al*., 2002), and this has been elegantly confirmed using temperature-sensitive alleles of KOG1 (Araki *et al*., 2005).

TCO89

The 89-kDa subunit of TOR complex one (TCO89) is a nonessential protein originally identified in a screen for mutations that block glycerol uptake under conditions of osmotic stress (Holst *et al*., 2000). Conserved only among fungi closely related to *S. cerevisiae*, TCO89 is very rich in Ser and Thr residues and heavily phosphorylated *in vivo* (unpublished). Cells lacking TCO89 are hypersensitive to rapamycin and caffeine, and in other ways resemble cells lacking TOR1, suggesting that TCO89 functions positively in TORC1 (Reinke *et al*., 2004).

TOR complex 2 (TORC2)

TORC2 contains LST8, AVO1, AVO2, AVO3, BIT2, BIT61 and TOR2, but not TOR1 (Figure 2 and Table 1; Loewith *et al*., 2002; Wedaman *et al*., 2003; Reinke *et al*., 2004; Fadri *et al*., 2005). TORC2 is likely dimeric, built on a TOR2–TOR2 dimer (Wullschleger *et al*., 2005). Consistently, metazoan TOR can also dimerize (Wullschleger *et al*., 2005; Zhang *et al*., 2006). TOR2, when functioning in TORC2, cannot be bound by FKBP12-rapamycin, perhaps because a TORC2 component occludes the FRB domain. This failure of FKBP12-rapamycin to bind explains why TORC2 function is insensitive to rapamycin (Loewith *et al*., 2002). Analogously, mTORC2 is also insensitive to rapamycin because mTOR in mTORC2 cannot be bound by FKBP12-rapamycin (Jacinto *et al*., 2004; Sarbassov *et al*., 2004), although very recent data suggest that *de novo* formation of mTORC2 may eventually be compromised after extended exposure to rapamycin (Sarbassov *et al*., 2006). A complex resembling TORC2 has also recently been described in the slime mould *Dictyostelium discoideum* (Lee *et al*., 2005).

TORC2 localization

Indirect immunofluorescence imaging of tagged AVO2 and AVO3 suggest that TORC2 localizes in discrete punctae at the plasma membrane (R Shioda and MN Hall, personal communication). Although the significance of these punctae remains to be determined, this localization pattern is very similar to those of SLM1 and SLM2, homologous PH domain containing proteins that are direct substrates of TORC2 (described further below; Audhya *et al*., 2004). The mechanism(s) that tethers TORC1 or TORC2 to membranes is unknown.

Table 1 Human orthologues of *S. cerevisiae* TOR signalling network components[a]

S. cerevisiae	*H. sapiens*[b]	*Function (in yeast)*
TORC1, - 2		
TOR1, -2	mTOR	PI-kinase related protein kinase, TOR1 is in TORC1, TOR2 is in TORC1 and TORC2
LST8	mLST8	Binds TOR kinase domain, required for complex stability and TORC1/TORC2 kinase activity
KOG1	Raptor	TORC1 component, couples TOR to substrates?
AVO1	hSIN1	TORC2 component, required for complex stability, heavily phosphorylated
AVO3	Rictor	TORC2 component, required for complex stability, heavily phosphorylated
Phosphatase modules		
CDC55	PPP2R2A-D	Regulatory subunit B of PP2A
CNA1, CMP2	PPP3CA-C	Catalytic subunit of calcineurin A, Ca^{2+}/calmodulin-regulated protein phosphatase
CNB1	PPP3R1, -R2, CHP, -2	Calcineurin B; regulatory subunit of calcineurin A
PPH21, -22	PPP2CA, -BA	Catalytic subunit of protein phosphatase 2A (PP2A)
PPH3	PPP4C	Catalytic subunit of protein phosphatase (PP2A-like)
RRD1	PPP2R4	Subunit of the TAP42-SIT4-RRD1 complex
RRD2	PPP2R4	Subunit of the TAP42-PPH21-RRD2 complex
RTS1	PPP2R5A-E	Regulatory subunit B' of PP2A
SAP4, -155, -185, -190	SAPS1-3	SIT4-associated proteins
SIT4	PPP6C	PP2A-related serine-threonine phosphatase
TAP42	IGBP1	Regulation of the catalytic activity of PP2A-related serine/threonine phosphatases
TIP41	TIPRL	Binds and regulates TAP42
TPD3	PPP4R1, PPP2R1A, -B	Regulatory subunit A of PP2A
Protein kinases		
NPR1	CHEK1	Antagonizes ubiquitin-mediated degradation of amino acid transporters
GCN2	EIF2AK1, -2, -4	eIF2α kinase
SCH9	RPS6KB1, AKT1, -2, SGK2, STK32B	Regulates G_1 progression, G_0 entry, ribosome biosynthesis, cell size, and life span in response to nutrient availability
RIM15	NDR-/LATS-related kinases[c]	Orchestrates G_0 entry in response to various nutrients
TPK1, -2, -3	PRKX, -Y, PRKACA, -B, -G,	Catalytic subunit of cAMP-dependent protein kinase (PKA)
YAK1	DYRK1A, - 1B, -2, -3	Involved in growth control in response to glucose availability
YPK1, -2	RPS6KB1, -2, AKT3, SGK, -2, -3, STK32B	Required for receptor-mediated endocytosis; involved in sphingolipid-mediated and cell integrity signaling pathways
PKC1	PRKCE, -CH, -CA, -CB1,-CG, -CD, -CQ, PKN2	Protein kinase C (PKC), Essential for cell wall remodeling during growth, phosphorylates BCK1
BCK1	YSK4, MAP3K2, -14	MAP kinase kinase kinase phosphorylates downstream kinases MKK1, -2
MKK1, -2	MAP2K1, -2, -5	MAP kinase kinase, phosphorylates MPK1 following activation by BCK1
MPK1	MAPK1, - 4, -6, -7, -11-15	MAP kinase regulates maintenance of cell wall integrity and the cell cycle progression
ATG1	ULK1, -2, -3, STK35, PDIKL1	Required for autophagy and cytoplasm-to-vacuole targeting pathway
Transcription		
ESA1	MYST1-4	Histone acetyltransferase catalytic subunit of the native multisubunit complex (NuA4)
FHL1	FOXN1, -4[d]	Transcriptional regulator
GCN4	JUN, -B, - C	Transcriptional activator of amino acid biosynthetic genes
GLN3	GATA1-3, -5, TRPS1	Transcriptional activator of genes regulated by nitrogen catabolite repression (NCR)
RPD3	HDAC1, -2, - 3, -8	Histone deacetylase; regulates transcription and silencing
HMO1	HMGB1-4; HMG20A, SP100, GCX1, TFAM, UBTF, TOX	Chromatin associated high mobility group (HMG) family member; rDNA-binding component of the Pol I transcription system
RTG3	USF1, -2, TFE3, -B, -C, MITF	bHLH/Zip transcription factor, activates retrograde response in complex with RTG1
SFP1	JAZF1	Transcription factor, controls expression of ribosome biogenesis genes
SIN3	SIN3A, -B	DNA binding subunit of SIN3-RPD3 histone deacetylase complex
URI/BUD27	URI/RMP	Involved in bud-site selection and TORC1-controlled gene expression
Miscellaneous		
GTR1	RRAGA, -B	GTP-binding protein involved in exit from rapamycin-induced growth arrest

Table 1 (*continued*)

S. cerevisiae	*H. sapiens*[b]	*Function (in yeast)*
GTR2	RRAGC, -D	GTP-binding protein involved in exit from rapamycin-induced growth arrest
MSS4	PIP5K1A, -B, -C, K2A, -B	Phosphatidylinositol-4-phosphate 5-kinase, controls actin cytoskeleton/morphogenesis
RHO1	RHOA, -B, -C[d]	GTP-binding protein of the rho subfamily regulates PKC1 and FKS1
ROM2	ARHGEF3, NET1	GDP/GTP exchange protein for RHO1 and RHO2
SAC7	ARHAGAP6, -11A, -29, HMHA1, GMIP	GTPase activating protein (GAP) for RHO1
SSD1	DIS3, MGC4562, -42174	Role in maintenance of cellular integrity, interacts with TOR pathway components

[a]Human orthologues were identified with the web-based YOGY resource (http://www.sanger.ac.uk/PostGenomics/S_pombe/YOGY). Gene names correspond to the nomenclature at EXPASY (http://www.expasy.org). [b]At present, no human orthologues could be identified for the following yeast proteins: ATG13, AVO2, BIT2, BIT61, CRF1, EGO1, EGO3, FKS1, GIS1, IFH1, IME1, MKS1, RTG1, RTG2, SLM1, SLM2, TCO89. CRZ1, MSN2, and MSN4 may have human orthologues, but the regions of homology are generally restricted to the zinc-finger domain. [c]RIM15 belongs to the family of nuclear DBF2-related (NDR) and large tumour suppressor (LATS) ser/thr kinase sublasses of AGC kinases. [d]RHO1 and FHL1 may have many more human orthologues; the selection was reduced to the best hits as assessed by Basic Local Alignment Search Tool (BLAST) analysis at the National Center for Biotechnology Information (NCBI).

