BJ www.biochemj.org

BJ Signal

REVIEW ARTICLE

TOR regulation of AGC kinases in yeast and mammals

Estela JACINTO*[1] and Anja LORBERG†[1]

*Department of Physiology and Biophysics, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854, U.S.A., and †SFB 431 Nachwuchsgruppe, Barbarastr.13, Erweiterungsbau Biologie, Universität Osnabrück, D-49076 Osnabrück, Germany

The TOR (target of rapamycin), an atypical protein kinase, is evolutionarily conserved from yeast to man. Pharmacological studies using rapamycin to inhibit TOR and yeast genetic studies have provided key insights on the function of TOR in growth regulation. One of the first *bona fide* cellular targets of TOR was the mammalian protein kinase p70 S6K (p70 S6 kinase), a member of a family of kinases called AGC (protein kinase A/protein kinase G/protein kinase C-family) kinases, which include PKA (cAMP-dependent protein kinase A), PKG (cGMP-dependent kinase) and PKC (protein kinase C). AGC kinases are also highly conserved and play a myriad of roles in cellular growth, proliferation and survival. The AGC kinases are regulated by a common scheme that involves phosphorylation of the kinase activation loop by PDK1 (phosphoinositide-dependent kinase 1), and phosphorylation at one or more sites at the C-terminal tail. The identification of two distinct TOR protein complexes, TORC1 (TOR complex 1) and TORC2, with different sensitivities to rapamycin, revealed that TOR, as part of either complex, can mediate phosphorylation at the C-terminal tail for optimal activation of a number of AGC kinases. Together, these studies elucidated that a fundamental function of TOR conserved throughout evolution may be to balance growth versus survival signals by regulating AGC kinases in response to nutrients and environmental conditions. This present review highlights this emerging function of TOR that is conserved from budding and fission yeast to mammals.

Key words: AGC kinase, kinase regulation, mammalian target of repamycin (mTOR), rapamycin, TOR complex 2 (TORC1/2).

INTRODUCTION

Nutrients fuel the growth and development of living organisms. The eukaryotic machinery that allows unicellular and multicellular organisms to utilize nutrients efficiently involves the highly conserved protein TOR (target of rapamycin). The presence of abundant nutrients allows cells to undergo synthesis of proteins and other macromolecules that are critical for increase in cellular mass and number. In unicellular eukaryotes, such as yeast, the cellular responses triggered by nutrients are coupled to TOR signalling. Under nutrient limitation or other adverse environmental conditions, these responses are down-regulated by TOR to favour energy-saving mechanisms and cell survival. Nutrient starvation, which can be mimicked by the inhibition of TOR, down-regulates translation initiation, up-regulates genes involved in starvation, leads to accumulation of storage carbohydrates and induces autophagy. In multicellular organisms multiple inputs, in addition to nutrients, converge to regulate cellular responses to growth signals in order to accommodate the needs of different tissues and organs of the body. mTOR (mammalian TOR) integrates these multiple growth cues to orchestrate cell growth. Recently, mTOR has also been linked to the regulation of cell survival in response to stress by mechanisms that remain to be elucidated [40,222].

Rapamycin, a bacterial macrolide that potently inhibits TOR, was instrumental in the initial identification of downstream effectors of the TOR pathway that are critical for growth regulation both in yeast and mammals. S6K (S6 kinase), a protein kinase belonging to the AGC (protein kinase A/protein kinase G/protein kinase C) kinase family and a critical regulator of protein synthesis, was first to be identified as a rapamycin-sensitive target of mTOR [84,149]. Since structural and biochemical studies have hinted at a common regulatory mode for regulation of the AGC family kinases, the identification of S6K as an mTOR target also suggested that TOR/mTOR may also regulate other members of this family. Genetic and biochemical

Abbreviations used: AGC, protein kinase A/protein kinase G/protein kinase C-family; aPKC, atypical protein kinase C; AST, active-site tether; ATM, ataxia-telangiectasia; CaMK, Ca^{2+}/calmodulin-dependent protein kinase; cPKC, conventional protein kinase C; CWI, cell-wall integrity; 4E-BP, eukaryotic initiation factor 4E-binding protein; eIF3, eukaryotic initiation factor 3; ePK, eukaryotic protein kinase; ES, embryonic stem; FAT, FRAP/TOR, ATM, TRRAP; FATC, FRAP/TOR, ATM, TRRAP C-terminal; DBF, dumbbell forming; ERK, extracellular-signal-regulated-protein kinase; FRAP, FKBP12-rapamycin-associated protein; FRB, FKBP12/rapamycin-binding; Gad8, G_1-arrest defective 8; HM, hydrophobic motif; HOG1, high osmolarity glycerol response 1; Hsp, heat-shock protein; hVps34, human vacuolar protein sorting 34; IGF-1, insulin-like growth factor 1; KOG1, kontroller of growth 1; Ksg, kinase responsible for sporulation and growth; (m)LST8, (mammalian) lethal with sec thirteen; MAPK, mitogen-activated protein kinase; MEF, murine embryonic fibroblast; MSK1/2, mitogen- and stress-activated kinase 1/2; MK2, MAPK-activated protein kinase 2; mTOR, mammalian target of rapamycin; NLT, N-lobe tether; nPKC, novel protein kinase C; PDK1, phosphoinositide-dependent kinase; PH, pleckstrin homology; PHLPP, PH domain leucine-rich repeat protein phosphatase; PIF, PDK-interacting fragment; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A (cAMP-dependent protein kinase); PKC, protein kinase C; PKH, PKB-activating kinase homologue; PP, protein phosphatase; PRAS40, proline-rich Akt substrate of 40 kDa; Protor, protein observed with rictor; PRR5, proline-rich protein 5; PRR5L, PRR5-like; PX, Phox homology; raptor, regulatory-associated protein of mTOR; rictor, rapamycin-insensitive companion of mTOR; ROCK, Rho-associated kinase; RIM15, regulator of IME2; Rsk, p90 ribosomal S6K; Sck1/2, suppressor of loss of cAMP-dependent protein kinase 1/2; SGK, serum- and glucocorticoid inducible kinase; S6K, S6 kinase; SLM1/2, synthetic lethal with MSS4 1/2; TAP42, type 2A phosphatase-associated protein 42; TCO89, TOR complex one 89 kDa subunit; TM, turn motif; TOR, target of rapamycin; TORC1/2, TOR complex 1/2; TOS, TOR signalling; TRRAP, transactivation/tranformation-domain-associated protein; Tsc, tuberous sclerosis; YPK, yeast protein kinase.

Note on nomenclature: for the sake of clarity, we did not conform to the standard nomenclature used by the yeast field to name genes and proteins. Budding yeast gene/protein names are all capitalized, whereas fission yeast gene/protein names are only initially capitalized.

[1] Correspondence can be addressed to either author (email jacintes@umdnj.edu or anja.lorberg@biologie.uni-osnabrueck.de).

© The Authors Journal compilation © 2008 Biochemical Society

Biochemical Journal

Table 1 Comparison of TORC1 and TORC2 in *S. cerevisiae*, *S. pombe* and *Homo sapiens*

Conserved core components of each TORC and non-conserved interactors or proteins that associate with TOR directly or indirectly are included. Also listed are the AGC kinase targets linked to the TOR pathway either directly or indirectly and other non-AGC kinase targets that have been demonstrated to be phosphorylated in a TOR-dependent manner. (?) indicates orthologues that have not been shown to be part of or targeted by TORCs.

	S. cerevisiae		*S. pombe*		*H. sapiens*	
	TORC1	TORC2	TORC1	TORC2	TORC1	TORC2
Conserved components	TOR1	TOR2	Tor2	Tor1	mTOR	mTOR
	TOR2					
	KOG1		Mip1		raptor	
	LST8	LST8	Wat1	Wat1	mLST8	mLST8
		AVO1		Sin1		SIN1
		AVO3		Ste20		rictor
Non-conserved interactors	TCO89					
		AVO2				
		BIT61				
		BIT2				
		SLM1/2	SPAC637.13 (?)			
				Mei2		
				Ste11		
					PRAS40	
						PRR5/PRR5L
AGC kinase target	SCH9		Sck1/2 (?)		S6K	
		PKC1		Pck1/2 (?)		cPKC
		YPK1/2		Gad8		SGK (?)
						Akt
Other targets	NPR1		Oca2 (?)			
	GLN3					
	TAP42		SPCC63.05 (?)			
		SLM1/2	SPAC637.13 (?)			
					4E-BP	
					eEF2	

studies in budding (*Saccharomyces cerevisiae*) and fission (*Schizosaccharomyces pombe*) yeast confirmed that a conserved signalling module consisting of TOR–PDK1 (phosphoinositide-dependent kinase 1)–AGC kinase exists. More importantly, the discovery that TOR/mTOR exists as a part of distinct protein complexes with different rapamycin sensitivities catalysed a new line of inquiry on this conserved signalling mechanism.

TOR STRUCTURE AND COMPLEX COMPONENTS

TOR belongs to a family of protein kinases, termed the PIKKs [PI3K (phosphoinositide 3-kinase)-related kinases], a subgroup of the atypical protein kinases [1]. This family comprises a small subset of protein kinases that do not share sequence homology with conventional protein kinases, but has been demonstrated experimentally to possess protein kinase activity [2,3]. TOR consists of conserved domains that are believed to mediate protein–protein interactions. Its N-terminus is made up of HEAT repeats [4] [Huntingtin, Elongation Factor 3, A subunit of PP2A (protein phosphatase 2A), TOR1], which adopt a large extended superhelical structure [5]. Electron microscopy and structural analysis revealed that these HEAT repeats form a curved tubular-shaped domain that associates with the C-terminal WD40 repeat domain of KOG1 (kontroller of growth 1), a TORC1 interactor [6]. The HEAT repeats are followed by the FAT [FRAP (FKBP12-rapamycin-associated protein)/TOR, ATM (ataxia-telangiectasia), TRRAP (transactivation/tranformation-domain-associated protein)] region, a domain that is conserved among PIKK proteins. The FAT domain occurs in tandem with the FATC domain at the tail end. Together, the FAT and FATC (FRAP, ATM, TRRAP C-terminal) domains of mTOR are important for its catalytic activity, as mutations in these domains abolish mTOR autophosphorylation activity as well as mTOR-dependent phosphorylation of 4E-BP (eukaryotic initiation factor 4E-binding protein) and S6K [6–8]. These domains were proposed to play a role in the folding or proper organization of the kinase domain [9]. Consistent with this proposal, solution structure and mutational studies on the conserved cysteine residues that form a redox-sensitive disulfide-bonded loop on the TOR1 FATC domain revealed that the protein levels of TOR can be diminished by mutation of the conserved cysteine residue [10]. Adjacent to the kinase domain of TOR is the FRB (FKBP12/rapamycin-binding) domain, where rapamycin, in complex with the peptidyl prolyl isomerase FKBP12, binds and inhibits TOR. The FRB can bind phosphatidic acid and binding of this lipid has been speculated to mediate membrane localization of mTOR [11–14].

In budding yeast, TOR is encoded by two genes, *TOR1* and *TOR2* (Table 1). TOR1 is part of TORC1 (TOR complex 1) whereas TOR2 is a component of TORC2 in budding yeast. In the absence of TOR1, TOR2 can substitute for its function. Under these conditions, TOR2 forms a complex with the TORC1 components, which may explain why *TOR1* deletion is not lethal. However, TOR2 performs an essential function that cannot be replaced by TOR1 [15]. In fission yeast, there are also two TORs, Tor1 and Tor2. In this organism, Tor1 is part of TORC2. Tor2 may form a complex with both TORC1 and TORC2 [16]. Speculatively, the presence of two TOR genes in both the budding and fission yeast may have occurred by gene duplication. Eventually, the two TORs may have acquired specific functions. In other yeast genera and organisms including mammals, there is only one TOR gene. Nevertheless, mammalian TOR can form two distinct protein complexes, mTORC1 and mTORC2, similar to its yeast counterparts.

© The Authors Journal compilation © 2008 Biochemical Society

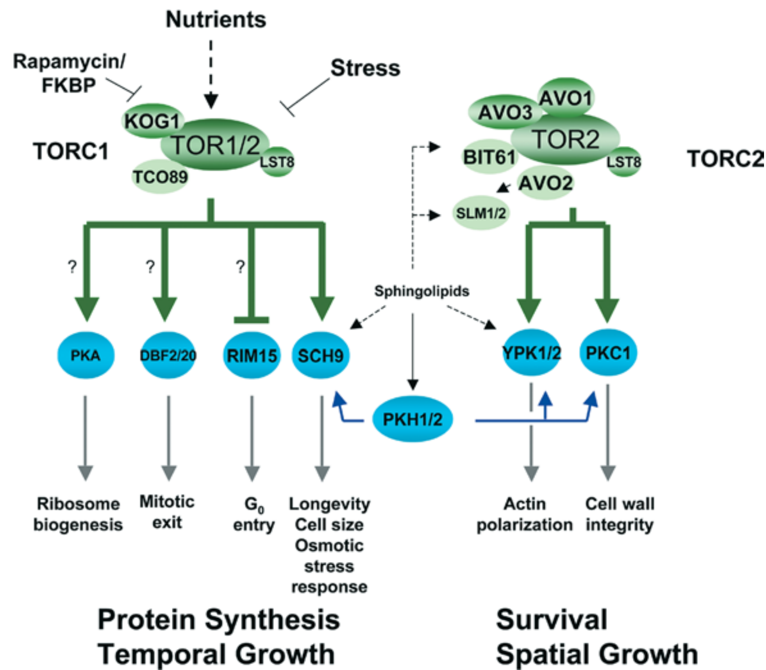

Figure 1 TOR complex signalling to AGC kinases in the budding yeast *S. cerevisiae*

Thick green lines/arrows indicate TOR regulation of an AGC kinase, which are shaded in light blue. Thin indigo lines indicate PKH (PDK) regulation of an AGC kinase. ? denotes the lack of experimental data on direct regulation. Conserved TOR core complex components are in dark green whereas non-conserved interactors are in light green.