AVO1–3

The AVOs adhere voraciously to TOR2 (Loewith *et al*., 2002). AVO1 (131 kDa) and AVO3 (164kDa) are essential proteins, but AVO2 (47 kDa) is not. AVO1 and AVO3 bind cooperatively to the N-terminal half of TOR2 and, like LST8 (described above), are required for TORC2 stability. The AVO designation of AVO2 is somewhat of a misnomer as this protein associates indirectly with TOR2 via AVO1 and AVO3 (Wullschleger *et al*., 2005). The AVOs appear to be phosphorylated by TOR2, but kinase activity of TOR2 is not required for TORC2 stability (Wullschleger *et al*., 2005). Although mammalian AVO3, now known as rictor, binds to mTOR in the context of mTORC2 (Jacinto *et al*., 2004; Sarbassov *et al*., 2004; Wullschleger *et al*., 2006), binding of mammalian AVO1 (SIN1) to mTOR was not observed previously (Loewith *et al*., 2002). However, since that report, the existence of multiple splice variants of SIN1 have been described, raising the possibility that a SIN1 variant (other than the SIN1β isoform tested) may interact with mTOR (Schroder *et al*., 2004). AVO2 homologues are found only in fungi closely related to *S. cerevisiae*.

All of the AVOs act positively in TORC2 (Loewith *et al*., 2002; Audhya *et al*., 2004; Fadri *et al*., 2005; Ho *et al*., 2005), but the specific molecular functions of these proteins are unknown. AVO1 has no obvious domains, whereas AVO2 contains several ankyrin repeats and may function as an adaptor linking TOR2 to substrates (Audhya *et al*., 2004; Fadri *et al*., 2005). AVO3 contains a RasGEFN domain, which is found N-terminal to the catalytic GDP/GTP exchange domain in some guanine nucleotide exchange factors for Ras-like small GTPases. However, this domain is not well conserved among AVO3 homologues and its functional significance in yeast is also not clear (Ho *et al*., 2005).

BIT61 and BIT2

The BITs are related (45% identical) 61 kDa proteins that bind TOR2 (Reinke *et al*., 2004; Fadri *et al*., 2005). The BITs are not essential, either alone or in combination (RL, unpublished) and their molecular function is not known. They appear to function positively with TOR2 in TORC2. Like AVO2, they bind to TORC2 substrates and thus may serve an adaptor function (Fadri *et al*., 2005). Obvious BIT homologues appear to be confined to fungi closely related to *S. cerevisiae*; *hbrB*, a BIT-related gene from *Aspergillus nidulans*, is required for polarized growth (Gatherar *et al*., 2004), which is consistent with TORC2 function as described below.

TOR regulates cell growth

Similarly to nitrogen- or carbon-source withdrawal, exposure to rapamycin arrests actively growing yeast within one generation in a G_0-like state. Although not immediately appreciated, this observation was the first clue that rapamycin-sensitive TOR signals may function by coupling nutrient cues to the cell growth machinery. Consistent with this idea, subsequent studies demonstrated that rapamycin treatment results in a dramatic downregulation of anabolic processes, and an upregulation of catabolic and stress-response processes (diagrammed in Figure 1).

Phenotypes associated with loss of TOR2 function demonstrate that rapamycin-insensitive TOR2 signals also regulate growth; but, unlike the temporal aspects of growth regulated by rapamycin-sensitive TOR signals, rapamycin-insensitive TOR2 signals regulate spatial aspects of cell growth. It is now generally accepted that TOR is a central regulator of cell growth and these functions are conserved from yeast to man (Schmelzle and Hall, 2000; De Virgilio and Loewith, 2006; Wullschleger *et al*., 2006). The many growth-related readouts of rapamycin-sensitive (i.e. TORC1) and rapamycin-insensitive (i.e. TORC2) signals are detailed below.

TORC1 promotes anabolic processes

The most obvious anabolic process regulated by TORC1 is protein synthesis. TORC1 positively controls protein synthesis at multiple levels, specifically by

regulating (i) translation initiation, (ii) expression and assembly of the translation machinery, (iii) mRNA turnover and (iv) the activity of high-affinity amino-acid permeases that pump amino acids for immediate use by the translation machinery.

Translation initiation

A critical insight into TOR function was made with the observation that rapamycin treatment elicits a rapid and pronounced inhibition of translation initiation (Barbet *et al*., 1996). This finding was the first hint that TOR is part of a signalling pathway that couples nutrient cues to cell growth. TORC1 appears to regulate translation initiation at multiple levels although the intricacies of these signalling pathways remain to be elucidated. The mechanisms by which TORC1 regulates translation initiation are now better characterized in mammalian systems and are reviewed elsewhere in this issue. In yeast, TORC1 translation targets include eIF4E, eIF4G and eIF2.

In budding yeast, eIF4E, the 5′-mRNA cap-binding protein, is encoded by *CDC33*. *cdc33* and *tor* mutants display similar phenotypes, which, among other observations, suggested that TORC1 may maintain translation initiation via eIF4E (Barbet *et al*., 1996; Danaie *et al*., 1999). In mammalian cells, mTORC1 phosphorylates the eIF4E-binding proteins (4E-BPs), which prevents the 4E-BPs from binding and inhibiting eIF4E. Consistently, deletion of *EAP1*, a gene encoding a putative yeast orthologue of the 4E-BPs, confers partial resistance to rapamycin (Cosentino *et al*., 2000). EAP1 is a phosphoprotein, but whether or not its phosphorylation is regulated by TORC1 has not been determined.

eIF4G is an adaptor protein that binds to eIF4E and recruits additional initiation factors to the 5′ cap. In yeast, rapamycin treatment and nutrient depletion both result in enhanced degradation of eIF4G (Berset *et al*., 1998). Studies in mammalian cells suggest that mTORC1 regulates both the phosphorylation of eIF4G and the association of eIF4G with eIF3, suggesting that regulation of eIF4G may also be an important mechanism by which TORC1 regulates translation initiation (Raught *et al*., 2000; Harris *et al*., 2006).

TORC1 has also been shown to regulate translation initiation via eIF2 (Cherkasova and Hinnebusch, 2003; Kubota *et al*., 2003). GTP-loaded eIF2 is required to deliver an initiator methionyl-tRNA to the 40S ribosomal subunit and eventually to the translation initiation codon of an mRNA. The α-subunit of eIF2 (eIF2α) is phosphorylated by GCN2 and this inhibits general translation initiation (Hinnebusch, 2005). The kinase activity of GCN2 is stimulated by uncharged tRNAs, which bind to a histidyl-tRNA synthetase-related domain in GCN2. GCN2 is negatively regulated by phosphorylation of Ser-577, which reduces the tRNA binding activity of GCN2 and subsequently inhibits kinase activity. Although the upstream kinase is unknown, phosphorylation of Ser-577 is promoted by TORC1: rapamycin treatment results in a rapid loss of Ser-577 phosphorylation, activation of GCN2 kinase activity, increased eIF2α phosphorylation and ultimately decreased general translation initiation (Hinnebusch, 2005). Regulation of translation by GCN2 is widely conserved; however, the involvement of TORC1 in this process has currently only been reported in budding yeast.

Expression and assembly of the translation machinery

Yeast ribosomes consist of the 5S, 5.8S, 18S and 25S RNAs in addition to 137 ribosomal proteins (RPs). Robust translation additionally requires the gene products of the ribosome biogenesis (Ribi) regulon (Jorgensen *et al*., 2002). The more than 200 *Ribi* genes encode accessory factors that assemble and modify rRNA and RPs in the nucleolus, translation factors, tRNA synthetases, subunits of RNA polymerases I and III, and enzymes involved in ribonucleotide metabolism. Expression of this suite of genes is coordinately regulated with the *rRNA* gene locus and the *RP* and *tRNA* genes. This requires the action of all three RNA polymerases and, in growing cells, accounts for approximately 95% of total transcription. Thus, fabrication of the translation machinery in budding yeast consumes an extraordinary fraction of the cell's resources and consequently is tightly regulated in response to nutrient and energy conditions (Warner, 1999). Rapamycin treatment, like nutrient limitation, results in a dramatic reduction in expression of *RP*, *Ribi*, *rRNA* and *tRNA* genes (Zaragoza *et al*., 1998; Cardenas *et al*., 1999; Hardwick *et al*., 1999; Powers and Walter, 1999), and TORC1 is also required for efficient processing of the 35S precursor *rRNA* (Powers and Walter, 1999). Although the mechanisms by which TORC1 regulates *rRNA* processing are not understood, a number of recent studies have begun to elucidate the signalling pathways that couple TORC1 to the transcriptional regulation of *RP*, *Ribi*, *rRNA* and *tRNA* genes.

Several transcription factors regulate *RP* gene expression in a TORC1-dependent fashion. FHL1 contains a fork head DNA-binding domain and is found localized to the promoters of most *RP* genes (Lee *et al*., 2002). FHL1 binding to *RP* promoters appears to be constitutive, and is facilitated by HMO1, a high-mobility group protein, and RAP1, a protein required for expression of *RP* genes among many other activities (Hall *et al*., 2006). The mutually exclusive interaction of FHL1 with two other proteins, IFH1 and CRF1, appears to be regulated by TORC1 activity (Martin *et al*., 2004b; Schawalder *et al*., 2004; Wade *et al*., 2004; Rudra *et al*., 2005). IFH1–FHL1 complexes stimulate, whereas CRF1–FHL1 complexes suppress expression of RP genes. Both IFH1 and CRF1 are phosphoproteins with phosphorylation dictated by TORC1 activity. CRF1 is phosphorylated by YAK1 (Martin *et al*., 2004b), but how YAK1, or the yet unidentified IFH1-kinase, is regulated by TORC1 is not entirely clear; possible upstream kinases include protein kinase A (PKA) and casein kinase II (Krogan *et al*., 2004; Martin *et al*., 2004b; Rudra *et al*., 2005).