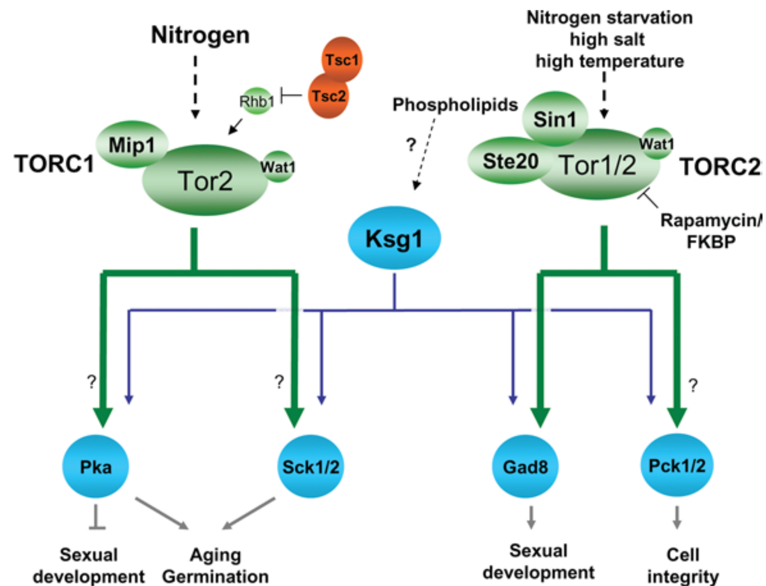

Figure 2 Tor complex signalling to AGC kinases in the fission yeast *S. pombe*

With the exception of Gad8, there is little evidence so far that the TORCs regulate the AGC kinases in fission yeast. See Figure 1 for colour-coding.

TORC1/mTORC1

In budding yeast and mammals, TORC1 signalling regulates the response to nutrients [15]. Budding yeast TORC1 forms a complex with the conserved KOG1 and LST8 (lethal with sec thirteen; in fission yeast Mip1 and Wat1) (Figures 1 and 2), whose mammalian counterparts are raptor (regulatory-associated protein of mTOR) and mLST8 (mammalian LST8; initially called GβL) respectively (Figure 3). Budding yeast and mammalian TORC1 mediate the rapamycin-sensitive functions of TORC1. In fission yeast, the analogous TORC1 is also responsive to nutrient conditions [16,17]. As in mammals, fission yeast TORC1 is controlled by Tsc1/Tsc2 (where Tsc is tuberous sclerosis complex) via negative regulation of the GTPase Rhb1 [18,19]. How TSCs are regulated in fission yeast are yet to be addressed. In mammals, several pathways convey the presence of growth signals such as insulin to mTORC1 via regulation of TSC1/TSC2 [20]. The TSCs do not have orthologues in budding yeast, although

© The Authors Journal compilation © 2008 Biochemical Society

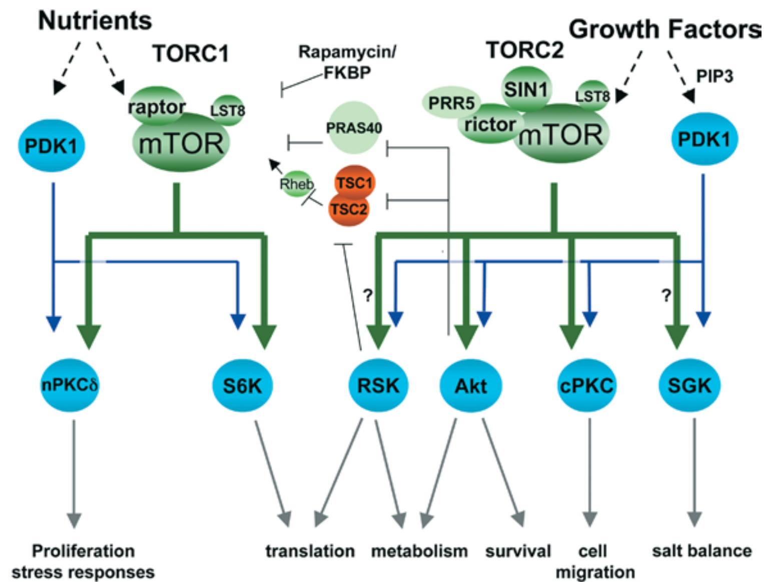

Figure 3 mTOR complex signalling to AGC kinases in mammals

In the presence of growth signals such as nutrients and growth factors, the AGC kinases become activated by phosphorylation at the T-loop by PDK1 and at other C-terminal tail sites by 'PDK2' (presumably mTOR). A model of regulation would be that it is the conformation and/or localization of the AGC kinase that determines when it will be phosphorylated by PDK1 and mTORCs. See Figure 1 for colour-coding. ? indicates that experimental evidence is lacking for a direct mechanism. Conserved mTOR core complex components are in dark green, whereas non-conserved interactors are in light green. Examples of AGC kinase cellular function are also included.

a Rheb orthologue, RHB1, is present [21,22]. Whether RHB1 regulates TOR in this organism, despite the absence of the TSCs, remains to be investigated. Due to the amenability of yeast to genetic studies, we will probably gain important insights into the relationship between the two TORCs and the significance of TSC/Rheb and FKBP/rapamycin signals on the TORC pathway using these simpler organisms.

Raptor (KOG1/Mip1)

KOG1, a product of an essential gene in budding yeast, co-purifies with TOR1 and contains four internal HEAT repeats and seven C-terminal WD40 repeats [23]. Fission yeast Mip1 forms a complex with and displays a genetic interaction with Tor2 (the functional counterpart of TOR1 in *S. cerevisiae*), as part of TORC1 [16,17].

In mammals, raptor also binds to mTOR [23–25], but this association, unlike the yeast counterpart, is sensitive to rapamycin and perhaps nutrients as well [24,26]. Raptor is thought to serve as a scaffold to present substrates to mTOR, but does not alter the intrinsic catalytic activity of mTOR. No viable null mice were obtained from heterozygous intercrosses of raptor$^{+/-}$ mice, similar to mTOR$^{+/-}$ mice, indicating that raptor is an essential gene [27,28].

mLST8 (LST8; Wat1)

LST8 from budding yeast is an essential, G_β-like protein made up entirely of seven WD40 repeats. It is found in both TORC1 and TORC2 and binds to the kinase domain of TOR2 [29]. LST8 is required for amino acid permease transport from the Golgi to the cell surface [30,31]. Consistent with these findings, LST8 localizes as a peripheral membrane protein to endosomal or Golgi membranes [32]. Overexpression of CCT6 (chaperonin containing TCP-1), a chaperonin complex subunit, suppresses the abnormal growth phenotype of an LST8 mutant (*lst8-2*). Interestingly, this chaperonin can also suppress the growth defect of a *tor2* mutant or a strain overexpressing the TORC1-regulated phosphatase SIT4 [33]. The mechanism for this suppression is not understood, but supports a role for the TORCs in protein folding and stabilization (see PKC below).

In fission yeast, the LST8 orthologue Wat1 binds both Tor1 and Tor2 [16,17] and is required for proper localization of F-actin [34], although another study found that neither TORC1 nor TORC2 play a significant role in actin organization in fission yeast [16]. mLST8 binds to mTOR and raptor when both are overexpressed [23,35]. mLST8 binds to the kinase domain of mTOR to stimulate mTOR kinase activity [35]. Its association with mTOR is insensitive to nutrients, but is required for the nutrient-sensitive interaction of raptor to mTOR. How mLST8 can promote mTOR kinase activity is still unclear, but it has been speculated that it could contribute to the stability and folding of the mTOR kinase domain or could also play a role in the recognition and recruitment of substrates to mTOR [35]. Knockdown of mLST8 leads to defects in actin cytoskeleton reorganization upon serum restimulation of fibroblasts, indicating that mLST8 may also function as part of mTORC2 [36]. More recent studies using mLST8-knockout cells indicate that mLST8 is required for mTORC2 but not mTORC1 functions [27].

TORC2/mTORC2

Budding yeast TOR2 forms a complex with the conserved proteins AVO1, AVO3 and LST8 to comprise TORC2. In fission yeast, TORC2 consists of Tor1, Ste20, Sin1 and Wat1. Despite the presence of only one mTOR in mammals, mTOR also forms a separate complex with rictor (rapamycin-insensitive companion of mTOR; AVO3 orthologue), SIN1 (AVO1 orthologue) and mLST8. Budding yeast and mammalian TORC2 signalling is not acutely sensitive to rapamycin. Curiously, in fission yeast, rapamycin does not inhibit TORC1 during vegetative growth, but appears to affect some of the TORC2-dependent functions [37,38]. Two-hybrid results also indicate that Fkh1 (FKBP12) can bind to Tor1, a component of TORC2, in the presence of

© The Authors Journal compilation © 2008 Biochemical Society

rapamycin [38]. Additionally, genetic data reveal that a TORC2-dependent function is inhibited by rapamycin [38], suggesting a more complicated mechanism for rapamycin action. In mammals, growth-factor or hormonal signals appear to regulate mTORC2 although the mechanism is not understood [39,40]. Since these signals are not conserved in yeast, mTORC2 may be modulated by signals that similarly regulate yeast TORC2. These signals are yet to be identified, although studies from yeast suggest that they are required for cell integrity and viability under stress conditions [41,42]. In fission yeast, TORC2 is also required for responses to starvation, sexual development and stress conditions [43,44].

Rictor (AVO3/Ste20)

AVO3 in budding yeast is required for TORC2 integrity, but is dispensable for the *in vitro* kinase activity of TOR2 [29]. Mutations in *AVO3* (formerly named *TSC11*) suppress *csg2* mutants that accumulate sphingolipids [45]. In this screen, mutants of *tor2* were also pulled out. It is currently unclear how TORC2 may be involved in sphingolipid metabolism or signalling, although newly identified TORC2 effectors SLM1/2 (synthetic lethal with MSS4 1/2) are targets of sphingolipid signalling during heat stress [42,46–48].

S. pombe Ste20 (different from *S. cerevisiae* STE20) shares homology with rictor [49]. Ste20 is probably part of TORC2, since it binds Tor1 more strongly than Tor2 upon overexpression [16]. Fission yeast *ste20* mutants are resistant to amiloride, which inhibits sodium uptake and cell growth in *S. pombe* [49]. In mammals, knockdown of rictor leads to defects in both actin cytoskeleton organization and in the phosphorylation of cPKCα (conventional protein kinase Cα) and Akt at the hydrophobic motif sites [36,39,50]. The most compelling evidence for a role of rictor (TORC2) in phosphorylation of these AGC kinases was demonstrated in studies using rictor-knockout cells (as discussed below). Rictor knockout is embryonic lethal [27,51,52]. MEFs (murine embryonic fibroblasts) isolated from null embryos did not display any remarkable defects in actin cytoskeleton nor morphology, although the growth rate of rictor-null cells was slower than that in the wild-type MEFs [51].

SIN1 (AVO1/Sin1)

A truncated form of SIN1 (JC310) was originally identified as a human protein that suppressed the heat-shock-sensitive phenotype of a *S. cerevisiae* mutant expressing the hyperactive RAS2 version, RAS2^{Val19} [53]. Sin1 binds *S. pombe* SAPK (stress-activated protein kinase) whereas, in mammals, it binds JNK (c-Jun N-terminal kinase) and MEKK2 [MAPK (mitogen-activated protein kinase)/ERK (extracellular-regulated-protein kinase) kinase kinase 2] [54–56]. Hence this protein participates in Ras and/or MAPK signalling in yeast and mammals by mechanisms that await further investigation. In budding yeast, AVO1 binds to yeast TORC2. Decreasing expression of *AVO1* in yeast leads to actin depolarization, a phenotype that is displayed by *tor2* mutants [23]. Mammalian SIN1 is found in the rapamycin-insensitive mTORC2 [40,52]. Knockout of SIN1 in mammals leads to embryonic lethality, loss of TORC2 complex formation and kinase activity [40,52]. In mammals, at least five alternatively spliced isoforms could be generated [55,57]. Three of these isoforms were reported to form three distinct mTORC2 complexes [58]. Most SIN1 orthologues contain a Raf-like RBD (Ras-binding domain) and PH (pleckstrin homology) domain, but the function of these domains *in vivo* awaits further examination [59].

Other TORC interactors

Other non-conserved proteins can form a complex with either TORC1 or TORC2. TCO89 (TOR complex one 89 kDa subunit), the product of a yeast-specific non-essential gene in *S. cerevisiae*, originally identified as a protein involved in glycerol uptake under osmotic stress conditions [60], associates with TORC1 [61]. Loss of TCO89 leads to rapamycin hypersensitivity and defects in CWI (cell-wall integrity). However, TCO89 does not exclusively associate with TORC1. It has distinct localization in vacuolar structures and associates with the vacuolar armadillo repeat protein VAC8 [62–64], and is therefore thought to perform an additional cellular role that is independent of TORC1 [61,63]. Since TORC1 was recently found at the vacuolar membrane [62], it remains to be examined whether TCO89 functions together with TORC1 in this compartment. TORC2 also physically interacts with the yeast-specific proteins AVO2 and BIT61 [23,61], both encoded by non-essential genes. Although their function is unknown, BIT61 can associate with SLM1 and SLM2, two proteins that mediate TORC2 function in actin cytoskeleton organization and responses to heat and oxidative stress [41,46–48,65]. The SLMs are phosphorylated in a sphingolipid-dependent manner that is counteracted by dephosphorylation by calcineurin [47,48]. TOR2 has been linked to the phosphorylation of the SLMs *in vivo* and *in vitro*, but it is unclear if this is via direct regulation [41,46].

PRAS40 (proline-rich Akt substrate of 40 kDa) can bind and negatively regulate mTORC1 [66–71]. It interferes with binding of substrates to mTOR and itself is phosphorylated by mTORC1 in a rapamycin-sensitive fashion [68]. Akt phosphorylates PRAS40 to negatively regulate this protein, thereby promoting mTORC1 signals. Unlike TSC2, which antagonizes the positive role of Rheb on mTORC1, PRAS40 can directly inhibit mTORC1 [69]. Another proline-rich protein, Protor (protein observed with rictor), also called PRR5 (proline-rich protein 5) binds to rictor, independently of mTOR [72,73]. The function of PRR5 is unknown, but it may play a role in PDGFR (platelet-derived-growth-factor receptor) signalling [72]. Knockdown of PRR5/Protor does not significantly reduce phosphorylation of Akt at Ser^{473} [72]. A PRR5L (PRR5-like) protein, which regulates apoptosis, was also identified to bind to mTORC2 [71]. Both PRR5 and PRR5L are not required for binding of rictor and SIN1 to mTOR. Whether these mammalian TORC2 interactors could mediate specific functions of mTORC2 remains to be investigated.