TOR-dependent regulation of *RP* genes is still observed in the absence of the FHL1/IFH1/CRF1 system, suggesting the existence of other mechanisms

by which TOR regulates *RP* expression. One candidate is the transcription factor SFP1. Under favourable growth conditions, SFP1 localizes to many *RP* and *Ribi* promoters, and inactivation of TORC1 with rapamycin results in a relocalization of SFP1 to the cytoplasm (Lee *et al*., 2002; Jorgensen *et al*., 2004; Marion *et al*., 2004). How TORC1 influences SFP1 localization is not known. The AGC-family kinase SCH9 also has been proposed to regulate *RP* and *Ribi* gene expression in a TORC1-dependent, but FHL1- and SFP1-independent fashion (Jorgensen *et al*., 2004). Lastly, a number of chromatin-modifying activities, including the remodel the structure of chromatin (RSC) complex, the RPD3 histone deacetylase and the ESA1 histone acetylase, have also been implicated in regulation of RP genes (Damelin *et al*., 2002; Ng *et al*., 2002; Rohde and Cardenas, 2003; Humphrey *et al*., 2004). Presumably, these chromatin-remodelling/modifying complexes are recruited by TORC1-regulated transcription factors, although RPD3 is apparently not required for inhibition of RNA polymerase I transcription by rapamycin (Oakes *et al*., 2006).

Although mechanistically unclear, TORC1 regulates *rRNA* transcription by influencing the activity of RRN3, an essential RNA polymerase I initiation factor (Claypool *et al*., 2004). The next big challenge is to ascertain how the expression of *RP* and *Ribi* genes is coordinated with the expression of *rRNA* and *tRNA* genes. Like the *RP* and *Ribi* genes (transcribed by RNA polymerase II), expression of the *35S rRNA* precursor (transcribed by RNA polymerase I) is also influenced by HMO1, RSC, RPD3 and possibly FHL1 (Hermann-Le Denmat *et al*., 1994; Damelin *et al*., 2002; Ng *et al*., 2002; Humphrey *et al*., 2004). Furthermore, both biochemical and genetic analyses in human and yeast cells suggested that TORC1 also regulates transcription via a protein known as URI/BUD27 (for unconventional prefoldin RBP5 interactor; Gstaiger *et al*., 2003). Although the exact nature of the mechanism by which URI controls transcription remains to be elucidated, what is of interest here is its reported interaction with RPB5, a shared subunit of all three RNA polymerases (Woychik *et al*., 1990). These observations suggest some possible mechanisms for co-regulation.

mRNA turnover

mRNA turnover also plays an important role in the control of gene expression. There are two general pathways of mRNA decay in eukaryotic cells (Coller and Parker, 2004; Parker and Song, 2004). Most frequently, mRNA decay begins with deadenylation, that is, a shortening of the poly(A) tail at the 3′ end of the mRNA. Subsequently, deadenylated mRNAs can be either degraded in a $3' \rightarrow 5'$ direction by the cytoplasmic exosome, or decapped and degraded in a $5' \rightarrow 3'$ direction by the XRN1 exonuclease. The deadenylation-dependent decapping pathway is the major mRNA decay mechanism in actively growing yeast. Both nutrient limitation and rapamycin treatment accelerate this major mRNA decay pathway, resulting in an enhanced turnover of some but not all mRNAs (Albig and Decker, 2001). However, the control of mRNA stability by TORC1 is complex and the relevant signalling mechanisms are not known. Some messages are degraded rapidly following rapamycin treatment, whereas others have much slower decay kinetics, suggesting that TORC1 controls decay by multiple mechanisms.

This complexity might be explained if TORC1 were to regulate the movement of mRNAs between polysomes and cytoplasmic processing bodies (P bodies). P bodies are sites where mRNA can be stored and/or decapped and degraded. The Parker group has recently demonstrated that there is a general translation repression machinery that evicts mRNAs from translation and targets them into P bodies (Brengues *et al*., 2005; Coller and Parker, 2005). When the rate of translation is low, for example during nutrient limitation or in the presence of noxious stress, mRNAs exit translation and P bodies accumulate. Conversely, recovery from stress results in a decline of P bodies and a return of the formerly sequestered mRNA to polysomes. As outlined below, it is thought that TORC1 activity is regulated by both nutrient and stress cues. Thus, although not yet tested, TORC1 signals could promote mRNA flux out of P bodies or alternatively inhibit the exit of mRNAs from translation and this could potentially explain the differential stability of mRNAs observed following rapamycin treatment.

Permease activity

Budding yeast can use a wide variety of compounds as carbon or nitrogen sources. However, when provided with a mixture, preferred carbon and nitrogen sources are exclusively utilized before non-preferred, suboptimal sources. How yeast perceives the quality and quantity of these (and other) nutrient sources and couples this information to the regulatory pathways governing the hierarchical consumption of these sources is not understood, but TORC1 appears to be involved.

Yeast encode many (>270) membrane transporters by which they can selectively transport specific nutrients (Van Belle and Andre, 2001). For example, there are approximately 20 hexose transporters and 19 amino-acid transporters, each with distinct substrate specificity, affinity and transport capacities; the expression and activity of many of these transporters is tightly regulated by nutrient availability (Ozcan and Johnston, 1999; Magasanik and Kaiser, 2002). Under optimal growth conditions, many high-affinity, substrate-selective permeases are expressed and targeted to the plasma membrane to pump nutrients that are used directly in ATP production, and in the case of amino-acid transporters, protein synthesis (Magasanik and Kaiser, 2002). In contrast, in poor nutrient conditions, a few low-affinity, broad-specificity permeases are expressed and targeted to the plasma membrane. These permeases pump a wider range of carbon and nitrogenous compounds as alternative nutrient sources that can be catabolized by the cell.

Upon downshift from good to poor growth conditions, high-affinity permeases, whether at the plasma membrane or at internal membranes along the secretory

route, are ubiquitinated, endocytosed and trafficked to the vacuole for degradation (Helliwell *et al*., 2001; Schmelzle *et al*., 2004). In many cases, turnover of these transporters is also induced by rapamycin treatment (Schmidt *et al*., 1998; Beck and Hall, 1999; Schmelzle *et al*., 2004). This suggests that TORC1 positively regulates the activity of these transporters as a component of the signalling pathway that couples nutrient cues to the post-translational sorting of high-affinity transporters. TORC1 may regulate the sorting of these transporters via the protein kinase NPR1 (Schmidt *et al*., 1998; Beck *et al*., 1999; De Craene *et al*., 2001). TORC1 activity mediates phosphorylation, and presumably activity of NPR1, which, in undefined ways, is thought to regulate the sorting of several amino-acid transporters (Schmidt *et al*., 1998; De Craene *et al*., 2001; Jacinto *et al*., 2001). Consistent with these yeast studies, mTORC1 also appears to maintain the activity of numerous nutrient transporters (reviewed in Edinger, 2005).

In contrast, TORC1 negatively regulates low-affinity transporters. Regulation of low-affinity transporters by TORC1 occurs primarily at the transcriptional level. Nutrient starvation or rapamycin treatment strongly induces expression of the ammonia permease gene, *MEP2*, and the general amino-acid permease gene, *GAP1*, via two GATA transcription factors, GLN3 and GAT1, which are negatively regulated by TORC1 (described below). The sorting of GAP1 is also regulated by NPR1 and nitrogen, particularly by levels of intracellular amino acids. However, these sorting decisions have (curiously) been reported to be independent of TORC1 (De Craene *et al*., 2001; Chen and Kaiser, 2002; Rubio-Texeira and Kaiser, 2006).

TORC1 inhibits catabolic processes

When nutrients are in ample quantity and sufficient quality, yeast cells rapidly grow and divide. However, introduction of any of a number of stresses, including starvation, elicits an abrupt cessation of mass accumulation and cell cycle arrest. Thus 'growth' and 'stress response' are generally incompatible and mutually exclusive states. As elaborated above, TORC1 promotes the accumulation of mass by regulating a number of growth-related readouts. Consistently and conversely, TORC1 inhibits growth arrest by negatively regulating a number of stress-related functions including autophagy and the activities of a number of stress-responsive transcription factors.

Autophagy

Autophagy is a ubiquitous process of controlled self-digestion, which is responsible for the degradation and recycling of nonessential cellular components (from macromolecules to whole-cell organelles). The primary role of autophagy in unicellular organisms is to liberate metabolites under conditions of nutrient starvation, thereby enabling the cells to adapt to and survive extended periods of starvation. Based on morphological characteristics, three types of autophagy are defined in yeast: macro-, micro-, and piecemeal microautophagy.