TOR and its targets

mTOR can phosphorylate the AGC kinase S6K (S6 kinase) and the translational regulator 4E-BP [74]. 4E-BP is a small regulatory protein with no enzymatic activity, but it is phosphorylated at multiple residues in a rapamycin-sensitive fashion to promote cap-dependent translation. A common recognition motif, TOS (TOR signalling), is found in S6K and 4E-BP [75], which could explain why mTOR can phosphorylate these two structurally diverse targets. mTOR, as part of the rapamycin-insensitive complex mTORC2, was also shown to regulate the phosphorylation of another AGC kinase, Akt [39,76]. In budding and fission yeast, TOR can mediate phosphorylation of yeast AGC kinases as well [62,77]. Other non-AGC kinase proteins in yeast are phosphorylated in a TOR-dependent manner, such as the phosphatase regulator TAP42 (type 2A phosphatase-associated protein 42) and TIP41 [78,79], transcription factor GLN3 [80], NPR1 (nutrient permease regulator) kinase [81] and phosphatidylinositol-binding proteins SLM1/2 [46]. Whether TOR can directly phosphorylate these targets via recognition of a common motif would need more rigorous scrutiny. Comparison

© The Authors Journal compilation © 2008 Biochemical Society

Table 2 Comparative list of AGC kinases from *S. cerevisiae*, *S. pombe* and *H. sapiens*

Budding yeast AGC kinases	Fission yeast AGC kinases	Mammalian AGC kinases
PKC1	Pck1/2	cPKC-$\alpha/\beta/\gamma$, nPKC-$\eta/\varepsilon/\delta/\theta$, aPKC-$\iota/\zeta$, PRK1/2, PKN3
		ROCK1/2, DMPK, MRCK
YPK1/2	Gad8	Akt1/2/3, SGK1/2/3
SCH9	Sck1/2	S6K1/2, RSK1/2/3/4, MSK1/2
TPK1/2/3	Pka1	PKAc-$\alpha/\beta/\gamma$, PRKY, PRKX
		PKG1/2
CBK1	Orb6	NDR1/2
RIM15	Ppk18/Cek1	(NDR1/2)
DBF2/DBF20	Ppk35/Sid2	LATS1/2
		MAST1/2/3/4, MASTL
		βARK1/2, GPR4/5/6/7, GPRK7
PKH1/2	Ksg1	PDK1
PKH3	Ppk21	
		YANK1/2/3, SGK494
IPL1	Ark1	Aurora kinases
BUB1	Bub1	
KIN82	Ppk22	
YNR047W	Ppk14	
YBR028C	Psk1	
YKL171W	Ppk31	
	Cmk2	

of all known and putative TOR/mTOR targets indicates that TOR may target a serine/threonine residue followed by a proline residue or by a hydrophobic, aromatic amino acid such as a phenylalanine residue. The TOR target site in AGC kinases lies at the conserved hydrophobic motif located at the C-terminal tail of the kinase domain. There are other conserved motifs in the C-terminal tail among the AGC kinases that contain phosphorylated residues, which can also be potentially regulated by TOR. Phosphorylation at the C-terminal tail critically regulates the catalytic activity, stability and localization of the AGC kinases. The importance of phosphorylation at this segment was revealed in early studies on PKC and S6K [82–84]. The structural divergence of the C-terminal tail of AGC kinases seems to coincide with the emergence of TOR. Interestingly, a parasitic unicellular eukaryote was recently reported to have primordial AGC kinases [related to PKA (protein kinase A) and NDR] but does not have TOR [85]. Hence, the TOR and AGC kinase C-terminal tail could have co-evolved to fulfill a basic function among eukaryotes, perhaps to balance growth versus survival signals in response to nutrients and environmental conditions.

REGULATION OF AGC KINASE DOMAIN IN YEAST AND MAMMALS

The AGC kinases belong to the conventional ePKs (eukaryotic protein kinases) (Table 2) [2]. Members of the AGC family share considerable homology in their kinase domains [86]. The catalytic core of AGC kinases, like other ePKs, has a bilobal composition [87]. The smaller N-terminal lobe binds nucleotides whereas the large C-terminal lobe participates in substrate binding and catalysis. Like other ePKs, the AGC kinases are activated by phosphorylation at the activation loop (also called the T-loop, consensus sequence T*FCGT, where * is a phosphorylated residue) that connects the N- and C-lobes of the kinase domain. Phosphorylation at a conserved serine/threonine residue in the T-loop can occur via autophosphorylation or can be catalysed by another protein kinase. Several members of the AGC family can be phosphorylated at the T-loop by PDK1. The AGC kinases are characterized by the presence of a C-terminal tail that is critical for interactions with the N- and C-lobes of the kinase domain. Three regions mediate these interactions, namely the NLT (N-lobe tether), which includes the conserved HM (hydrophobic motif), the AST (active-site tether) and the CLT (C-lobe tether) [88]. These regions could act as *cis*-acting regulatory modules to allow binding of *trans*-acting cellular molecules to further regulate AGC kinase activity [88].

In addition to phosphorylation at the T-loop, most AGC kinases are phosphorylated at the HM (consensus sequence FXXFS/T*Y/F) [86]. The HM is significant for the following reasons. First, the HM site serves as a docking site for PDK1, which catalyses phosphorylation of the T-loop [89,90]. PDK1, which lacks the HM, docks to the HM site of its substrate via its own hydrophobic pocket [referred to as the PIF (PDK-interacting fragment) pocket]. Secondly, phosphorylated HM folds back and interacts with the hydrophobic pocket of the N-lobe. Occupation of the hydrophobic pocket leads to optimal alignment and stabilization of the αC-helix conformation. Together with T-loop phosphorylation, binding of the phosphorylated HM site to the hydrophobic pocket significantly stabilizes the kinase conformation and allosterically activates the AGC kinase. Several kinases have been proposed as a possible ‘PDK2’, the HM kinase [91]. Whereas most AGC members share the same T-loop kinase, PDK1, it seems that the PDK2 is variant among the AGC kinases because there are subtle differences in the mechanism of this phosphorylation. In both yeast and mammals, accumulating data indicate that TOR/mTOR is the ‘PDK2’ and mediates the phosphorylation of the HM site of several AGC kinases. We will discuss the evidence that supports a role for TOR/mTOR as the PDK2 and alternative mechanisms as to how it can mediate or allow HM phosphorylation.

Apart from the HM site, most of the AGC kinases are phosphorylated at several sites in the C-terminal tail. Between the AST and NLT, there is a highly conserved region named the TM (turn motif). In PKA this is at the apex of a tight turn of the tail, hence the name [92]. Most of the AGC kinases are phosphorylated at this residue presumably by TOR/mTOR. S6K and budding yeast SCH9 are additionally phosphorylated at several sites in the C-terminal tail adjacent to the HM [62,93,94]. Phosphorylation of one or more of these sites is rapamycin-sensitive, and most probably directly regulated by TOR.

Budding yeast AGC kinases

The budding yeast (also known as baker’s yeast or *S. cerevisiae*) genome codes for 117 ePKs and 10 aPKs (atypical protein kinase) [95], with approx. 20 of the ePKs belonging to the AGC kinase family (Table 2 and Figure 4A) [3,95]. We will focus our discussion on the AGC kinases that have genetic or functional linkage to TOR (Figure 4A).

YPK (yeast protein kinase)

YPK1/2 [also known as YKR2 (yeast-kinase related)] have the highest homology with mammalian SGK (serum- and glucocorticoid inducible kinase) and Akt and can be functionally replaced by SGK, and partially by Akt1 [96]. Deletion of *YPK1* results in a slow growth phenotype and in rapamycin hypersensitivity, whereas the loss of *YPK2* shows no apparent phenotypic defect [97,98]. The simultaneous deletion of both genes is lethal, suggesting overlapping functions. *YPK*-deficient cells exhibit translation initiation arrest and in wild-type cells YPK1 itself is rapidly degraded upon nitrogen starvation [97]. These findings suggest that YPK may be a component of a nutrient-sensing pathway that can regulate translation initiation and point to the rapamycin-sensitive TORC1 as a probable YPK

© The Authors Journal compilation © 2008 Biochemical Society

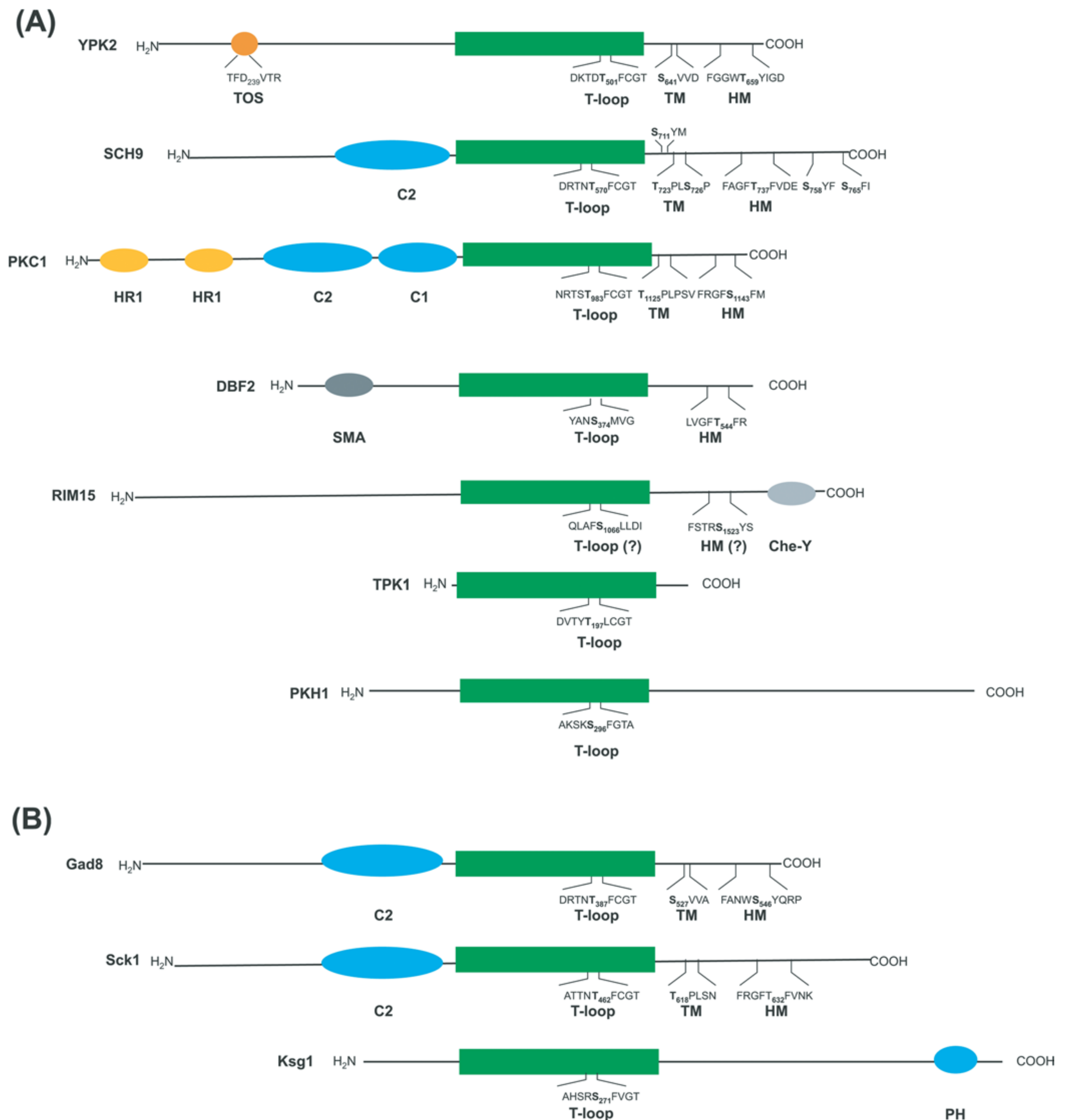

Figure 4 Yeast AGC kinase conserved regions and phosphorylation sites

(**A**) AGC kinases in budding yeast. YPK2 has a TOS (TOR signalling) motif in the N-terminus. SCH9 has a C2 (Ca^{2+}/lipid) domain of unknown function. PKC1 contains a Rho-binding domain (HR1) and C2 and C1 domains of unknown function but homologous with mammalian cPKC C1/C2 domains. DBF2 has a SMA (S100B and Mob1 association) domain in the N-terminus. RIM15 has a Che-Y (domain homologous with chemotaxis protein Che-Y in bacteria) [220] domain in the C-terminus. RIM15 T-loop and HM sites were predicted by alignment with NDR1 using CLUSTAL and SMART. All AGC kinases are activated by phosphorylation at the activation loop (T-loop) by PKH1. Except for PKH1 and PKA, the above AGC kinases contain an HM. (**B**) AGC kinases in fission yeast. Gad8 and Sck1 both possess C2 lipid domains of unknown function. Other conserved regions are as described in (**A**). Ksg1 is the PDK1 orthologue. The conserved kinase domain is green. Motifs (C1/C2, PH) that may potentially bind lipids or membranes are in blue. All other colours represent motifs that are not conserved among AGC kinases.

regulator. However, rapamycin does not affect the kinase activity and expression levels of YPK [97]. Instead, other evidence points to TORC2 as the upstream regulator of YPK.

In a genetic screen, a 5′-truncated version of the *YPK2* gene was identified as a suppressor of the lethality of a strain depleted for TOR2 [99]. Mutation of a conserved sequence ($T^{237}FDVT^{243}$)

© The Authors Journal compilation © 2008 Biochemical Society

in the N-terminal region, resulting in the *YPK2*^{D239A} allele, suppresses the defective actin organization of a *tor2* mutant and the impaired phosphorylation of MPK1, a downstream effector of TOR2 in the cell-integrity pathway. This N-terminal amino acid sequence, which resembles the TOS motif, negatively regulates YPK activity. TORC2 can phosphorylate YPK2 *in vitro* and this phosphorylation is decreased upon mutation of the HM (Thr^{659}) and the TM (Ser^{641}) sites to an alanine residue, suggesting that these sites are targeted by TORC2 [99]. The TORC2-mediated phosphorylation relieves the inhibition from the TOS-like regulatory region and allows phosphorylation of the activation loop. The kinase activity of YPK2 in a temperature-sensitive *tor2-21* mutant was extensively diminished when compared with YPK2 from wild-type cells, confirming that TOR2 as part of TORC2 regulates YPK2 activity [99]. YPK1 and TORC2 act in the same pathway since overexpression of the RHO1-GEF (RHO1-guanine-nucleotide-exchange factor), TUS1, suppresses *ypk* or *tor2* mutants [100,101].

YPK1 is activated by phosphorylation at the T-loop (Thr^{504}) of the kinase domain by PKH (PKB-activating kinase homologue) 1, the PDK1 orthologue in budding yeast [96]. This phosphorylation is essential for YPK1 function because mutation of Thr^{504} to an alanine residue cannot complement the sensitivity of a *ypk1*Δ mutant to myriocin, a sphingolipid biosynthesis inhibitor [102]. An analogous mutant allele of YPK2 also does not complement a *ypk*Δ mutant, suggesting that T-loop phosphorylation is also essential for YPK2 function [99]. YPK1 was isolated as a high-copy suppressor of the lethal sphingolipid depletion induced by myriocin [103]. This suppression may be accounted for by the sphingolipid-mediated activation of PKH [104], which in turn can activate YPK. YPK can also be activated by the sphingolipid signalling molecule, phytosphingosine, in a more direct manner via an unknown mechanism, but speculatively due to HM site phosphorylation by TORC2. A phosphomimetic mutant of YPK1 at the HM site T662D shows higher kinase activity than wild-type and restores myriocin resistance [102,105]. Whether YPK may be recruited to a membrane compartment where it binds sphingolipids is not clear, since YPK has no apparent lipid-binding domain, although it localizes to the membrane depending on the sphingolipid level [64,103,105]. Together, these findings corroborate that the YPKs are downstream effectors of TORC2. However, since other findings suggest a possible role of YPK in translation initiation, it is likely that there is significant crosstalk between the two TORC signals.

SCH9

SCH9 was isolated as a suppressor of the Ras/PKA signalling defects [106] and is required for longevity and cell size in budding yeast [107,108]. The closest mammalian homologue of SCH9 is Akt, although recent analysis revealed it has functional resemblance to S6K [62] and is directly targeted by TORC1. Phosphorylation of seven serine/threonine residues in the C-terminus of SCH9 occurs *in vivo* [62]. Six of them were rapamycin- and nutrient-sensitive, indicating that they are regulated by TORC1. Thr^{737} lies in a classical HM sequence whereas Ser^{758} and Ser^{765} share similarity to the HM due to the presence of hydrophobic residues surrounding the phosphorylation site (Figure 4A). Ser^{711} is also followed by a hydrophobic residue at position $+1$ and $+2$. Thr^{723} and Ser^{726} are followed by a proline residue, very similar to phosphorylated residues in the turn motif. TORC1 directly phosphorylates at least five of these sites *in vitro*. Phosphorylation of these residues is absolutely essential for SCH9 function. A combined alanine mutation of these TORC1 target sites (SCH9^{5A}) abolishes *in vitro* kinase activity of SCH9. Whether the C-terminal tail sites distal to the HM are analogous to the autoinhibitory domain in S6K remains to be examined.

Like other AGC kinases, SCH9 needs to be phosphorylated by PKH on residue Thr^{570} of the activation loop. Addition of phytosphingosine, which stimulates PKH1, also leads to phosphorylation and activation of SCH9 in the presence of PKH1 and PKH2 *in vitro* [102]. Like YPK, SCH9 is activated by phytosphingosine indirectly via PKH phosphorylation of the T-loop. Since a PKH kinase-dead allele also increases SCH9 phosphorylation in the presence of phytosphingosine, a more direct effect of this sphingolipid on SCH9 may also occur. This mechanism remains to be characterized, but speculatively involves the phosphorylation of the C-terminal tail sites by TORC1.

Unlike YPK, SCH9 has a predicted C2 lipid-binding domain. SCH9 is predominantly at the vacuolar membrane [109]. The vacuolar surface also contains active TORC1. Consistent with a model that TORC1 may regulate SCH9 in this compartment, vacuolar membrane targeting of SCH9 results in hyperphosphorylation of SCH9 in a rapamycin-sensitive manner [62]. The targets of SCH9 in this cellular compartment remain to be identified. Since the vacuole is an important reservoir of nutrients in yeast, TORC1 and/or SCH9 may play a role in sensing and/or remobilization of intracellular nutrients. Compatible with its structural similarity to S6K, SCH9 also regulates translation initiation, ribosome biogenesis and phosphorylates RPS6 (ribosomal protein S6) *in vitro* [62]. SCH9 phosphorylation is also reduced following osmotic, oxidative or thermal stress [62]. Another study provided evidence that SCH9 plays a role in the transcriptional activation of genes that are essential for osmotic stress responses [110]. This function of SCH9 is dependent on the MAPK HOG1 (high osmolarity glycerol response 1) and the ATF (activating transcription factor)/CREB (cAMP-response-element-binding protein) transcription factor SKO1, suggesting that SCH9 functions in a similar manner to the mammalian AGC kinase MSK1/2 (mitogen- and stress-activated kinase 1/2), a close homologue of Rsk1/2 (p90 ribosomal S6K 1/2) [110]. SCH9 does not contain the C-terminal kinase domain that is regulated by MAPK in MSK1/2, but the HOG1/SCH9 signalling mechanism may be ancestral to the mammalian p38 MAPK/MSK signalling and should yield insights on how nutrient and stress signals are integrated.

PKC1

The budding yeast genome encodes only one PKC, namely PKC1. PKC1 is considered an archetypal PKC [111], since it possesses all the domains found in the various mammalian PKC isotypes, including the PRKs (PKC-related kinases) and ROCKs (Rho-associated kinases). In a similar manner to the ROCK subfamily of AGC kinases, it binds to and is regulated by Rho1 (GTPase) [112]. The *pkc*Δ mutant can also be complemented by mammalian novel PKC [113]. PKC1 functions in the CWI signalling pathway and in regulating actin polarization via MAPK signalling [114]. TORC2 signals to the actin cytoskeleton via PKC1. Previous studies have demonstrated that the PKC regulation by TORC2 occurs via the activation of the Rho1 GTPase [115]. PKC1 associates with and becomes activated by GTP-bound Rho1 [112]. It is also phosphorylated by PKH1/2 at Thr^{983} of the activation loop [116]. *In vitro*, PKC1 phosphorylation by PKH1 is enhanced by the presence of phytosphingosine [104], suggesting that PKC1 may be activated by sphingolipids similar to YPK2. The phosphatidylinositol lipid PtdIns(4,5)P_2 can bind to the Rho-GTPase GEF Rom2 and may also be required to activate Rho1 and promote PKC activation [117]. The role of lipids

© The Authors Journal compilation © 2008 Biochemical Society

(sphingolipids and phospholipids) in PKC activation remains to be further examined, although addition of phosphatidylserine, in the presence of GTP-bound Rho can potently activate PKC1 *in vitro* [112]. Despite the presence of a lipid-binding domain, PKC1 does not seem to be activated by diacylglycerol or Ca^{2+}, unlike conventional mammalian PKC. Whether PKC1 can be directly regulated and activated by TORC2 remains to be examined. Recently, one of us found that phosphorylation of the conserved TM site in PKC1 is mediated by TORC2 (E. Jacinto, unpublished work). Yeast cells depleted for the TORC2 component AVO1, but not the TORC1 component KOG1, had decreased TM phosphorylation. Phosphorylation of the TM site in mammalian cPKC is important for stability of the kinase and in the absence of this phosphorylation, molecular chaperones such as Hsp (heat-shock protein) 70, were shown to bind and stabilize cPKC [118,119]. In line with these findings, the growth defect of yeast strains expressing a defective Hsp40 and temperature-sensitive allele of Hsp90 was suppressed by overexpression of *PKC1* [120]. These findings suggest that stabilization of AGC kinases via phosphorylation and chaperone action could play an important role in their signalling function. Other possible phosphorylation at the C-terminal tail of PKC1, such as the HM site, may also be regulated by TORC2, but this remains to be formally demonstrated.

Other yeast AGC kinases: are they regulated by the TORCs?

Several other AGC kinases in budding yeast have been linked to TOR signalling or at least function in a parallel pathway with common downstream targets. Aside from the AGC kinases discussed above, none of the other members of this family in yeast has been shown to be regulated by the TORCs at their conserved C-terminal tail.

PKA (cAMP-dependent protein kinase)

In yeast there are three coding genes for the catalytic subunit of PKA, *TPK1*, *TPK2* and *TPK3* and one for the regulatory subunit *BCY1*. The N-terminal region of these three catalytic isoforms is highly variable, but the conserved kinase domains have more than 75 % homology with each other. Like mammalian PKA, cAMP-binding leads to dissociation of BCY1 from the catalytic subunits to activate PKA. Addition of glucose to cells grown on a fermentable carbon source results in rapid and transient cAMP accumulation and PKA activation. Although there is no evidence that either TORC1 or TORC2 directly phosphorylate subunits of PKA, several lines of evidence indicate a functional linkage between TOR and PKA signalling. Both signalling pathways are key regulators of ribosome biogenesis [121]. Mutations that hyperactivate PKA signalling confer rapamycin resistance [122]. Furthermore, constitutive activation of the Ras/cAMP pathway prevents several rapamycin-induced responses such as nuclear translocation of the stress-related transcription factors MSN2/MSN4, induction of stress genes, induction of autophagy, glycogen accumulation and down-regulation of ribosome biogenesis [123]. Although these findings indicate that PKA is an effector of TOR signals, the mechanisms for PKA regulation by TOR is obscure. Upon rapamycin treatment, TPK1 accumulates in the nucleus, a response similar to cAMP deprivation [123], suggesting that TOR may regulate localization of TPK. BCY1 is mostly nuclear in glucose-growing cells, and localization of TPK to the nucleus upon rapamycin treatment may promote inhibition of PKA activity by association of TPK with BCY1. These findings imply that TOR maintains PKA activity in the cytoplasm. TPK1 undergoes phosphorylation in a glucose-dependent manner, but the sites that undergo phosphorylation have not been identified, although it has been surmised that possible sites other than the T-loop site become phosphorylated [124]. The TPKs do not contain a serine/threonine residue at the homologous turn motif, however. Therefore it is unlikely that TOR regulates phosphorylation of the C-terminal tail of TPKs.

DBF (dumbbell forming)

DBF2/20 are part of the NDR subfamily of AGC kinases. A unique feature of this subfamily is the presence of a 30–60-amino-acid insert between subdomains VII and VIII of their kinase domain. The function of this insert is poorly understood, but it is known in mammalian NDR1 to contain a nuclear localization signal [125]. Members of this kinase subfamily are mainly nuclear and regulate cell cycle and morphogenesis. Although so far no direct link between DBF2/DBF20 and the TOR complexes has been observed, a possible connection between the DBF2/20 and TOR could exist on the basis of genetic data. *dbf2* mutants show increased sensitivity to rapamycin and wortmannin in comparison with wild-type cells [126]. Interestingly, they also show increased glycogen accumulation in a similar manner to *tor1* mutants [127]. The T-loop site (Ser^{281} of NDR1) of this family of kinases becomes autophosphorylated in mammalian NDR1, whereas the HM site (Thr^{444}) could be phosphorylated by the STE20 family of kinases. In budding yeast, the DBF2 upstream kinase is the STE20-like CDC15 [128]. The amino acids surrounding the HM site diverge from the consensus sequence (Figure 4A), hence TOR may only indirectly regulate DBF.

RIM15 (regulator of IME2)

RIM15 is a distant member of the NDR family of AGC kinases. Regulation of RIM15 occurs via the regulation of its subcellular distribution. RIM15 seems to be a common target of TORC1 and PKA [129]. A PKA-dependent phosphorylation leads to nuclear exclusion of RIM15 because of binding to BMH1 and BMH2 [95,130]. In parallel, TOR also negatively regulates nuclear accumulation of RIM15 via unknown mechanisms [129].

PKH1/2

PKH1/2 encode orthologues of mammalian PDK1 since the lethality of a *pkh1*Δ*pkh2*Δ double mutant is suppressed by expression of human PDK1 [96,116]. PKH1/2 have no apparent PH domains and they are unlikely to be regulated by PtdIns(3,4,5)P_3 since budding yeast does not produce PtdIns(3,4,5)P_3 or PtdIns(3,4)P_2 [131]. In addition, PDK1 lacking a PH domain could rescue *pkh1*Δ*pkh2*Δ growth defects [96]. In contrast with mammalian PDK1, which is regulated by 3-phosphoinositides, PKHs need sphingolipids for full activation [104]. Deletion of PKH1, but not PKH2, also results in rapamycin hypersensitivity [97]. Although TOR and PKH share some overlapping functions, there is no evidence that PKH is directly regulated by TOR.

Fission yeast AGC kinases

The fission yeast (*S. pombe*) genome codes for 106 protein kinases [132]. Of these, 20 were classified as potential AGC kinases (Table 2 and Figure 4B) [3]. There is very little known on how these AGC kinases may be regulated at their kinase domain. Remarkably, the existence of a conserved TOR–PDK1–AGC kinase signalling module was first recognized in *S. pombe* studies [77].

© The Authors Journal compilation © 2008 Biochemical Society

Gad8 (G_1-arrest defective 8)

Gad8 is the fission yeast orthologue of budding yeast YPK and mammalian SGK, although rescue experiments with the heterologous proteins have yet to be performed. *gad8*$^+$ was first isolated as a high-copy suppressor of the sterility and the temperature-sensitive growth phenotype of a mutant expressing a C-terminally truncated Tor1 protein [77]. Site-directed mutagenesis of each of the AGC kinase conserved phosphorylation sites (Thr^{387}, Ser^{527} and Ser^{546}) to an alanine residue revealed that phosphorylation of Ser^{527} in the turn motif and Ser^{546} in the HM is Tor1-dependent. However, evidence that Ser^{527} and Ser^{546} are directly phosphorylated by Tor1 is missing. As in mammals and budding yeast, Thr^{387} within the activation loop of Gad8 is phosphorylated directly by the PDK1 orthologue, Ksg (kinase responsible for sporulation and growth) 1, *in vitro*. Analysis of *gad8* mutant alleles demonstrated that phosphorylation of all three residues (Thr^{387}, Ser^{527} and Ser^{546}) is essential for Gad8 activity and *in vivo* function. Gad8 also bears a C2 lipid-binding domain of unknown function.

Sck1/2 (suppressor of loss of cAMP-dependent protein kinase)

Sck1/2 represent the fission yeast orthologues of budding yeast SCH9 [133,134]. As in budding yeast, loss of PKA activity can be suppressed by overexpression of *sck1*$^+$ or *sck2*$^+$ [134]. Interestingly, overexpression of *sck2*$^+$ leads to decreased mating under starvation conditions [134]. Inhibition of sexual differentiation and mating also occurs in cells overexpressing *tor2*$^+$, a component of TORC1 [17]. The role of Ksg1 and Tor2 in phosphorylating the conserved sites in the T-loop and C-terminal tail respectively, remains to be demonstrated. Like SCH9, Sck1 has a C2 lipid-binding domain of unknown function.

Ksg1

Ksg1 is an essential protein and shows the typical domain structure known for PDK1-like enzymes, including a C-terminal PH domain [135]. This PH domain may bind lipids since *S. pombe* can synthesize PtdIns(3,4,5)P_3 [136]. *ksg* mutants show impaired mating and low sporulation efficiency [135,137]. Mutants also show defects in G_1 arrest in response to nitrogen starvation [137]. Ksg1 also controls CWI, since growth defects of *ksg1* mutants could be suppressed by the addition of osmotic stabilizers [138]. All phenotypic defects could be suppressed by the expression of human PDK1, confirming that Ksg1 is the fission yeast PDK1 orthologue [138].

Ksg1 interacts with the fission yeast orthologues of PKC, Pck1 and Pck2, in a two-hybrid assay. This interaction depends on the presence of the PH domain of Ksg1 [138]. The interaction of Ksg1 and Pck1 was also confirmed biochemically. Genetic evidence supports the view that Pck1 acts downstream of Ksg1, since *ksg1* mutants are suppressed by overexpression of Pck2. The PKA orthologue in fission yeast, Pka1, is also regulated by Ksg1. Pka displayed less phosphorylation in *ksg1* mutants. Phosphorylation of Thr^{356} of Pka within the activation loop is Ksg1-dependent [137]. However, evidence for direct regulation of Pka1 by Ksg1 is not available. In a similar manner to yeast PKH and mammalian PDK, there is currently no evidence linking fission yeast Tor in the regulation of Ksg1.