Macroautophagy. Macroautophagy (often simply referred to as autophagy) involves the formation of double-layered vesicles (autophagosomes), which enclose parts of the cytosol in their lumen. Upon fusion of the autophagosome with the lysosome or vacuole, the inner vesicle (autophagic body) is released into the lumen of the degradative compartment where its membrane and its cytoplasmic cargo are degraded and eventually recycled (Yorimitsu and Klionsky, 2005). Reduction of available nitrogen or inhibition of TORC1 with rapamycin causes a rapid induction of macroautophagy in yeast. Key to this process is the TORC1-controlled interaction of ATG13 with the Ser/Thr protein kinase ATG1 (Kamada *et al*., 2004). Under nutrient-rich conditions, TORC1 directly or indirectly causes hyperphosphorylation of ATG13, thereby reducing its affinity for and promoting dissociation from ATG1 (Funakoshi *et al*., 1997; Scott *et al*., 2000). Under these conditions, macroautophagy is minimal (Kamada *et al*., 2000). TORC1 inactivation results in partial dephosphorylation of ATG13, ATG13–ATG1 complex formation and subsequent induction of macroautophagy via recruitment of several additional proteins (e.g., ATG11, ATG17 and VAC8; Yorimitsu and Klionsky, 2005). At present, no *bona fide* targets of ATG1 are known and it is still a matter of debate whether ATG1 primarily controls maroautophagy via a phosphorylation event(s) or via a proposed structural role in autophagic complex formation (Matsuura *et al*., 1997; Kamada *et al*., 2000; Abeliovich *et al*., 2003). Notably, macroautophagy is also regulated by additional protein kinases, such as PKA (Budovskaya *et al*., 2004), SNF1 (Wang *et al*., 2001) and GCN2 (Talloczy *et al*., 2002).

Inhibiton of mTORC1 or withdrawal of growth factors or nutrients also elicits macroautophagy in mammalian cells. However, the physiological relevance of macroautophagy in metazoans is just beginning to be explored. Macroautophagy may be required for the survival of cells with damaged mitochondria (see also below) or to help clear protein aggregates associated with neurodegenerative disease. Conversely, macroautophagy has been observed in the non-apoptotic cell death of numerous cell types and may thus serve a tumour suppressor function (Lum *et al*., 2005). So far, no APG13 (or APG17) homologues have been identified in higher eukaryotes; however, the human UNC-51-like kinases ULK1 and ULK2 are putative ATG1 homologues (Table 1; Okazaki *et al*., 2000).

Microautophagy. Microautophagy is less well characterized than macroautophagy, but is also found in higher eukaryotes (Dean, 1977). Morphologically, the hallmarks of microautophagy are cytoplasm-sequestering vesicles formed by direct invagination of the lysosomal/vacuolar membrane (Yorimitsu and Klions-

ky, 2005). Interestingly, even though individual components of the ATG-dependent macroautophagic machinery appear not to be directly involved in microautophagy, induction of macroautophagy (by nutrient starvation or TORC1 inactivation) is a prerequisite for induction of microautophagy (Müller *et al*., 2000; Sattler and Mayer, 2000). A possible explanation for these findings is that microautophagy, which leads to uptake and degradation of vacuolar membrane, primarily serves to compensate the massive influx of membranous material towards the vacuolar membrane that occurs during continuous fusion of the outer membranes of macro-autophagosomes. Although this interdependency between macro- and microautophagy makes it difficult to assess the role of TORC1 for microautophagy, recently published *in vivo* and cell-free microautophagy assays suggest that TORC1 may in fact positively regulate microautophagy (Kunz *et al*., 2004; Dubouloz *et al*., 2005). In line with this interpretation, both re-growth of cells and microautophagy-mediated vacuolar membrane homeostasis following the release from rapamycin-induced growth arrest require the presence of the EGO protein complex (see below; Dubouloz *et al*., 2005).

Piecemeal microautophagy. A form of selective microautophagy aimed at degrading and recycling nonessential parts of the nucleus has recently been described (Roberts *et al*., 2003). Piecemeal microautophagy of the nucleus (PMN) occurs at specific velcro-like junctions formed between the vacuolar protein VAC8 and its nuclear counterpart NVJ1. At these sites, the nucleus bulges into the vacuole and parts of the nucleus are sequestrated into invaginations of the vacuolar membrane that are pinched off into the lumen for subsequent degradation. PMN occurs in vegetatively growing cells, but is highly induced following carbon or nitrogen starvation or rapamycin treatment. An attractive hypothesis is that PMN may serve to degrade and recycle nucleolar pre-ribosomes under conditions of nutrient starvation where they are no longer needed. Thus, ribosome biogenesis (see above) and ribosome/pre-ribosome degradation may both be tightly coordinated by TORC1. Presently, it is not known whether PMN is universal among eukaryotic cells, but the fact that yeast cells have a closed mitosis could make PMN unique to them.

Mitophagy and pexophagy
Although autophagy primarily serves as a response to nutrient starvation, homeostatic and/or housekeeping functions have recently been attributed to various autophagic processes. For example, peroxisomes (induced by growth on methanol or fatty acids) can be eliminated by either macro- or microautophagy (pexophagy) upon modification of the carbon source (Leao and Kiel, 2003). Moreover, while bulk degradation of mitochondria following nutrient starvation (or TORC1 inactivation) occurs via a selective macroautophagic process (i.e. mitophagy; Kissova *et al*., 2004), individual damaged mitochondria can also be specifically removed by mitophagy during vegetative growth (Priault *et al*., 2005). Interestingly, mitochondrial damage-induced mitophagy most likely relies on the classical macroautophagic machinery and may therefore also involve TORC1 signalling (Priault *et al*., 2005).

TORC1 blocks transcriptional stress responses

In addition to regulating autophagy, TORC1 also negatively regulates growth arrest by antagonizing the activities of a number of stress-responsive transcription factors. This became apparent in the analyses of several genome-wide expression studies of rapamycin-treated cells (Cardenas *et al*., 1999; Hardwick *et al*., 1999; Komeili *et al*., 2000; Shamji *et al*., 2000; Chen and Powers, 2006). The most striking set of genes whose expression is induced following TORC1 inhibition includes those involved in assimilation of alternative nitrogen sources, in protein degradation and in general stress protection. Thus, TORC1 negatively controls transcriptional programmes that normally operate under nutrient starvation and/or general stress conditions.

Activity of stress-responsive transcription factors
One common mechanism by which TORC1 controls the expression of nutrient- and stress-responsive genes is by sequestering specific transcription factors in the cytoplasm. For example, TORC1 inhibits transcription of genes normally activated following nitrogen limitation by promoting the association of the GATA transcription factor GLN3 with the cytoplasmic repressor protein URE2 (Beck and Hall, 1999). Accordingly, TORC1 inhibition, similar to nitrogen limitation, causes dephosphorylation, dissociation from URE2 and nuclear import (via the SRP1 importin) of GLN3. Subsequent GLN3-dependent activation and expression of *nitrogen-catabolite repression (NCR)*-sensitive genes enables the cells to import and catabolize poor nitrogen sources such as proline and allantoin (Beck and Hall, 1999; Carvalho and Zheng, 2003). TORC1 additionally controls the phosphorylation state of URE2 by a still unknown mechanism (Cardenas *et al*., 1999; Hardwick *et al*., 1999). TORC1 also prevents the access of the GATA transcription factor GAT1 to the nucleus, although the underlying control mechanism likely differs from the one observed for GLN3 (Beck and Hall, 1999; Kuruvilla *et al*., 2001; Crespo *et al*., 2002).

TORC1 further promotes cytoplasmic retention of a heterodimeric transcription factor complex composed of the bZip/HLH proteins RTG1 and RTG3, which are central elements of the mitochondria-to-nucleus signalling pathway (also known as retrograde response pathway) that activates genes whose products (e.g., mitochondrial and peroxisomal enzymes) are required for biosynthesis and homeostasis of glutamate and glutamine (Komeili *et al*., 2000; Butow and Avadhani, 2004). TORC1 antagonizes RTG1/RTG3 function by promoting their association with MKS1 and the 14-3-3

proteins BMH1 and/or BMH2 (Dilova *et al*., 2002; Sekito *et al*., 2002; Tate *et al*., 2002; Liu *et al*., 2003; Dilova *et al*., 2004). Dephosphorylation of MKS1 following TORC1 inactivation, or as a result of mitochondrial dysfunction, causes disassembly of this inhibitory complex, association of MKS1 with its inhibitor RTG2, and subsequent translocation of RTG1/RTG3 into the nucleus (Liao and Butow, 1993; Komeili *et al*., 2000; Sekito *et al*., 2000; Liu *et al*., 2003). The mechanism by which TORC1 impinges on MKS1 (and/or RTG2) is still a matter of debate, but involves, at least in part, the TORC1 subunit LST8 (Giannattasio *et al*., 2005).

TORC1 further controls both the nucleocytoplasmic distribution and activity of the partially redundant Zn^{2+}-finger transcription factors MSN2 and MSN4. MSN2/4 regulate *stress-responsive element (STRE)*-dependent transcription in response to a wide range of stresses (including nutrient limitation; Mager and De Kruijff, 1995; Ruis and Schüller, 1995). TORC1 regulates MSN2/4 by promoting their phophorylation and cytoplasmic accumulation, which may (Beck and Hall, 1999) or may not (Santhanam *et al*., 2004) require cytoplasmic 14-3-3 anchor proteins. Following nutrient limitation, transcriptional activation by MSN2/4, and GIS1, a closely related transcription factor that activates expression of *post-diauxic-shift (PDS)* element-controlled genes, also requires the Ser/Thr protein kinase RIM15 (Reinders *et al*., 1998; Pedruzzi *et al*., 2003; Cameroni *et al*., 2004). Intriguingly, the nucleocytoplasmic distribution of RIM15 is also controlled by TORC1 in a 14-3-3-dependent fashion (Reinders *et al*., 1998; Pedruzzi *et al*., 2003; Wanke *et al*., 2005). RIM15 is required to orchestrate key aspects of the G_0 programme although the mechanisms by which it influences MSN2/4 and GIS1 activity are not known (Cameroni *et al*., 2004; Swinnen *et al*., 2006).

TORC1 prevents both the nuclear localization and stabilization of an additional transcription factor, IME1 (Colomina *et al*., 2003). In response to nutrient availability, diploid cells proceed through various developmental pathways. For example, in the absence of both fermentable carbon and good nitrogen sources, diploid cells undergo meiosis and sporulation. Meiosis requires the activity of IME1, which initiates a transcriptional cascade of sporulation-specific genes involved in different steps of meiosis and spore formation. Thus, TORC1 appears to not only couple nutrient cues to growth and stress responses but also to developmental programmes.

Cell wall integrity pathway

Yeast cells regulate the expression of many cell wall biosynthetic enzymes via the cell wall integrity (CWI) pathway (Levin, 2005). These enzymes help to remodel the cell wall both during normal growth and in response to stress. The CWI pathway is centred around a small G-protein, RHO1 (reviewed in Levin, 2005). Several cell surface sensors (belonging to the WSC family of proteins; for cell wall integrity and stress response component) regulate RHO1 via the guanine-nucleotide exchange factors ROM1/2. RHO1 itself has numerous effectors including the single yeast protein kinase C homologue, PKC1. PKC1 also likely has multiple effectors, the best characterized being a mitogen-activated protein kinase (MAPK) cascade composed of BCK1 (a MAPKKK), MKK1/2 (redundant MAPKKs) and SLT2/MPK1 (a MAPK). This MAPK cascade regulates many physiological processes including cell-wall synthesis and actin polarization. As discussed below, genetic studies have demonstrated a clear link between TORC2 and this pathway. Intriguingly, several groups have found that TORC1 also regulates this pathway (Ai *et al*., 2002; Krause and Gray, 2002; Torres *et al*., 2002; Reinke *et al*., 2004; Araki *et al*., 2005).

Mutations in *PKC1*, *BCK1* and *MPK1* cause cells to rapidly die upon carbon or nitrogen starvation, demonstrating that the CWI pathway is required for viability in G_0 (Krause and Gray, 2002; Torres *et al*., 2002). Consistently, entry into stationary phase, carbon starvation, nitrogen starvation or rapamycin treatment all elicit activation of the CWI pathway as observed by an increased phosphorylation of MPK1 at sites required for its activation (Ai *et al*., 2002; Krause and Gray, 2002). These results suggest that in growing cells TORC1 negatively regulates the CWI pathway. Indeed, in some strains, rapamycin-induced hyperactivation of the CWI pathway is also manifested as depolymerization of the actin cytoskeleton (Torres *et al*., 2002; Araki *et al*., 2005). The level at which TORC1 signals impinge upon the CWI pathway is not clear. One hypothesis is that TORC1 inhibition results in a membrane stress that activates WSC family members which in turn activate downstream components of the pathway (Torres *et al*., 2002).

TORC1 regulates lifespan

A growing body of evidence from different model systems indicates that TORC1 plays a role in lifespan regulation (Vellai *et al*., 2003; Jia *et al*., 2004; Kapahi *et al*., 2004). The general picture that emerges from these studies is that deficiencies in TORC1 signalling likely extend lifespan by mimicking calorie restriction (CR), an intervention that can slow aging and extend lifespan in virtually every biological system examined (Weindruch and Walford, 1988; Kennedy *et al*., 2005; Masoro, 2005). Yeast is used as a model for aging of mitotic cells (replicative aging) and postmitotic cells (chronological aging), which are determined by the number of mitotic events a mother cell undergoes before senescence and by the time a nondividing population remains viable in liquid media, respectively (Mortimer and Johnston, 1959; Fabrizio *et al*., 2001; MacLean *et al*., 2001). The histone deacetylase SIR2 is a critical factor that slows down replicative aging (Kennedy *et al*., 1995; Kaeberlein *et al*., 1999), by repressing the recombination of repetitive ribosomal DNA (rDNA) and the subsequent formation of extrachromosomal rDNA circles (ERCs). In parallel, SIR2 likely accelerates chronological aging by promoting DNA damage, inhibiting stress resistance and/or inhibiting the activation of alcohol dehydrogen-

ase (ADH2; Fabrizio *et al*., 2005; Kennedy *et al*., 2005). Surprisingly, two recently published large-scale analyses of single-gene deletion strains revealed that TORC1 is another critical determinant of both replicative and chronological aging in yeast, which appears (at least with respect to replicative aging) to act independently of SIR2 (Kaeberlein *et al*., 2005; Powers *et al*., 2006). A likely scenario is, therefore, that CR-mediated downregulation of TORC1 induces at least a partial nutrient-stress response (see above) from which cells benefit in terms of prolongation of both replicative and chronological lifespan.

Interestingly, similar to downregulation of TORC1, defective signalling from two other key nutrient-sensory kinases, namely PKA and the PKB/Akt homologue SCH9, increases both replicative and chronological aging (Fabrizio *et al*., 2001; Kaeberlein *et al*., 2005). Although the epistatic relationship between TORC1 and SCH9 remains to be elucidated (see below), several reports support the notion that PKA acts in parallel to both SCH9 and TORC1 to control common downstream targets (Görner *et al*., 2002; Pedruzzi *et al*., 2003; Jorgensen *et al*., 2004; Roosen *et al*., 2005; Wanke *et al*., 2005; Chen and Powers, 2006). It is therefore likely that regulation of replicative and chronological aging results from proper integration of at least two different nutrient signals.

Potential signalling pathways downstream of TORC1

Owing largely to the rapid and specific inhibition of TORC1 with rapamycin, a large number of TORC1-regulated readouts have been elucidated as described above. However, understanding of the signalling pathways that couple TORC1 to these readouts remains limited. Although some groups have reported substrates phosphorylated *in vitro* by immunoprecipitated TOR1 or TOR2 (Jiang and Broach, 1999; Bertram *et al*., 2000), *bona fide* substrates of purified TORC1 have not been reported.

TORC1 regulated protein phosphatases

Some, but not all, TORC1 readouts are mediated by the type 2A and 2A-related protein phosphatases. Protein phosphatase 2A (PP2A) exists predominately as a heterotrimeric complex consisting of a catalytic (PP2Ac) subunit, one of two regulatory (B or B′) subunits and a scaffold (A) subunit (reviewed in Düvel and Broach, 2004). There are three catalytic PP2A subunits in yeast: PPH21 and PPH22 are redundant whereas PPH3 appears to have distinct functions. The B and B′ regulatory subunits, CDC55 and RTS1, respectively, have little or no functional overlap and influence catalytic activity both positively and negatively. The single A subunit, TPD3, provides a scaffold for interaction between the catalytic and regulatory subunits. Genetic analyses demonstrated that PP2A performs multiple roles in the cell, at least one of which is essential for growth. Budding yeast additionally encode several PP2A-related catalytic subunits, including SIT4. Four regulatory subunits, SAP155, SAP185, SAP190 and SAP4, individually associate with SIT4 and appear to act semiredundantly to activate SIT4 function. Like PP2A, SIT4 has a range of cellular targets. Analysis of SIT4 function is further complicated by the presence of strain-dependent alleles of a polymorphic gene, *SSD1*. SSD1 is a putative RNA-binding protein but its precise functions are not understood (Uesono *et al*., 1997). Disruption of *SIT4* is synthetically lethal in *ssd1-d* backgrounds, but viable in *SSD1-v* backgrounds. Given that SIT4 is an important mediator of TORC1 signalling (see below), it is not surprising that *SSD1* allele status accounts for some of the important (e.g., loss of actin polarization) strain-specific responses to rapamycin (Reinke *et al*., 2004).

In addition to these heterotrimeric (ABC) PP2A and SIT4-SAP complexes, a fraction of PP2Ac and SIT4 can be found in distinct complexes containing TAP42 and either RRD1 or RRD2 (Di Como and Arndt, 1996; Jiang and Broach, 1999; Zheng and Jiang, 2005). It is these TAP42-containing complexes that appear to be regulated by TORC1. Although there is evidence implicating the SAPs, CDC55 and TPD3 in rapamycin responses, it is not known if these proteins are involved directly or if their deletion effects rapamycin responses indirectly by altering the abundance and/or activity of TAP42-containing complexes (Jacinto *et al*., 2001; Rohde *et al*., 2004; Santhanam *et al*., 2004). In rapidly growing cells, TAP42 is phosphorylated and tightly associated with both SIT4-RRD1 and PP2Ac-RRD2 (Zheng and Jiang, 2005). Carbon or nitrogen starvation or treatment with rapamycin results in dephosphorylation of TAP42 and a reduced association of TAP42 with SIT4-RRD1 and PP2Ac-RRD2 (Di Como and Arndt, 1996; Jiang and Broach, 1999; Zheng and Jiang, 2005). How TORC1 regulates TAP42 is not entirely clear. TORC1 may directly phosphorylate TAP42 (Jiang and Broach, 1999; Düvel and Broach, 2004), although the physiological relevance of this phosphorylation has not been investigated. Alternatively (or additionally), TORC1 may regulate TAP42 via TIP41 (Jacinto *et al*., 2001). Like TAP42, TIP41 is also dephosphorylated following rapamycin treatment. TIP41 dephosphorylation correlates with an increased association of TIP41 with TAP42, but here too, the physiological relevance of TIP41 phosphorylation in this process has not been confirmed (Jacinto *et al*., 2001). Presumably, the phosphorylation status of TAP42 and/or its association with TIP41 alters the substrate specificity of associated phosphatases (Düvel *et al*., 2003; Düvel and Broach, 2004; Van Hoof *et al*., 2005; Zheng and Jiang, 2005). The latest model (Yan *et al*., 2006) posits that TORC1 regulates TAP42-associated phosphatases via direct physical interaction, which sequesters the phosphatases with TORC1 at membranes. Inactivation of TORC1, by rapamycin or nutrient deprivation, elicits the release of phosphatase complexes into the cytoplasm where they are transiently active until TAP42 becomes dephosphorylated, likely by heterotrimeric (ABC) PP2A holoenzymes (Yan *et al*., 2006). However, as noted above, characterization of these phosphatases is not trivial and this model,

although appealing, requires further testing. Additionally, the roles of RRD1/2 in this process are only beginning to be elucidated, even though it has been known for some time that deletions of the corresponding genes confer resistance to rapamycin (Rempola *et al*., 2000).