Mammalian AGC kinases

Out of approx. 500 protein kinases in the human genome, there are 63 kinases grouped into the AGC family (Table 2 and Figure 5). Apart from the catalytic domain, the AGC kinases show little similarity and not surprisingly, are controlled via distinct mechanisms. Nevertheless, the structural similarity of their catalytic domain suggests overlapping substrate specificity and regulation of this domain.

S6K

Initial studies identified S6K as insulin- or interleukin 2-activated protein kinase that is potently inhibited by rapamycin [139,140]. S6K is encoded by two genes, *S6K1* and *S6K2* [141]. Active S6K phosphorylates the ribosomal protein S6 to promote protein synthesis. S6K can be divided into four modular domains (Figure 5): the N-terminal domain, which contains the TOS motif, the catalytic domain, which includes the activation loop, a linker domain, which contains two important regulatory phosphorylation sites namely the TM and HM sites, and the autoinhibitory pseudosubstrate domain that is unique to S6K among AGC kinases and is phosphorylated at multiple sites [142]. In S6K2, a proline-rich region is also present following the autoinhibitory domain [141]. Phosphorylation of S6K occurs at multiple sites in a hierarchical manner and is sensitive to nutrient or growth-factor withdrawal and rapamycin treatment. The TOS motif in the N-terminus facilitates association of S6K1 with the mTORC1 partner raptor to allow efficient phosphorylation of S6K1 [143]. Truncation of the N-terminus including the TOS motif inhibits S6K activation [144,145]. This motif consists of a five-amino-acid sequence (FDIDL) that is conserved among S6K1 and S6K2 across species [75]. A similar motif (FEMDI) is also found at the C-terminus of the translational regulator 4E-BP, another well-characterized mTORC1 substrate. This motif may not exclusively be a TORC1-recognition motif, since a similar sequence is found in the yeast YPK2, which is regulated by TORC2 [99].

The phosphorylation of Thr^{229} at the activation loop by PDK1 is mandatory for S6K activation. In PDK1$^{-/-}$ ES (embryonic stem) cells, Thr^{229} phosphorylation and kinase activity are abrogated [146]. PDK1 docks to the phosphorylated HM site (Thr^{389}) of S6K. In cells with a knockin mutation of the PH domain of PDK1 that abolishes PtdIns(3,4,5)P_3 binding, S6K can be activated and phosphorylated at Thr^{389} by expression of Rheb or by the addition of amino acids, but not by IGF-1 (insulin-like growth factor 1) [147,148]. However, the PIF pocket of PDK1 was found to be required for the amino-acid-induced activation. These findings support the model in which PtdIns(3,4,5)P_3 binding to PDK1 is not required for the nutrient-induced S6K activation but PDK1 docking to the HM site is critical for S6K phosphorylation. The failure of IGF-1 to stimulate S6K in the PH domain mutant knockin cells could be explained by the defective Akt activation in these cells, which leads to down-regulation of Rheb and mTORC1 [148] (Figure 3).

S6K phosphorylation at Thr^{389} of the HM and Ser^{404} at the linker domain is highly rapamycin-sensitive, indicating that S6K is regulated by mTORC1 [84]. Indeed, immunopurified mTOR can phosphorylate S6K1 at Thr^{389} and Ser^{412} (Ser^{411}) *in vitro* [149,150]. Knockdown of the mTORC1 component, raptor, also led to decreased phosphorylation of Thr^{389} [24] and Ser^{411} [151] and correlated with decreased cell size and growth rate. However, other kinases were also implicated to phosphorylate Thr^{389} such as PDK1 [152], NEK [NIMA (never in mitosis in *Aspergillus nidulans*)]-related kinase] 6 and 7 [153], and S6K itself [154]. It is possible that there are several inputs that promote or enhance the phosphorylation of the HM site to prolong the activation state or enhance stability of S6K. S6K is also phosphorylated at seven other residues with a serine/threonine–proline motif [142]. One of these sites, Ser^{371}, is at the TM, located at the linker region, close to the HM site. Phosphorylation at the TM

© The Authors Journal compilation © 2008 Biochemical Society

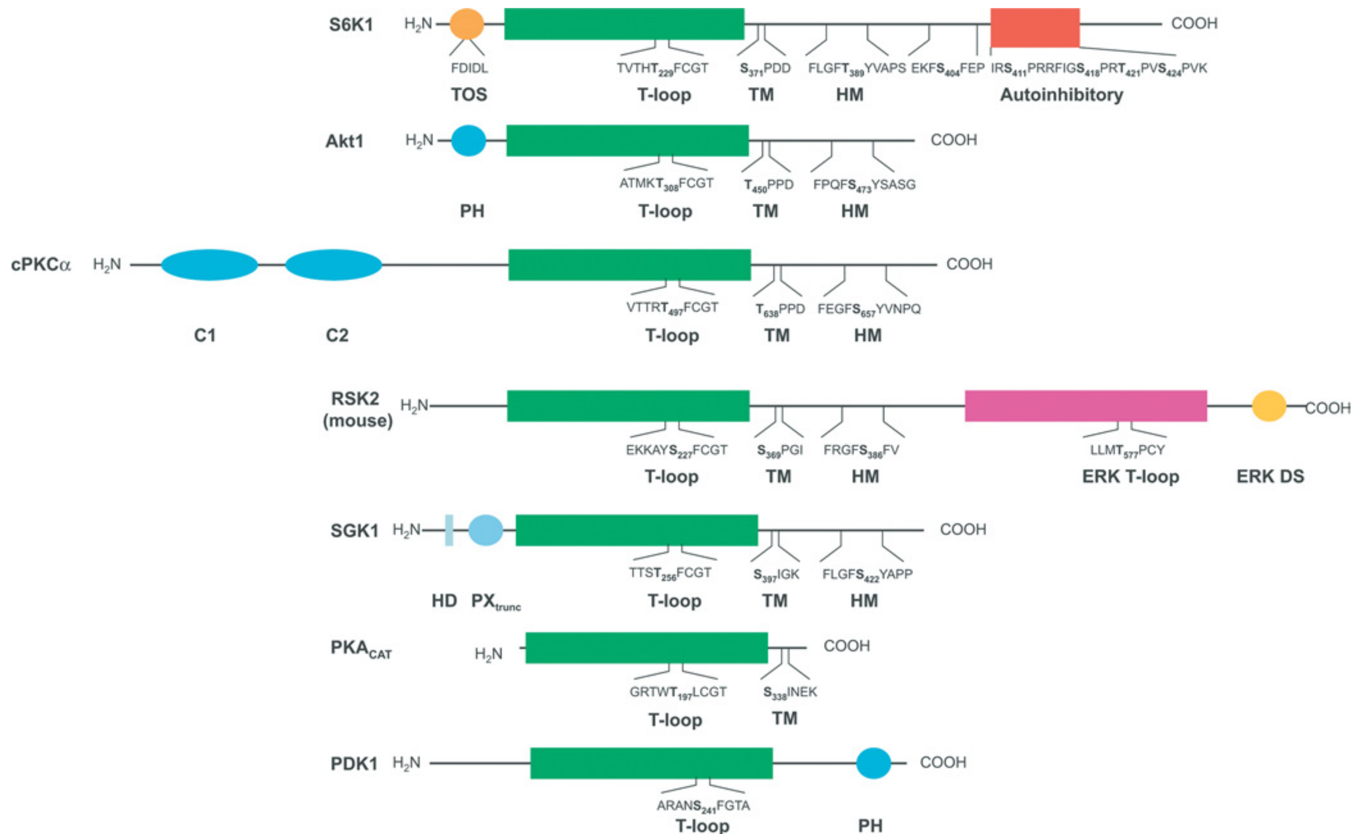

Figure 5 Mammalian AGC kinases conserved regions and phosphorylation sites

S6K1 has a TOS motif, Akt has a PH domain, cPKC has the Ca^{2+}/lipid binding domains C1 and C2, SGK1 has a truncated PX domain and a hydrophobic degradation (HD) domain [221] in the N-terminus. S6K1 has a multiphosphorylated autoinhibitory domain in the C-terminus. Rsk1/2 has a C-terminal kinase domain that is homologous with the CaMK family of kinases. The C-terminal kinase domain is phosphorylated by ERK1/2 upon binding of ERK to the ERK docking site (ERK DS) in the C-terminus. PDK1 also contains a PH domain in the C-terminus. Except for PDK1, all kinases above possess the conserved TM. Except for PDK1 and PKA, all kinases contain the HM. Motifs (C1/C2, PH) that may potentially bind lipids or membranes are in blue (PX-truncated is in pale blue). All other colours represent motifs that are not conserved among AGC kinases.

is mitogen-inducible and is required for kinase activity along with HM and T-loop phosphorylation [93]. Mutation of the TM site to an alanine or aspartate residue abolished serum-induced activation of S6K. However, a phosphomimetic mutant of the HM site did not rescue the activity of the TM alanine mutant, indicating that phosphorylation at the TM site plays a more independent role in regulating S6K activity [155]. There are conflicting reports as to whether this phosphorylation is rapamycin-sensitive [93,155,156], although it was shown to be phosphorylated by mTOR *in vitro* [93].

Four phosphorylation sites (Ser^{411}, Ser^{418}, Thr^{421} and Ser^{424}), all of which contain a proline residue at the $+1$ position and a hydrophobic residue at the -2 position, are found in the autoinhibitory domain. These sites are hypophosphorylated in quiescent cells and undergo hyperphosphorylation upon serum stimulation. Mutation of these sites to an alanine residue suppresses activation of S6K, but phosphomimetic mutants still require phosphorylation at the activation loop and linker region to fully activate the kinase [94]. Deletion of the C-terminus, including the autoinhibitory domain, or substitution of acidic residues for all four residues, reversed the effect of truncation of the S6K1 N-terminus and led to kinase activation [144,145,157]. This active mutant allele of S6K1 is also rapamycin-insensitive, i.e. Thr^{389} of the HM remains phosphorylated. Surprisingly, this phosphorylation depends on mTORC2 [151]. An extended C-terminal tail containing multiple phosphorylation sites also exists in SCH9, a TORC1 target [62].

These findings suggest that the structure of the C-terminal tail may play an important role in determining the requirement for mTOR partners. Phosphomimetic mutation of the four residues (D3E) in the autoinhibitory domain does not enhance T-loop phosphorylation, although this mutant S6K is catalytically more active. Therefore these four sites co-operate with HM phosphorylation to synergistically activate the kinase. T-loop phosphorylation can occur, albeit weakly, in the absence of Thr^{389} phosphorylation, suggesting that the HM phosphorylation although not absolutely required, increases the affinity for PDK1 to enhance T-loop phosphorylation. Intriguingly, in $PDK1^{-/-}$ ES cells, Thr^{389} phosphorylation is also absent [146], suggesting that PDK1 may influence HM site phosphorylation as well.

How is mTORC1 activity towards S6K regulated? It was previously demonstrated that, depending on the activation state of S6K, it could associate with different regulatory complexes. In the inactive state, it is bound to eIF3 (eukaryotic initiation factor 3) [158]. Upon cell stimulation, mTOR/raptor binds to eIF3 and subsequently phosphorylates S6K at Thr^{389}. The phosphorylation of S6K by mTORC1 leads to its dissociation from the eIF3–mTORC1 complex and it becomes available to phosphorylate its downstream targets. These findings raise the possibility that the activity of mTOR/raptor is dependent on the signalling complex it associates with and most probably on the cellular compartment it localizes to. The nutrient-dependent cellular signals that promote mTORC1 phosphorylation of S6K remain to be

© The Authors Journal compilation © 2008 Biochemical Society

characterized. Previously, a class III PI3K, hVps34 (human vacuolar protein sorting 34), was identified to regulate mTOR in the presence of nutrients [159,160]. The product of hVps34 kinase, PtdIns3P, mediates recruitment of proteins containing FYVE or PX (Phox homology) domains to endosomal membranes. S6K does not have discernible FYVE, PX or other lipid-binding domains, but an attractive model would be that these phospholipids may provide a platform for mTORC1 phosphorylation of S6K in a membrane compartment.

Akt

Three Akt genes have been identified in mammals: *Akt1* (*PKBα*), *Akt2* (*PKBβ*), and *Akt3* (*PKBγ*) [161]. Akt phosphorylates a number of diverse targets and functions in cell survival, growth, proliferation and migration. A unique feature of Akt not found in other AGC kinases is the presence of a PH domain in its N-terminus. This domain in Akt specifically binds PtdIns(3,4)P_2 and/or PtdIns(3,4,5)P_3 and plays a negative regulatory role. First, when the Akt PH domain is deleted, PDK1 can phosphorylate this mutant Akt in the absence of phosphoinositides [162,163]. Secondly, the PH and kinase domains were found to interact intramolecularly to prevent PDK1 phosphorylation of the activation loop despite the physical association of PDK1 with Akt [164]. PDK1 also contains a PH domain in its C-terminus and exhibits a high affinity for PtdIns(3,4,5)P_3 [165]. Thus the presence of PtdIns(3,4,5)P_3 could enhance phosphorylation of Akt by binding of PtdIns(3,4,5)P_3 on the PH domain of PDK1. Indeed, knockin mutation of the PH domain of PDK1 that fails to bind PtdIns(3,4,5)P_3 abolished Akt activation by IGF-1 [148]. Therefore PtdIns(3,4,5)P_3 binding to both Akt and PDK1 PH domains are required for Akt activation. This requirement could co-localize these two kinases at the membrane where they bind the lipids. The finding that membrane-associated Akt mutants become constitutively active and phosphorylated at Thr^{308} supports this view [166–168]. Since these mutants are also constitutively phosphorylated at Ser^{473} of the HM site, phosphorylation of the HM is also believed to occur in the membrane.

The kinase that phosphorylates the HM site of Akt has been termed 'PDK2' [169]. Among the kinases that were identified as a PDK2 in addition to mTOR are DNA-PK (DNA protein kinase), ILK (integrin-linked kinase), cPKC, ATM and Akt itself [91,170–173]. HM phosphorylation is induced by growth factors such as insulin and is sensitive to inhibitors of PI3Ks such as wortmannin. Previous studies provide compelling evidence that mTORC2 mediates Akt phosphorylation, strongly suggesting it is the *bona fide* PDK2 [39,76]. mTORC2 promotes phosphorylation of Ser^{473} *in vitro* in a serum-inducible and wortmannin-sensitive manner [39]. In another study using 3T3 L1 adipocytes, the kinase activity was not induced by serum stimulation but was increased by exogenous addition of PtdIns(3,4,5)P_3 [76], consistent with the previously held proposition that the PDK2 is constitutively active at the membrane and that insulin enhances Ser^{473} phosphorylation via the effect on Akt itself and not on PDK2 [76,174,175]. It is noteworthy that although Ser^{473} phosphorylation is highly inducible *in vivo* upon growth-factor or serum stimulation, its induction *in vitro* using the immunoprecipitated mTOR complex as a kinase source is not as dramatic. Although there could be several technical explanations for this, such as high basal phosphorylation of exogenous Akt, it is also possible that the spatial context is amiss in these assays. The presence of negative regulators that can associate with the mTOR complex may also inhibit mTOR activity *in vitro* [69].