TORC1 readouts regulated by PP2Ac and/or SIT4

As diagrammed in Figure 1, many TORC1 readouts are regulated via PP2A and/or PP2A-like phosphatases. Key to these studies has been the generation of semidominant, rapamycin-resistant alleles of TAP42, which in many cases block rapamycin-induced effects (Di Como and Arndt, 1996; Düvel and Broach, 2004). However, as elegantly demonstrated by Chen and Kaiser (2003), caution is advised when interpreting these studies, as the use of these types of alleles can lead to misinterpretations owing to dramatic alterations in the composition of the intracellular metabolome. Alterations in the metabolome can then independently influence TORC1 readouts and thus confuse conclusions. That said, readouts including autophagy, HXT1 (a glucose transporter) activity and transcription of *RP*, *Ribi*, *rRNA* and *tRNA* genes appear to be independent of PP2Ac and SIT4 (Düvel *et al*., 2003; Schmelzle *et al*., 2004). In contrast, TORC1 promotes nuclear export of MSN2 via TAP42-dependent inhibition of PPH21 and PPH22 (Görner *et al*., 2002; Düvel *et al*., 2003; Santhanam *et al*., 2004). Similarly, PP2Ac and/or SIT4 are required for rapamycin-induced dephosphorylation of NPR1 (Schmidt *et al*., 1998), GCN2 (Cherkasova and Hinnebusch, 2003; Rohde *et al*., 2004), RTG1/3 (Düvel and Broach, 2004) and GLN3 (Beck and Hall, 1999; Düvel and Broach, 2004). Conversely, the inhibition of SIT4 by TORC1 and TAP42 maintains MPK1 activity at a basal level (Torres *et al*., 2002; Reinke *et al*., 2004).

It is important to note that PP2A, PP2A-related phosphatases, TIP41, TAP42, SSD1 and RRD1/2 are all evolutionarily conserved (Table 1) and in many cases, mammalian orthologues have also been implicated in mTORC1 signalling (reviewed in Harris and Lawrence, 2003; Jacinto and Hall, 2003; Düvel and Broach, 2004). However, as in yeast, more detailed biochemical studies using biologically relevant substrates are required to fully appreciate the exact roles of phosphatase complexes in TORC1 signalling.

Signalling pathways acting in parallel to TORC1

A major challenge in the TOR field is to understand how TORC1-mediated signals are integrated with signals from other nutrient signalling pathways. In this context, some progress has recently been made in the understanding of how TORC1 and the glucose-responsive PKA signalling pathway jointly control a number of growth-related cellular responses (e.g., ribosome biogenesis and stress responses). A recurrent theme that emerges is that both pathways impinge on common target proteins, as demonstrated by various examples. For instance, TORC1 and PKA (via direct phosphorylation) independently regulate nuclear export and import, respectively, of MSN2/4 (Görner *et al*., 1998, 2002; Santhanam *et al*., 2004). In a similar vein, TORC1 prevents nuclear accumulation of RIM15 by modulating (directly or indirectly) the phosphorylation status within its critical 14-3-3-binding domain, whereas PKA-dependent phosphorylation inhibits RIM15 kinase activity (Pedruzzi *et al*., 2003; Wanke *et al*., 2005). Yet, another example is the transcription factor SFP1, whose subcellular localization appears to be controlled by PKA as well as by a PKA-independent TORC1 effector branch (Marion *et al*., 2004). In agreement with the fact that all of these TORC1- and PKA-regulated proteins are either directly (MSN2/4 and SFP1) or indirectly (RIM15) involved in transcriptional control, transcriptional profile studies support a model in which TORC1 and PKA provide separate inputs to positively control various gene clusters (Zurita-Martinez and Cardenas, 2005; Chen and Powers, 2006). Nevertheless, these findings do not exclude the possibility that signal integration between TORC1 and PKA may rely on additional layers of regulation. One such example is the recent finding that inactivation of TORC1 causes rapid nuclear accumulation of at least one of the three partially redundant PKA catalytic subunits (i.e., TPK1; Schmelzle *et al*., 2004). Notably, the regulatory PKA subunit BCY1 resides predominantly in the nucleus and might, therefore, favour engagement of incoming TPK1 subunits into the formation of inactive TPK1-BCY1 holoenzymes (Griffioen *et al*., 2000; Martin *et al*., 2004b; Schmelzle *et al*., 2004). At present, however, the functional significance of the observed changes in TPK1 subcellular localization remains speculative.

TORC1 also shares common target proteins with the intracellular glucose-sensory protein kinase SNF1, the yeast homologue of the AMP-activated protein kinase (AMPK). The transcription factor GLN3, for instance, is independently and oppositely regulated by the activities of TORC1 and SNF1: TORC1 promotes cytoplasmic retention of GLN3 (see above), whereas SNF1 (via direct phosphorylation) promotes nuclear accumulation of GLN3 (Bertram *et al*., 2002). In addition, TORC1 and SNF1 also converge on MSN2. Accordingly, TORC1 positively controls nuclear export of MSN2 (see above), whereas SNF1 – likely as part of an adaptive programme to prolonged glucose starvation – slows down nuclear import of MSN2 (Mayordomo *et al*., 2002; De Wever *et al*., 2005).

Recent evidence suggests that the nutrient-sensitive protein kinase SCH9 significantly overlaps in function with TORC1 with respect to the control of lifespan, ribosome biogenesis and cell size (Toda *et al*., 1988; Fabrizio *et al*., 2001; Jorgensen *et al*., 2002; Jorgensen *et al*., 2004; Kaeberlein *et al*., 2005). SCH9 function requires phosphorylation of a serine residue within its activation loop, which is carried out by PKH1 and PKH2, the orthologues of the mammalian PDK1 protein kinase (Roelants *et al*., 2004). Presumed components, other than PKH1 and PKH2, which lie upstream of SCH9 remain elusive. Similarly, *bona fide* downstream effectors of SCH9 have not yet been

identified, but may include RIM15 and/or GIS1 (Pedruzzi *et al*., 2003; Roosen *et al*., 2005; Wanke *et al*., 2005). Even though the epistatic relationship between TORC1 and SCH9 remains to be addressed, one possible model would have TORC1 converge with PKH1/2 and perhaps additional regulators on SCH9. The discovery that TORC1 inactivation results in rapid disappearance of SCH9 phospho-isoforms (Jorgensen *et al*., 2004) is in line with this presently speculative, but intriguing model.

For the sake of completeness, the mechanistic cross talk between TORC1 and the general amino-acid control response pathway (see above), which involves dephosphorylation of a specific residue in the GCN2 protein kinase, followed by increased phosphorylation of the GCN2 target eIF2α (and subsequent specific translational derepression of *GCN4* mRNA message; Figure 1), may also be recalled at this point (Cherkasova and Hinnebusch, 2003; Kubota *et al*., 2003).

TORC2 readouts

The readouts downstream of TORC2 are much less well characterized compared to those of TORC1, owing to the fact that there is no rapamycin-equivalent with which to inhibit and interrogate TORC2 function. However, continuing genetic screens, in a variety of systems, suggest that TORC2 will regulate as rich an array of processes as does TORC1. Currently, the best-characterized function of TORC2 is its regulation of cell polarity.

Cell polarity

Cell growth is often polarized; for example, in budding yeast, the majority of growth occurs at a discrete locus – the bud. In yeast, it is believed that this polarized growth is directed by an underlying polarization of the actin cytoskeleton: actin cables orient from the mother cell to the bud and cortical actin patches concentrate in the bud. Actin cables are thought to orient the secretory pathway, thus facilitating partitioning of organelles and targeting of macromolecules synthesized in the mother cell to the bud (Pruyne *et al*., 2004).