In vivo analyses also support mTORC2 as a critical regulator of HM phosphorylation. Knockdown of the mTORC2 component rictor, but not the mTORC1 component raptor, led to defective phosphorylation of Akt at Ser^{473} [39]. This decrease in Ser^{473} phosphorylation was also accompanied by defective Thr^{308} phosphorylation, in agreement with previous claims that Ser^{473} phosphorylation is important for subsequent Thr^{308} phosphorylation [168,176,177]. Genetic studies using MEFs from mice that have deficient mTORC components confirm that mTORC2 is required for HM site phosphorylation. In rictor-, mLST8- or SIN1-knockout cells, Ser^{473} phosphorylation was abolished. Phosphorylation at Thr^{308} was not grossly abnormal in $rictor^{-/-}$ cells [51,52] and was normal in $mLST8^{-/-}$ [27] and in $SIN1^{-/-}$ cells [40]. These findings support the alternative view that PDK1 docking to the phosphorylated HM site of Akt is not critical for T-loop phosphorylation (see also S6K and PKC). Studies from $PDK1^{-/-}$ ES cells have also revealed that in the absence of Thr^{308} phosphorylation, the HM site phosphorylation at Ser^{473} still occurs [146]. Thus phosphorylation of the T-loop and HM site of Akt seems to occur independently of each other. Akt remained partly active in mTORC2-disrupted cells and phosphorylated a subset of its known substrates but was defective in the phosphorylation of the apoptosis promoting proteins Foxo1/3a [40]. These findings suggest that the phosphorylation of Ser^{473} may play a role in cell survival and that HM site phosphorylation may regulate substrate specificity in Akt and possibly other AGC kinases as well.

Akt is also constitutively phosphorylated at Ser^{124} and Thr^{450} [176]. Both residues are immediately followed by a proline residue. Ser^{124} is located in the linker region between the PH domain and the catalytic domain, but this residue is not conserved in *Drosophila* Akt [176]. The significance of this phosphorylation site remains unknown. Thr^{450} is part of the TM. Structural and biochemical studies on cPKC have suggested that the phosphorylation at the TM stabilizes the protein presumably by enhancing the HM interaction at the C-terminal tail. Regulation of the TM site in Akt is quite different from the HM site. Although HM phosphorylation is growth-factor-inducible, TM phosphorylation is constitutive, but is dependent on the integrity of the PH domain [166]. Since TM phosphorylation in S6K and yeast AGC kinases has been shown to be TOR-dependent [62,77,93,99], it is probable that mTOR also regulates this site in Akt. Why the TM and HM sites could be regulated very differently by mTOR is quite intriguing and implies that it is not the direct regulation of mTOR activity itself that leads to phosphorylation of these two sites. Regulation of the mTORC components or compartmentalization of mTORCs are possible mechanisms to distinctly regulate these sites. If mTOR is not the direct TM or HM kinase, mTORC2 may facilitate autophosphorylation, or activate the TM/HM kinases. Other possible mechanisms include inhibition of phosphatases, and/or mediating compartmentalization, both of which are discussed below.

Phosphatases that dephosphorylate and inactivate Akt probably play an equally important role as well. PP2A was proposed to dephosphorylate Akt based on the effect of the PP2A-specific inhibitor okadaic acid [178]. Another type of phosphatase of the PP2C family, PHLPP (PH domain leucine-rich repeat protein phosphatase) [179] which is okadaic-acid-insensitive, was identified as more specific for the dephosphorylation of Ser^{473} of the HM site of Akt. The two PHLPP isoforms display preference towards different Akt isoforms to regulate Akt substrate specificity. Thus, in addition to the mTORC2 regulation of Akt substrate specificity, PHLPP also regulates Akt substrate specificity via dephosphorylation of Akt at the HM site. It would be interesting to see whether mTORC2 may also regulate PHLPP. Studies in yeast have emphasized a critical role for TOR in the negative regulation of phosphatases. The

© The Authors Journal compilation © 2008 Biochemical Society

yeast phosphatase regulator TAP42 was shown to associate with TORC1 on membrane structures and the TAP42–phosphatase complex is released into the cytosol upon rapamycin treatment and nutrient deprivation [180]. These findings suggest that the phosphatases are kept inactive by TORC1 in the membrane and imply that compartmentalization plays an important role in the regulation of TORC targets.

PKC

In mammals, there are three classes of PKC, grouped according to the domains present in the regulatory moiety present in the N-terminus. The conventional type of PKC (cPKC) includes α, β1, βII and γ; the nPKC (novel type) consists of δ, ε, θ and η/L; and the aPKC (atypical PKC) consists of ζ and ι/λ [181]. The activation loop of these different PKC classes is phosphorylated by PDK1. T-loop phosphorylation by PDK1 is constitutive and does not require phosphoinositides [182]. Newly synthesized PKC associates with a membrane compartment where it is in an 'open' conformation and can be phosphorylated at the T-loop and C-terminal tail. T-loop phosphorylation by PDK1 critically regulates C-terminal tail phosphorylation. In the absence of T-loop phosphorylation, the TM and HM sites remain unphosphorylated and the protein is highly unstable. In PDK1$^{-/-}$ ES cells, protein levels of different PKC isotypes were largely diminished [183]. This is consistent with the requirement for prior T-loop phosphorylation followed by TM and HM site phosphorylation to stabilize newly synthesized PKC. The mRNA level of nPKCδ was also diminished 2-fold in the absence of PDK1 [183]. Hence the decreased protein levels may be a combined defect of the lack of phosphorylation by PDK1 at the T-loop site and defective mRNA processing or stability in the knockout cells.

In earlier studies, phosphorylation of the HM sites of the cPKCα and nPKCδ was reported to be rapamycin-sensitive, since the kinase activity of a membrane-associated protein that phosphorylates the HM site *in vitro* was suppressed by rapamycin treatment [184]. Purification of this kinase activity identified the aPKCζ as the possible HM kinase, and identified that the activated mutant of aPKCζ phosphorylated the HM site of nPKCδ in a rapamycin-independent manner. Upon overexpression of nPKCδ and nPKCε, the HM site phosphorylation of these isoforms was also shown to be rapamycin-sensitive [185]. Furthermore, the serum-induced phosphorylation of this site in nPKCδ is inhibited by amino acid deprivation, suggesting a role for mTOR in phosphorylating the HM site [185]. Using pharmacological inhibitors, the HM site of nPKCε was dephosphorylated by a phosphatase that is not mediated through PP1, PP2A or PP2B during cell passage of quiescent cells [186]. The phosphatase may be regulated by mTOR since dephosphorylation occurs upon rapamycin treatment. In nPKCθ, HM site (Ser^{695}) phosphorylation is increased by PMA or anti-CD3/CD28 stimulation of Jurkat T lymphocytes, but is not sensitive to rapamycin [187]. Taken together, the HM site of nPKC may also be regulated by mTOR, but whether this is via a direct mechanism would need to be evaluated.

Genetic studies using rictor- and SIN1-knockdown and knockout cells provide new insights into the regulation of PKC phosphorylation at the HM site. Knockdown of rictor and mTOR expression revealed diminished phosphorylation of cPKCα [50]. In rictor-, SIN1- and mLST8-knockout cells, phosphorylation of cPKCα at the HM site (Ser^{657}) was abolished, but this was also accompanied by a dramatic reduction in cPKCα protein expression [27]. However, the decrease in cPKCα expression was not evident in rictor-knockdown cells [50]. It is possible that the T-loop and TM site phosphorylation in cPKC are also defective. Earlier *in vitro* studies have demonstrated that, without TM-site phosphorylation, cPKCα is sensitive to oxidation, cleavage and dephosphorylation, whereas kinase activity is intact [119]. Mutation of the TM site, together with adjacent compensating phosphorylation sites in cPKCβII generates an inactive kinase [188]. Phosphorylation at the TM is necessary for subsequent HM site autophosphorylation [188,189] and occurs via an intramolecular mechanism [190]. Whether TM phosphorylation can also occur via an mTORC2-dependent autophosphorylation of PKC is not currently clear. Based on the *in vitro* studies, TM-site phosphorylation could play a role in stabilization of the protein. Consistent with this interpretation, the absence of TM-site phosphorylation specifically increased the binding of cPKC to the folding chaperone Hsp70. The binding of Hsp70 to TM-site-mutated PKC stabilizes the protein and prolongs the signalling capacity of the kinase [118,191]. The amino acid sequences of all PKCs align very well in the TM and probably adopt the same structure. Analysis of aPKCι and cPKCβII structure implies that TM phosphorylation is critical in stabilizing the kinase domain by fixing the C-terminus at the top of the upper lobe of the kinase core [192,193]. The TM phosphate of PKC contacts at least three basic residues, which are also conserved in Akt [194]. Given the constitutive nature of the phosphorylation of the HM and TM sites in PKC, it is puzzling how mTORC2 may regulate these similar sites in Akt, particularly that of the Akt HM site, which requires induction of PI3K signalling. This paradox may be explained by a model wherein the HM/TM kinase (or mTOR) is constitutively active and that the proper conformation or localization of the AGC kinase determines when it will be phosphorylated.

Other mammalian AGC kinases: evidence of mTOR regulation?

Although evidence is lacking for a direct regulation by mTOR of the C-terminal tail of the mammalian AGC kinases discussed below, these kinases have been linked to mTOR signalling, either functionally or indirectly by regulation of a common downstream target.

p90 Rsk

In mammals, there are four Rsk (*Rsk1–Rsk4*) and two related MSK (*MSK1*/*MSK2*) genes and, although there are orthologues in flies and worms, there is none in yeast (but see SCH9). Rsk is a downstream effector of ERK in response to numerous cellular stimuli [195]. Among its cellular targets are proteins involved in translation, metabolism and cellular stress responses. Rsk contains two kinase domains connected by a regulatory linker sequence. The N-terminal kinase domain shares homology with other AGC kinases and phosphorylates cytoplasmic and nuclear substrates. The C-terminal kinase domain is similar to the CaMK (Ca^{2+}/calmodulin-dependent protein kinase) family of kinases and functions to activate the N-terminal kinase. The activation of Rsk requires co-ordinate regulation by the Ras/MAPK pathway and PDK1. Four major phosphorylation sites are critical for Rsk activation. Ser^{369} (human Rsk2 numbering) at the linker, followed by Thr^{577} of the C-terminal kinase domain are phosphorylated by ERK. Ser^{386} of the HM site is also phosphorylated in an ERK1/2-dependent manner and, finally, Ser^{227} of the T-loop is phosphorylated by PDK1 [196,197]. MK2 (MAPK-activated protein kinase 2) was previously shown to phosphorylate the HM site of Rsk via p38 in dendritic cells [198]. Notably, MK2 can also phosphorylate the HM site of Akt in neutrophils [199]. Phosphorylation of the HM site of Rsk lacks rapamycin sensitivity and is therefore unlikely to be regulated by mTORC1, although a role for mTORC2 remains to be examined. The TM site is also phosphorylated indirectly by ERK [200]. C-terminal truncation

© The Authors Journal compilation © 2008 Biochemical Society

of Rsk resulting in the loss of the ERK-docking site, abolishes Rsk activity [200]. Intriguingly, upon membrane targeting of this C-terminally truncated form, Rsk becomes activated and phosphorylated at the TM and HM sites. Hence it is possible that ERK may escort or mediate association of Rsk to the membrane, where it becomes phosphorylated at this linker region speculatively by mTOR.

Active Rsk is known to phosphorylate a number of substrates, most of which overlap with other AGC kinases such as S6K. Rsk phosphorylates the S6K substrates rpS6 and eIF4B, but in a rapamycin-insensitive manner [201,202]. It is interesting to note that in $S6K1^{-/-}/S6K2^{-/-}$ mice, phosphorylation of rpS6 and eIF4B are normal [203]. Hence in the absence of S6K, Rsk may compensate to allow phosphorylation of these substrates, which are critical for translation.

SGK

SGK is highly regulated on different levels: its expression, its kinase activity and subcellular localization [204]. Three SGK paralogues exist in mammals and mainly function to modulate salt balance. SGKs activate ion channels, carriers and the Na^+/K^+-ATPase. It also phosphorylates other cellular substrates that are involved in stress responses and some of these targets overlap with Akt substrates. The SGKs are structurally highly similar to Akt. Although they do not contain a PH domain, they require PI3K activation for function. SGK3, but not the other two SGK paralogues, contain a PX domain [205]. PX domains are known to bind monophosphorylated lipids and to direct PX domain proteins to endosomal membranes [206]. SGK1 and SGK2 contain a truncated PX domain, although this domain in SGK1 can bind phosphoinositides *in vitro* and is required for its function [207]. PDK1 phosphorylates SGK on its T-loop [208,209] and this requires the prior PI3K-dependent phosphorylation of SGK1 at the HM site by an unidentified kinase. Studies on SGK3 hint that localization of SGK3 at the endosomes via a PI3K-dependent and staurosporine-sensitive HM kinase is important for SGK3 activation [204]. The hyperphosphorylation of SGK upon serum stimulation is not blocked by rapamycin treatment [210], suggesting that mTORC2 may mediate phosphorylation of the HM site. SGK is a very attractive candidate for mTOR regulation given the reported functions of SGK in the modulation of nutrient transporters and ion channels.

PKA

PKA is the principal target of the intracellular second messenger cAMP. The cAMP/PKA signalling pathway is activated by a number of different receptors that couple to G-proteins upon binding of their respective ligands. Mammalian PKA, like the yeast counterpart, is made up of the regulatory (R) and catalytic (C) subunits and is activated in a similar manner. Phosphorylation at two sites at the C-terminus, Thr^{197} of the T-loop and Thr^{338} of the TM, are important for catalysis and stabilization. PKA does not contain an HM. In contrast with other AGC kinases, phosphorylation of the C-subunit occurs before assembly into an inactive holoenzyme complex. PDK1 is not required for T-loop phosphorylation *in vivo* [146] although it can recognize it *in vitro* [211]. Structural and biochemical studies have shown that phosphorylation at Ser^{338} at the TM stabilizes the kinase core. Phosphorylation of this site is resistant to phosphatases and appears to be required for stabilization [92]. There is currently no evidence linking mTOR to phosphorylation of PKA, but the TM site may be a possible regulatory site. As in yeast, it is not entirely clear whether PKA directly mediates one or more of the functions of mTOR in growth and stress responses. Some findings indicate that PKA can phosphorylate LKB1, a tumor suppressor protein, to suppress cell growth [212,213]. Although PKA activation appears to be associated with decreased mTORC1 signalling [213], it remains to be examined whether mTORC2 could play a role in PKA regulation.