A link between TOR2 and actin was established when it was observed that overexpression of CCT6, a protein required for assembly of actin structures, suppresses the growth defect caused by overexpression of a dominant-negative (kinase-dead) allele of TOR2 (Schmidt *et al*., 1996). Subsequent studies demonstrated that disruption of TORC2 results in depolarization of the actin cytoskeleton and loss of viability and that these phenotypes are suppressed by hyperactivation of the CWI pathway (introduced above; Figure 2). Specifically, overexpression of ROM2, RHO2, PKC1, BCK1, MKK1 or MPK1 can restore viability and actin polarization to mutants compromised in TORC2 function (Schmidt *et al*., 1996, 1997; Bickle *et al*., 1998; Helliwell *et al*., 1998b; Loewith *et al*., 2002). These genetic suppression studies clearly place TORC2 either upstream of, or parallel to, the CWI pathway in the control of actin organization. Somewhat weaker biochemical data suggest that TORC2, in an enigmatic way, stimulates ROM2 GEF activity (Schmidt *et al*., 1997; Levin, 2005). This would place TORC2 upstream of the CWI pathway and ultimately cell polarization. Consistent with these results, slime mould TORC2 is required for polarized growth (i.e. chemotaxis; Lee *et al*., 2005) and mammalian TORC2 appears to regulate actin structures via RHO family GTPases and PKCα (Jacinto *et al*., 2004; Sarbassov *et al*., 2004). How TORC2 communicates with ROM2 remains a mystery, but direct substrates of TORC2 have recently been identified, and these substrates may narrow the signalling gap between TORC2 and ROM2.

YPKs

YPK2 is an AGC family kinase thought to be most closely related to mammalian serum and glucocorticoid-stimulated kinases (SGKs). YPK2 functions redundantly with its close homologue, YPK1, to regulate actin polarization, and, like loss of TORC2 function, loss of YPK function can be suppressed by overexpression of components of the CWI pathway (Schmelzle *et al*., 2002). These observations suggested that YPKs and TORC2 may function in the same pathway. Consistently, Kamada *et al*. (2005) recently identified hyperactive alleles of YPK1 and YPK2 that suppress the lethality caused by disruption of TORC2 but not TORC1. TOR2, and presumably TORC2, directly phosphorylates residues in the turn and hydrophobic motifs (Ser^{641} and Thr^{659}, respectively) of YPK2 and this phosphorylation promotes YPK2 activity *in vivo*. Importantly, expression of an activated allele of YPK2 appears to restore the ability of TORC2-deficient cells to signal to the MAPK cascade of the CWI pathway (Kamada *et al*., 2005). Thus, it appears that TORC2 signals to ROM2 via the YPKs; however, as no YPK substrates have been reported, the mechanistic details connecting YPKs to ROM2 remain foggy.

Work done in *Schizosaccharomyces pombe* has also linked Gad8p (the fission yeast YPK orthologue) to Tor function (Matsuo *et al*., 2003). Specifically, overexpression of Gad8 suppresses the sterility of fission yeast *tor1* mutants, and Tor1p is required for kinase activity of Gad8. However, these observations are somewhat puzzling. Fission yeast, like budding yeast, encodes two TOR homologues (*Tor1* and *Tor2*). As in budding yeast, fission yeast Tor2, but not Tor1, is essential (Weisman, 2004). Therefore, one would predict that Gad8 functions downstream of Tor2/TorC2. The likely explanation is that the Tors are switched in fission yeast; that is, Tor1 and Tor2 behave like budding yeast TOR2 and TOR1, respectively. More specifically, fission yeast Tor1 functions in a TORC2-like complex and the activity of this complex is not essential in this yeast. This idea is based on the observation that fission yeast strains harbouring mutations in other putative TorC2 components such as Ste20 (=ScAVO3) and Sin1 (=ScAVO1), like Tor1 mutants, are sterile and hypersensitive to various stresses (Hilti *et al*., 1999; Wilkinson

et al., 1999; Weisman, 2004). Alternatively, it is possible that Gad8p/YPK1 also functions downstream of TORC1: budding yeast *ypk1* mutants, like *tor1* mutants, are hypersensitive to rapamycin and display a reduced efficiency of translation initiation (Gelperin *et al*., 2002).

TORC2 also contributes to actin polarization via a homologous, essential pair of PH domain proteins, SLM1 and SLM2 (Audhya *et al*., 2004; Fadri *et al*., 2005). The SLMs were identified in a screen for mutants that are synthetically lethal with a hypomorphic allele of the PI4P-5 kinase *mss4* (Audhya *et al*., 2004). Additional studies demonstrated that the SLMs localize to the plasma membrane via interactions with both TORC2 and $PI4,5P_2$ produced by MSS4. The SLMs are phosphoproteins and both *in vitro* and *in vivo* studies suggest that they are directly phosphorylated by TORC2. Simultaneous depletion of both SLM1 and SLM2 is lethal, and like loss of TORC2, results in perturbation of the actin cytoskeleton (Audhya *et al*., 2004; Fadri *et al*., 2005). The SLMs are not obviously conserved in higher eukaryotes, and neither the mechanism by which the SLMs signal to actin nor their relationship to the YPKs and the CWI pathway is clear. Very recent work suggests that the SLMs may couple TORC2 to the Ca^{2+}/calmodulin-regulated protein phosphatase, calcineurin.

Calcineurin

Global two-hybrid screens suggested that the SLMs interact with both CNA1 and CMP2, the two catalytic isoforms of yeast calcineurin (Uetz *et al*., 2000; Ito *et al*., 2001). Building on this, the Cyert and Emr groups (Bultynck *et al*., 2006; Tabuchi *et al*., 2006) have confirmed that the SLMs interact with, and are dephosphorylated by, calcineurin. Loss of SLM function results in increased expression of CRZ1 targets (CRZ1 is a transcription factor negatively regulated by calcineurin) and genetic or chemical inhibition of calcineurin activity suppresses the lethality and actin depolarization of *slm* mutants (Tabuchi *et al*., 2006). These results suggest that SLMs inhibit calcineurin. Consitently, previous work had suggested that hyperactive calcineurin causes actin depolarization and is toxic (Shitamukai *et al*., 2004). Importantly, inactivation of TORC2 also results in apparent hyperactivation of calcineurin (Martin *et al*., 2004a; and J-M Mulet, MN Hall personal communication). How calcineurin antagonizes actin polarization is currently unknown; but it is tempting to speculate that TORC2 in higher eukaryotes may also regulate calcineurin activity.

Endocytosis

Work from the Hicke group has demonstrated that TORC2 and YPK1 play important roles in the internalization step of both ligand-stimulated and fluid-phase endocytosis (deHart *et al*., 2002, 2003). Efficient endocytosis *per se* is closely associated with a functional actin cytoskeleton and consistently, the regulation of endocytosis by TORC2 and YPK1 is dependent on RHO1 (deHart *et al*., 2003). Surprisingly, however, RHO1 controls endocytosis not via its actin-regulating effector, PKC1, but via another effector, the β-1,3-glucan synthase FKS1 (deHart *et al*., 2003). The means by which FKS1 regulates endocytosis has not been established, but it seems that glucan-synthase activity may not be required (deHart *et al*., 2003). Mammalian RHO family GTPases are known to mediate endocytic traffic (discussed in deHart *et al*., 2003), but a role for mTORC2 in endocytosis has not been reported.

TORC2 may also influence endocytosis by regulating the production of sphingolipids, which together with sterols, have been proposed to play a role in this process (Pichler and Riezman, 2004). A selection for temperature-sensitive suppressors of the calcium sensitivity of *csg2* mutants (*tsc*s) identified alleles of *mss4*, *tor2* and *avo3* among others (Beeler *et al*., 1998). CSG2 is required for the mannosylation of a sphingolipid, inositolphosphoceramide (IPC), and *csg2* mutants accumulate excessive amounts of a particular IPC (IPC-C), which for unknown reasons is toxic in the presence of Ca^{2+}. The majority of *tsc*s were alleles of genes involved upstream in sphingolipid synthesis and suppressed by reducing the accumulation of IPC-C. This observation suggests that MSS4 and TORC2 may regulate some aspect of sphingolipid synthesis, and perhaps consequently endocytosis. This hypothesis has indeed found recent support: SLM function is required for proper sphingolipid metabolism and heat stress-induced endocytosis of the uracil permease (Bultynck *et al*., 2006; Tabuchi *et al*., 2006). Calcineurin is also involved in these processes but mechanistic details remain to be established.

Why does TORC2 regulate endocytosis? When yeast cells grow they must extensively remodel their cell wall to accommodate their increased size, and endocytosis may be required for this remodelling. Indeed, like growth, sites of endocytosis (cortical actin patches) are predominantly confined to the bud (Ayscough, 2005). Furthermore, endocytosed material must be efficiently trafficked, presumably in an actin dependent process, to specific endosomal destinations. Therefore, it seems logical for TORC2 to also regulate processes such as endocytosis that require the physical establishment of cell polarity.

Stress-activated MAP kinase signalling

Sin1 (=ScAVO1) was identified in a two-hybrid selection for proteins that interact with the *S. pombe* stress-activated MAP kinase (SAPK), Sty1/Spc1 (Wilkinson *et al*., 1999). These authors demonstrated that Sin1 is required for activation of Sty1-regulated transcription factors and proper induction of the stress-induced transcription programme. As mentioned above, Sin1 and other fission yeast TorC2 components are hypersensitive to multiple stresses. Furthermore, the α-isoform of human SIN1 was observed to bind, and potentially regulate, the activity of the mammalian SAPK, c-Jun NH_2 terminal kinase (JNK) (Schroder *et al*., 2005). Assuming that fission yeast and human SIN1 function in TORC2-like complexes, these observa-

tions suggest that TORC2 performs a conserved role in SAPK signalling.

How are the TORCs regulated?

Yeast growth is primarily, but not exclusively, regulated by the available nutrients and the absence of noxious stress. Consistently and as alluded to above, TORC1 activity appears to be sensitive to both nutrient and stress cues. The mechanisms by which these cues are sensed and how this is communicated to TORC1 are presently unknown, but potential mechanisms are discussed below. In addition to nutrient and stress cues, metazoans must also regulate cell growth in conjunction with overall organismal growth. This is achieved via circulating growth factors and hormones. The pathways that couple signals from growth factors and hormone receptors to mTORC1 have recently become much clearer and are discussed elsewhere in this issue. In contrast, the cues that regulate TORC2/mTORC2 are not known.

Regulation of TORC1 by nutrients

Rapamycin treatment, transfer of yeast cells from good- to poor-quality carbon or nitrogen sources or starvation for carbon or nitrogen elicit very similar responses, suggesting that TORC1 is regulated by the abundance and/or quality of the available carbon and nitrogen source. However, how nutrient abundance/quality is sensed and how this information is transmitted to TORC1 remains elusive. Notably, mTOR, while integrating growth factor signals, has also been proposed to (directly or indirectly) integrate information on the abundance of nutrients. Accordingly, branched-chain amino acids, especially leucine, intracellular levels of ATP, phosphatidic acid and inorganic polyphosphate are among the potential regulators of mTOR activity (Wang *et al*., 1998, 2003; Xu *et al*., 1998; Dennis *et al*., 2001; Fang *et al*., 2001; Peng *et al*., 2002).

Recent evidence further suggests that glutamine may play a particularly important role in regulation in TOR signalling in both yeast and mammalian cells (Iiboshi *et al*., 1999; Crespo *et al*., 2002; Peng *et al*., 2002). As glutamine can be readily converted to α-ketoglutarate (for use in tricarboxylic acid (TCA) cycle), or serve as an immediate precursor for the biosynthesis of other amino acids, nucleotides and nitrogen-containing molecules (such as nicotinamide adenine dinucleotide $(NAD)^+$), it is not only a key intermediate in nitrogen metabolism but also an important indicator of the cell's general nutrient status (Magasanik and Kaiser, 2002). In line with a model in which glutamine activates TORC1, starvation for glutamine results in a phenotype similar to TORC1 inactivation, inasmuch as it causes nuclear localization and activation of GLN3 and RTG1/RTG3 (Crespo *et al*., 2002; Butow and Avadhani, 2004). Importantly, other TORC1 readouts (such as subcellular distribution of MSN2 and GAT1) remain unaffected by glutamine starvation, indicating that TORC1 may (i) respond to additional nutrients and (ii) process different nutrient signals to elicit nutrient-specific responses. The mechanisms by which nutrient cues are signalled to TORC1 remain a mystery.

One major intracellular nutrient reservoir in yeast, which may have an important yet largely overlooked role in growth control, is the vacuolar compartment. In this context, the recently discovered, vacuolar membrane-associated EGO (exit from rapamycin-induced growth arrest) protein complex (EGOC), which consists of EGO1/MEH1, EGO3/NIR1/SLM4, GTR1 and GTR2, could transmit (directly or indirectly) critical nutrient signals to TORC1 (Dubouloz *et al*., 2005 and CDV and RL, unpublished data). Several observations are in line with such a (currently speculative) model. First, a significant amount of TORC1 localizes to the vacuolar membrane (Reinke *et al*., 2004; Araki *et al*., 2005; and CDV and RL, unpublished data). Secondly, *egoc* mutants very closely resemble cells compromised in TORC1 function and overproduction of EGOC subunits increases the cell's resistance to rapamycin (Dubouloz *et al*., 2005). Thirdly, a recent study identified EGO3/NIR1 as the target of an engineered molecule (SMIR4) that suppresses the effects of rapamycin, possibly by causing a gain of function of EGO3/NIR1 (Huang *et al*., 2004). Fourthly, the transcript profile of exponentially growing *ego3*Δ/*nir1*Δ cells was reported to be strikingly similar to the one of rapamycin-treated wild-type cells (Huang *et al*., 2004). Finally, the discovery that chronological lifespan extension can result from either loss of individual ECOC subunits (i.e. EGO1/MEH1, EGO3/NIR1/SLM4, GTR1, or GTR2) or a decrease in TORC1 signalling (Powers *et al*., 2006, see also above) provides further circumstantial evidence for an intriguing model in which EGOC relays vacuolar nutrient signals to TORC1. Although the function of EGO1/MEH1 and EGO3/NIR1/SLM4 is not yet clear, GTR1 and GTR2 are small GTPases and the yeast orthologues of the mammalian RagA, -B, -C and -D proteins (Sekiguchi *et al*., 2001). Notably, RagC is upregulated in metastatic mammalian cells and suggested to be involved in malignant progression (Nakaji *et al*., 1999).

Regulation of TORC1 by noxious stress

As described above, TORC1 represses the activity of a number of stress-responsive transcription factors. TORC1 activity would be predicted to be sensitive to one, or likely many, stressors. Indeed, mTORC1 activity appears to be reduced in response to stress. As detailed elsewhere in this issue (and reviewed in Wullschleger *et al*., 2006), a variety of stresses activate AMPK in mammalian cells. AMPK negatively regulates TORC1 by directly phosphorylating TSC2. AMPK-phosphorylated TSC2 has an enhanced GAP activity, which leads to increased hydrolysis of GTP by Rheb and consequently reduced TORC1 activity. An AMPK orthologue is found in yeast, SNF1, but to date no evidence has been reported suggesting that SNF1 regulates TORC1. Furthermore, budding yeast do not appear to encode a TSC2 orthologue. Therefore, if TORC1 signals

are regulated by stress – which they almost certainly are – the mechanisms of this regulation are not the same as those reported for mTORC1.

Future directions

The most obvious void in our models of TOR signalling is upstream of TORC2. Monitoring phosphorylation of the recently identified TORC2 substrates should provide an *in vivo* proxy of TORC2 activity and thus help elucidate both the cues and the signal transduction pathways that regulate TORC2. Perhaps TORC2, like TORC1, is regulated by nutrients and/or noxious stress. Consistently, many stresses elicit transient actin depolarization (Beck *et al*., 2001). Given that actin polarization changes during the cell cycle, it is also possible that the cell cycle machinery influences TORC2 signalling.

It is clear that nutrients and stresses influence TORC1 signalling; however, the precise nature of these cues, how they are sensed and how this information is communicated is still unclear. In mammalian cells, the PI-3 kinase hVPS34 and the small GTPase Rheb have both been proposed to participate in coupling nutrient cues to mTORC1 (Wullschleger *et al*., 2006), but it appears unlikely that the budding yeast orthologues of these proteins perform similar functions (Loewith and Hall, 2004; RL, unpublished). Notably, *S. pombe* may be a more appropriate system to investigate connections between nutrients, TSC1/2, Rheb and TORC1. Unlike budding yeast, fission yeast encodes apparent orthologues of mammalian TSC1 and TSC2. Furthermore, genetic studies in fission yeast are consistent with TSC and Rheb orthologues functioning, respectively, to antagonize and stimulate a TORC1-like activity (Mach *et al*., 2000; Yang *et al*., 2001; Tabancay *et al*., 2003; van Slegtenhorst *et al*., 2004; Urano *et al*., 2005; Nakase *et al*., 2006). There is considerable debate regarding the mechanisms by which Rheb regulates mTORC1 (Wullschleger *et al*. (2006) and described elsewhere in this issue); perhaps studies in a genetically tractable organism like *S. pombe* will help settle these controversies.

Phosphatases are a complex and underappreciated aspect of TOR signalling. In addition to being TORC effectors (as described above, both TORC1 and TORC2 appear to regulate phosphatase activities), phosphatases may play a more central role in regulating TORC signalling. Although it is very convenient to monitor the *in vivo* phosphorylation of TORC substrates (phosphorylation of T389 in S6K1 is the best example), this is in fact a readout of both TORC activity and phosphatase activity. The importance of this is highlighted by the observation that very few studies have been able to present *in vitro* TORC activity changes that correlate with *in vivo* substrate phosphorylation. Although there could be many explanations, one interpretation of this failure to recapitulate *in vitro* activity with predicted *in vivo* activity is that phosphatases rather than TORCs are regulated. Admittedly, this hypothesis is controversial but it is not without precedence: initial characterization of 'PDK2' – the enzyme activity required to phosphorylate the hydrophobic motif in AKT, thought now to be mTORC2 – demonstrated that this activity is constitutive and not regulated by extracellular signals (Hresko and Mueckler (2005) and references within). Yeast-based genetic screens should in principle provide an unbiased approach to identify additional inputs that regulate TORC signals.

TOR is a central controller of cell growth (Schmelzle and Hall, 2000) and although many TORC readouts have been identified, it is likely that many more readouts remain to be discovered. Also of importance will be the identification of additional TORC substrates and signalling pathways that couple TORCs to these cell growth readouts. To these ends, several groups have performed large-scale 'chemical-genetic' screens which have implicated numerous gene products in rapamycin-sensitive TOR signalling (Chan *et al*., 2000; Huang *et al*., 2004; Parsons *et al*., 2004; Xie *et al*., 2005; Butcher *et al*., 2006). Analyses of these data will undoubtedly expose many new and astonishing facets of TOR signalling.

Conclusions

Many of the advances in the field of cell growth were first described in yeast. The observations that TOR is the target of rapamycin, has rapamycin-insensitive functions, regulates growth and functions as a component of distinct complexes were all first made in yeast. Given the still unsurpassed genetic tools available to yeast researchers, studies in yeast will undoubtedly continue to make significant contributions to our understanding of TOR signalling.

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