PDK1

Unlike other AGC kinases, PDK1 is encoded by a single gene in mammals. It contains an N-terminal kinase domain and a C-terminal PH domain. The PH domain binds to PtdIns(3,4,5)P_3 and localizes PDK1 to the membrane. Although binding of PtdIns(3,4,5)P_3 does not increase PDK1 autophosphorylation, sphingosine appears to activate PDK1 by promoting its autophosphorylation [214]. However, the mechanism of this is not understood. PDK1 is phosphorylated at several sites but only Ser^{241}, the T-loop phosphorylation site, is conserved in other PDK1 orthologues [215]. As discussed above, the T-loop phosphorylation of AGC kinases is mediated by PDK1. The importance of this phosphorylation is underscored by the finding that knockout of PDK1 or knockin mutations in the PH domain or the PIF pocket is embryonic lethal [147,148,216]. PDK1 hypomorphic mice displayed normal phosphorylation of Akt, S6K and Rsk at the T-loop but these mice had a smaller body and organ size [216]. Hence, in addition, PDK1 could regulate growth independently of Akt, S6K and Rsk. These PDK1 hypomorphic mice also displayed reduced intestinal and renal amino acid transport and could therefore contribute to the growth defects observed in these mice [217]. PDK1 is also required for expression of key nutrient receptors and transporters in T-cell progenitors [218]. Studies on $PDK1^{-/-}$ ES cells also reveal that there is a more general defect in mRNA translation in these cells [219]. Taken together these studies suggest that PDK1, like mTOR, plays diverse functions in growth regulation. There is currently no direct evidence linking mTOR to the regulation of PDK1.

CONCLUSION

The TOR–AGC kinase signalling module has been conserved during the evolution of eukaryotes to co-ordinately regulate the growth and survival of cells. Regulation of the kinase domain including the C-terminal tail of the AGC kinases are providing clues on the functional diversity of these kinases despite common upstream cellular signals. Although a number of studies have now demonstrated a role for PDK1 in the phosphorylation of the activation loop, we are only beginning to understand how the phosphorylation of the C-terminal tail of the AGC kinases can be regulated by TOR and other protein kinases. Although T-loop phosphorylation is mandatory for kinase activity, C-terminal tail phosphorylation imparts another level of regulation to increase kinase activity, protein stability, control substrate specificity and protein–protein interaction, and other functions that remain to be discovered. How can TOR promote phosphorylation at the C-terminal tail? The emerging picture is that growth signals can regulate the AGC kinases such that by a change in conformation and/or localization, the activation loop and C-terminal tail of most of these kinases become accessible for phosphorylation by PDK1 and by TOR (or TOR-regulated kinase) respectively. The signal allowing changes in conformation and/or localization is variable among AGC kinases, for example PI3K-generated phosphoinositides, membrane localization via a lipid-binding motif or relief from autoinhibition. Whether these signals can also regulate TOR or its complex partners remains to be elucidated. But, given the variability of these signals, it seems more reasonable to speculate that these signals

© The Authors Journal compilation © 2008 Biochemical Society

do not regulate TOR kinase activity themselves. The recent discovery of other TORC partner proteins in both yeast and mammals indicates that there are other non-conserved organism-specific proteins that couple TORC signalling to specific cellular pathways and most probably in different cellular compartments, suggesting the presence of several distinct complexes. Thus further identification of TORC components that can specifically mediate phosphorylation of AGC kinases would provide answers as to how TOR can be involved in the phosphorylation of several AGC kinases and in promoting multiple cellular functions. Another outstanding question is why multiple kinases, including TOR, can phosphorylate the HM site, particularly in mammals. It would be of interest to determine in which context TOR can mediate phosphorylation of this site in several AGC kinases and how HM kinases may differentially affect the AGC kinase activity. It is also intriguing why the TORCs are essential for the phosphorylation of specific AGC kinases despite the high conservation of the TM and HM regions. What is the basis of this specificity? Lastly, why are there two distinct TOR core complexes and what is the reason for the difference in rapamycin sensitivities between the complexes? Comparative studies on how the TOR complexes can regulate the AGC kinases in different organisms should yield important answers to this question.

We would like to thank Christian Ungermann, Hans-Peter Schmitz and Alexandra Newton for helpful discussions and critical reading of the manuscript before its submission. This work was funded in part by grants from the American Heart Association, New Jersey Commission on Cancer Research, and American Cancer Society (to E. J.) and by Deutsche Forschungsgemeinschaft Grant SFB 431/P19 (to A. L.).

REFERENCES

1 Manning, G., Whyte, D. B., Martinez, R., Hunter, T. and Sudarsanam, S. (2002) The protein kinase complement of the human genome. Science **298**, 1912–1934

2 Hanks, S. K. and Hunter, T. (1995) Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. FASEB J. **9**, 576–596

3 Miranda-Saavedra, D. and Barton, G. J. (2007) Classification and functional annotation of eukaryotic protein kinases. Proteins **68**, 893–914

4 Perry, J. and Kleckner, N. (2003) The ATRs, ATMs, and TORs are giant HEAT repeat proteins. Cell **112**, 151–155

5 Groves, M. R. and Barford, D. (1999) Topological characteristics of helical repeat proteins. Curr. Opin. Struct. Biol. **9**, 383–389

6 Adami, A., Garcia-Alvarez, B., Arias-Palomo, E., Barford, D. and Llorca, O. (2007) Structure of TOR and its complex with KOG1. Mol. Cell **27**, 509–516

7 Peterson, R. T., Beal, P. A., Comb, M. J. and Schreiber, S. L. (2000) FKBP12–rapamycin-associated protein (FRAP) autophosphorylates at serine 2481 under translationally repressive conditions. J. Biol. Chem. **275**, 7416–7423

8 Takahashi, T., Hara, K., Inoue, H., Kawa, Y., Tokunaga, C., Hidayat, S., Yoshino, K., Kuroda, Y. and Yonezawa, K. (2000) Carboxyl-terminal region conserved among phosphoinositide-kinase-related kinases is indispensable for mTOR function *in vivo* and *in vitro*. Genes Cells **5**, 765–775

9 Bosotti, R., Isacchi, A. and Sonnhammer, E. L. (2000) FAT: a novel domain in PIK-related kinases. Trends Biochem. Sci **25**, 225–227

10 Dames, S. A., Mulet, J. M., Rathgeb-Szabo, K., Hall, M. N. and Grzesiek, S. (2005) The solution structure of the FATC domain of the protein kinase target of rapamycin suggests a role for redox-dependent structural and cellular stability. J. Biol. Chem. **280**, 20558–20564

11 Veverka, V., Crabbe, T., Bird, I., Lennie, G., Muskett, F. W., Taylor, R. J. and Carr, M. D. (2008) Structural characterization of the interaction of mTOR with phosphatidic acid and a novel class of inhibitor: compelling evidence for a central role of the FRB domain in small molecule-mediated regulation of mTOR. Oncogene, in the press

12 Fang, Y., Vilella-Bach, M., Bachmann, R., Flanigan, A. and Chen, J. (2001) Phosphatidic acid-mediated mitogenic activation of mTOR signaling. Science **294**, 1942–1945

13 Avila-Flores, A., Santos, T., Rincon, E. and Merida, I. (2005) Modulation of the mammalian target of rapamycin pathway by diacylglycerol kinase-produced phosphatidic acid. J. Biol. Chem. **280**, 10091–10099

14 Foster, D. A. (2007) Regulation of mTOR by phosphatidic acid? Cancer Res. **67**, 1–4

15 Wullschleger, S., Loewith, R. and Hall, M. N. (2006) TOR signaling in growth and metabolism. Cell **124**, 471–484

16 Matsuo, T., Otsubo, Y., Urano, J., Tamanoi, F. and Yamamoto, M. (2007) Loss of the TOR kinase Tor2 mimics nitrogen starvation and activates the sexual development pathway in fission yeast. Mol. Cell. Biol. **27**, 3154–3164

17 Alvarez, B. and Moreno, S. (2006) Fission yeast Tor2 promotes cell growth and represses cell differentiation. J. Cell Sci. **119**, 4475–4485

18 Urano, J., Comiso, M. J., Guo, L., Aspuria, P. J., Deniskin, R., Tabancay, Jr, A. P., Kato-Stankiewicz, J. and Tamanoi, F. (2005) Identification of novel single amino acid changes that result in hyperactivation of the unique GTPase, Rheb, in fission yeast. Mol. Microbiol. **58**, 1074–1086

19 Uritani, M., Hidaka, H., Hotta, Y., Ueno, M., Ushimaru, T. and Toda, T. (2006) Fission yeast Tor2 links nitrogen signals to cell proliferation and acts downstream of the Rheb GTPase. Genes Cells **11**, 1367–1379

20 Li, Y., Corradetti, M. N., Inoki, K. and Guan, K. L. (2004) TSC2: filling the GAP in the mTOR signaling pathway. Trends Biochem. Sci. **29**, 32–38

21 Urano, J., Tabancay, A. P., Yang, W. and Tamanoi, F. (2000) The *Saccharomyces cerevisiae* Rheb G-protein is involved in regulating canavanine resistance and arginine uptake. J. Biol. Chem. **275**, 11198–11206

22 Aspuria, P. J. and Tamanoi, F. (2004) The Rheb family of GTP-binding proteins. Cell Signaling **16**, 1105–1112

23 Loewith, R., Jacinto, E., Wullschleger, S., Lorberg, A., Crespo, J. L., Bonenfant, D., Oppliger, W., Jenoe, P. and Hall, M. N. (2002) Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Mol. Cell **10**, 457–468

24 Kim, D. H., Sarbassov dos, D., Ali, S. M., King, J. E., Latek, R. R., Erdjument-Bromage, H., Tempst, P. and Sabatini, D. M. (2002) mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell **110**, 163–175

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

26 Oshiro, N., Yoshino, K., Hidayat, S., Tokunaga, C., Hara, K., Eguchi, S., Avruch, J. and Yonezawa, K. (2004) Dissociation of raptor from mTOR is a mechanism of rapamycin-induced inhibition of mTOR function. Genes Cells **9**, 359–366

27 Guertin, D. A., Stevens, D. M., Thoreen, C. C., Burds, A. A., Kalaany, N. Y., Moffat, J., Brown, M., Fitzgerald, K. J. and Sabatini, D. M. (2006) Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCα, but not S6K1 Dev. Cell **11**, 859–871

28 Murakami, M., Ichisaka, T., Maeda, M., Oshiro, N., Hara, K., Edenhofer, F., Kiyama, H., Yonezawa, K. and Yamanaka, S. (2004) mTOR is essential for growth and proliferation in early mouse embryos and embryonic stem cells. Mol. Cell Biol. **24**, 6710–6718

29 Wullschleger, S., Loewith, R., Oppliger, W. and Hall, M. N. (2005) Molecular organization of target of rapamycin complex 2. J. Biol. Chem. **280**, 30697–30704

30 Roberg, K. J., Bickel, S., Rowley, N. and Kaiser, C. A. (1997) Control of amino acid permease sorting in the late secretory pathway of *Saccharomyces cerevisiae* by SEC13, LST4, LST7 and LST8. Genetics **147**, 1569–1584

31 Liu, Z., Sekito, T., Epstein, C. B. and Butow, R. A. (2001) RTG-dependent mitochondria to nucleus signaling is negatively regulated by the seven WD-repeat protein Lst8p. EMBO J. **20**, 7209–7219

32 Chen, E. J. and Kaiser, C. A. (2003) LST8 negatively regulates amino acid biosynthesis as a component of the TOR pathway. J. Cell Biol. **161**, 333–347

33 Kabir, M. A., Kaminska, J., Segel, G. B., Bethlendy, G., Lin, P., Della Seta, F., Blegen, C., Swiderek, K. M., Zoladek, T., Arndt, K. T. and Sherman, F. (2005) Physiological effects of unassembled chaperonin Cct subunits in the yeast *Saccharomyces cerevisiae*. Yeast **22**, 219–239

34 Kemp, J. T., Balasubramanian, M. K. and Gould, K. L. (1997) A wat1 mutant of fission yeast is defective in cell morphology. Mol. Gen. Genet. **254**, 127–138

35 Kim, D. H., Sarbassov dos, D., Ali, S. M., Latek, R. R., Guntur, K. V., Erdjument-Bromage, H., Tempst, P. and Sabatini, D. M. (2003) GβL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. Mol. Cell **11**, 895–904

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

37 Weisman, R., Choder, M. and Koltin, Y. (1997) Rapamycin specifically interferes with the developmental response of fission yeast to starvation. J. Bacteriol. **179**, 6325–6334

38 Weisman, R., Roitburg, I., Nahari, T. and Kupiec, M. (2005) Regulation of leucine uptake by tor1+ in *Schizosaccharomyces pombe* is sensitive to rapamycin. Genetics **169**, 539–550

39 Sarbassov, D. D., Guertin, D. A., Ali, S. M. and Sabatini, D. M. (2005) Phosphorylation and regulation of Akt/PKB by the rictor–mTOR complex. Science **307**, 1098–1101

© The Authors Journal compilation © 2008 Biochemical Society

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

41 Mulet, J. M., Martin, D. E., Loewith, R. and Hall, M. N. (2006) Mutual antagonism of target of rapamycin and calcineurin signaling. J. Biol. Chem. **281**, 33000–33007

42 Daquinag, A., Fadri, M., Jung, S. Y., Qin, J. and Kunz, J. (2007) The yeast PH domain proteins Slm1 and Slm2 are targets of sphingolipid signaling during the response to heat stress. Mol. Cell Biol. **27**, 633–650

43 Weisman, R., Finkelstein, S. and Choder, M. (2001) Rapamycin blocks sexual development in fission yeast through inhibition of the cellular function of an FKBP12 homolog. J. Biol. Chem. **276**, 24736–24742

44 Kawai, M., Nakashima, A., Ueno, M., Ushimaru, T., Aiba, K., Doi, H. and Uritani, M. (2001) Fission yeast tor1 functions in response to various stresses including nitrogen starvation, high osmolarity, and high temperature. Curr. Genet. **39**, 166–174

45 Beeler, T., Bacikova, D., Gable, K., Hopkins, L., Johnson, C., Slife, H. and Dunn, T. (1998) The *Saccharomyces cerevisiae* TSC10/YBR265w gene encoding 3-ketosphinganine reductase is identified in a screen for temperature-sensitive suppressors of the Ca^{2+}-sensitive csg2Δ mutant. J. Biol. Chem. **273**, 30688–30694

46 Audhya, A., Loewith, R., Parsons, A. B., Gao, L., Tabuchi, M., Zhou, H., Boone, C., Hall, M. N. and Emr, S. D. (2004) Genome-wide lethality screen identifies new PI4,5P2 effectors that regulate the actin cytoskeleton. EMBO J. **23**, 3747–3757

47 Tabuchi, M., Audhya, A., Parsons, A. B., Boone, C. and Emr, S. D. (2006) The phosphatidylinositol 4,5-biphosphate and TORC2 binding proteins Slm1 and Slm2 function in sphingolipid regulation. Mol. Cell. Biol. **26**, 5861–5875

48 Bultynck, G., Heath, V. L., Majeed, A. P., Galan, J. M., Haguenauer-Tsapis, R. and Cyert, M. S. (2006) Slm1 and slm2 are novel substrates of the calcineurin phosphatase required for heat stress-induced endocytosis of the yeast uracil permease. Mol. Cell. Biol. **26**, 4729–4745

49 Hilti, N., Baumann, D., Schweingruber, A. M., Bigler, P. and Schweingruber, M. E. (1999) Gene ste20 controls amiloride sensitivity and fertility in *Schizosaccharomyces pombe*. Curr. Genet. **35**, 585–592

50 Sarbassov, D., Ali, S. M., Kim, D. H., Guertin, D. A., Latek, R. R., Erdjument-Bromage, H., Tempst, P. and Sabatini, D. M. (2004) Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Curr. Biol. **14**, 1296–1302

51 Shiota, C., Woo, J. T., Lindner, J., Shelton, K. D. and Magnuson, M. A. (2006) Multiallelic disruption of the rictor gene in mice reveals that mTOR Complex 2 is essential for fetal growth and viability. Dev. Cell **11**, 583–589

52 Yang, Q., Inoki, K., Ikenoue, T. and Guan, K. L. (2006) Identification of Sin1 as an essential TORC2 component required for complex formation and kinase activity. Genes Dev. **20**, 2820–2832

53 Colicelli, J., Nicolette, C., Birchmeier, C., Rodgers, L., Riggs, M. and Wigler, M. (1991) Expression of three mammalian cDNAs that interfere with RAS function in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. **88**, 2913–2917

54 Wilkinson, M. G., Pino, T. S., Tournier, S., Buck, V., Martin, H., Christiansen, J., Wilkinson, D. G. and Millar, J. B. (1999) Sin1: an evolutionarily conserved component of the eukaryotic SAPK pathway. EMBO J. **18**, 4210–4221

55 Cheng, J., Zhang, D., Kim, K., Zhao, Y. and Su, B. (2005) Mip1, an MEKK2-interacting protein, controls MEKK2 dimerization and activation. Mol. Cell. Biol. **25**, 5955–5964

56 Schroder, W., Bushell, G. and Sculley, T. (2005) The human stress-activated protein kinase-interacting 1 gene encodes JNK-binding proteins. Cell. Signalling **17**, 761–767

57 Schroder, W., Cloonan, N., Bushell, G. and Sculley, T. (2004) Alternative polyadenylation and splicing of mRNAs transcribed from the human Sin1 gene. Gene **339**, 17–23

58 Frias, M. A., Thoreen, C. C., Jaffe, J. D., Schroder, W., Sculley, T., Carr, S. A. and Sabatini, D. M. (2006) mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s. Curr. Biol. **16**, 1865–1870

59 Schroder, W. A., Buck, M., Cloonan, N., Hancock, J. F., Suhrbier, A., Sculley, T. and Bushell, G. (2007) Human Sin1 contains Ras-binding and pleckstrin homology domains and suppresses Ras signalling. Cell. Signalling **19**, 1279–1289

60 Holst, B., Lunde, C., Lages, F., Oliveira, R., Lucas, C. and Kielland-Brandt, M. C. (2000) GUP1 and its close homologue GUP2, encoding multimembrane-spanning proteins involved in active glycerol uptake in *Saccharomyces cerevisiae*. Mol. Microbiol. **37**, 108–124

61 Reinke, A., Anderson, S., McCaffery, J. M., Yates, J. 3rd, Aronova, S., Chu, S., Fairclough, S., Iverson, C., Wedaman, K. P. and Powers, T. (2004) TOR complex 1 includes a novel component, Tco89p (YPL180w), and cooperates with Ssd1p to maintain cellular integrity in *Saccharomyces cerevisiae*. J. Biol. Chem. **279**, 14752–14762

62 Urban, J., Soulard, A., Huber, A., Lippman, S., Mukhopadhyay, D., Deloche, O., Wanke, V., Anrather, D., Ammerer, G., Riezman, H. et al. (2007) Sch9 is a major target of TORC1 in *Saccharomyces cerevisiae*. Mol. Cell **26**, 663–674

63 Tang, F., Peng, Y., Nau, J. J., Kauffman, E. J. and Weisman, L. S. (2006) Vac8p, an armadillo repeat protein, coordinates vacuole inheritance with multiple vacuolar processes. Traffic **7**, 1368–1377

64 Huh, W. K., Falvo, J. V., Gerke, L. C., Carroll, A. S., Howson, R. W., Weissman, J. S. and O'Shea, E. K. (2003) Global analysis of protein localization in budding yeast. Nature **425**, 686–691

65 Fadri, M., Daquinag, A., Wang, S., Xue, T. and Kunz, J. (2005) The pleckstrin homology domain proteins Slm1 and Slm2 are required for actin cytoskeleton organization in yeast and bind phosphatidylinositol-4,5-bisphosphate and TORC2. Mol. Biol. Cell **16**, 1883–1900

66 Vander Haar, E., Lee, S. I., Bandhakavi, S., Griffin, T. J. and Kim, D. H. (2007) Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. Nat. Cell Biol. **9**, 316–323

67 Wang, L., Harris, T. E., Roth, R. A. and Lawrence, Jr, J. C. (2007) PRAS40 regulates mTORC1 kinase activity by functioning as a direct inhibitor of substrate binding. J. Biol. Chem. **282**, 20036–20044

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

69 Sancak, Y., Thoreen, C. C., Peterson, T. R., Lindquist, R. A., Kang, S. A., Spooner, E., Carr, S. A. and Sabatini, D. M. (2007) PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. Mol. Cell **25**, 903–915

70 Fonseca, B. D., Smith, E. M., Lee, V. H., MacKintosh, C. and Proud, C. G. (2007) PRAS40 is a target for mammalian target of rapamycin complex 1 and is required for signaling downstream of this complex. J. Biol. Chem. **282**, 24514–24524

71 Thedieck, K., Polak, P., Kim, M. L., Molle, K. D., Cohen, A., Jeno, P., Arrieumerlou, C. and Hall, M. N. (2007) PRAS40 and PRR5-like protein are new mTOR interactors that regulate apoptosis. PLoS **2**, e1217

72 Woo, S. Y., Kim, D. H., Jun, C. B., Kim, Y. M., Haar, E. V., Lee, S. I., Hegg, J. W., Bandhakavi, S. and Griffin, T. J. (2007) PRR5, a novel component of mTOR complex 2, regulates platelet-derived growth factor receptor β expression and signaling. J. Biol. Chem. **282**, 25604–25612

73 Pearce, L. R., Huang, X., Boudeau, J., Pawlowski, R., Wullschleger, S., Deak, M., Ibrahim, A. F., Gourlay, R., Magnuson, M. A. and Alessi, D. R. (2007) Identification of Protor as a novel Rictor-binding component of mTOR complex-2. Biochem. J. **405**, 513–522

74 Mamane, Y., Petroulakis, E., LeBacquer, O. and Sonenberg, N. (2006) mTOR, translation initiation and cancer. Oncogene **25**, 6416–6422

75 Schalm, S. S. and Blenis, J. (2002) Identification of a conserved motif required for mTOR signaling. Curr. Biol. **12**, 632–639

76 Hresko, R. C. and Mueckler, M. (2005) mTOR.RICTOR is the Ser473 kinase for Akt/protein kinase B in 3T3-L1 adipocytes. J. Biol. Chem. **280**, 40406–40416

77 Matsuo, T., Kubo, Y., Watanabe, Y. and Yamamoto, M. (2003) *Schizosaccharomyces pombe* AGC family kinase Gad8p forms a conserved signaling module with TOR and PDK1-like kinases. EMBO J. **22**, 3073–3083

78 Jiang, Y. and Broach, J. R. (1999) Tor proteins and protein phosphatase 2A reciprocally regulate Tap42 in controlling cell growth in yeast. EMBO J. **18**, 2782–2792

79 Jacinto, E., Guo, B., Arndt, K. T., Schmelzle, T. and Hall, M. N. (2001) TIP41 interacts with TAP42 and negatively regulates the TOR signaling pathway. Mol. Cell **8**, 1017–1026

80 Bertram, P. G., Choi, J. H., Carvalho, J., Ai, W., Zeng, C., Chan, T. F. and Zheng, X. F. (2000) Tripartite regulation of Gln3p by TOR, Ure2p, and phosphatases. J. Biol. Chem. **275**, 35727–35733

81 Schmidt, A., Beck, T., Koller, A., Kunz, J. and Hall, M. N. (1998) The TOR nutrient signalling pathway phosphorylates NPR1 and inhibits turnover of the tryptophan permease. EMBO J. **17**, 6924–6931

82 Keranen, L. M., Dutil, E. M. and Newton, A. C. (1995) Protein kinase C is regulated *in vivo* by three functionally distinct phosphorylations. Curr. Biol. **5**, 1394–1403

83 Tsutakawa, S. E., Medzihradszky, K. F., Flint, A. J., Burlingame, A. L. and Koshland, Jr, D. E. (1995) Determination of *in vivo* phosphorylation sites in protein kinase C. J. Biol. Chem. **270**, 26807–26812

84 Pearson, R. B., Dennis, P. B., Han, J. W., Williamson, N. A., Kozma, S. C., Wettenhall, R. E. and Thomas, G. (1995) The principal target of rapamycin-induced p70s6k inactivation is a novel phosphorylation site within a conserved hydrophobic domain. EMBO J. **14**, 5279–5287

85 Miranda-Saavedra, D., Stark, M. J., Packer, J. C., Vivares, C. P., Doerig, C. and Barton, G. J. (2007) The complement of protein kinases of the microsporidium *Encephalitozoon cuniculi* in relation to those of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. BMC Genomics **8**, 309

86 Gold, M. G., Barford, D. and Komander, D. (2006) Lining the pockets of kinases and phosphatases. Curr. Opin. Struct. Biol. **16**, 693–701

87 Nolen, B., Taylor, S. and Ghosh, G. (2004) Regulation of protein kinases; controlling activity through activation segment conformation. Mol. Cell **15**, 661–675

88 Kannan, N., Haste, N., Taylor, S. S. and Neuwald, A. F. (2007) The hallmark of AGC kinase functional divergence is its C-terminal tail, a *cis*-acting regulatory module. Proc. Natl. Acad. Sci. U.S.A. **104**, 1272–1277

© The Authors Journal compilation © 2008 Biochemical Society

89 Biondi, R. M. and Nebreda, A. R. (2003) Signalling specificity of Ser/Thr protein kinases through docking-site-mediated interactions. Biochem. J. **372**, 1–13

90 Biondi, R. M. (2004) Phosphoinositide-dependent protein kinase 1, a sensor of protein conformation. Trends Biochem. Sci. **29**, 136–142

91 Dong, L. Q. and Liu, F. (2005) PDK2: the missing piece in the receptor tyrosine kinase signaling pathway puzzle. Am. J. Physiol. Endocrinol. Metab. **289**, E187–E196

92 Yonemoto, W., McGlone, M. L., Grant, B. and Taylor, S. S. (1997) Autophosphorylation of the catalytic subunit of cAMP-dependent protein kinase in *Escherichia coli*. Protein Eng. **10**, 915–925

93 Saitoh, M., Pullen, N., Brennan, P., Cantrell, D., Dennis, P. B. and Thomas, G. (2002) Regulation of an activated s6 kinase 1 variant reveals a novel mammalian target of rapamycin phosphorylation site. J. Biol. Chem. **277**, 20104–20112

94 Dennis, P. B., Pullen, N., Pearson, R. B., Kozma, S. C. and Thomas, G. (1998) Phosphorylation sites in the autoinhibitory domain participate in p70(s6k) activation loop phosphorylation. J. Biol. Chem. **273**, 14845–14852

95 Rubenstein, E. M. and Schmidt, M. C. (2007) Mechanisms regulating the protein kinases of *Saccharomyces cerevisiae* Eukaryot. Cell **6**, 571–583

96 Casamayor, A., Torrance, P. D., Kobayashi, T., Thorner, J. and Alessi, D. R. (1999) Functional counterparts of mammalian protein kinases PDK1 and SGK in budding yeast. Curr. Biol. **9**, 186–197

97 Gelperin, D., Horton, L., DeChant, A., Hensold, J. and Lemmon, S. K. (2002) Loss of ypk1 function causes rapamycin sensitivity, inhibition of translation initiation and synthetic lethality in 14-3-3-deficient yeast. Genetics **161**, 1453–1464

98 Chen, P., Lee, K. S. and Levin, D. E. (1993) A pair of putative protein kinase genes (YPK1 and YPK2) is required for cell growth in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **236**, 443–447

99 Kamada, Y., Fujioka, Y., Suzuki, N. N., Inagaki, F., Wullschleger, S., Loewith, R., Hall, M. N. and Ohsumi, Y. (2005) Tor2 directly phosphorylates the AGC kinase Ypk2 to regulate actin polarization. Mol. Cell. Biol. **25**, 7239–7248

100 Roelants, F. M., Torrance, P. D., Bezman, N. and Thorner, J. (2002) Pkh1 and pkh2 differentially phosphorylate and activate ypk1 and ykr2 and define protein kinase modules required for maintenance of cell wall integrity. Mol. Biol. Cell. **13**, 3005–3028

101 Schmelzle, T., Helliwell, S. B. and Hall, M. N. (2002) Yeast protein kinases and the RHO1 exchange factor TUS1 are novel components of the cell integrity pathway in yeast. Mol. Cell. Biol. **22**, 1329–1339

102 Liu, K., Zhang, X., Lester, R. L. and Dickson, R. C. (2005) The sphingoid long chain base phytosphingosine activates AGC-type protein kinases in *Saccharomyces cerevisiae* including Ypk1, Ypk2, and Sch9. J. Biol. Chem. **280**, 22679–22687

103 Sun, Y., Taniguchi, R., Tanoue, D., Yamaji, T., Takematsu, H., Mori, K., Fujita, T., Kawasaki, T. and Kozutsumi, Y. (2000) Sli2 (Ypk1), a homologue of mammalian protein kinase SGK, is a downstream kinase in the sphingolipid-mediated signaling pathway of yeast. Mol. Cell. Biol. **20**, 4411–4419

104 Friant, S., Lombardi, R., Schmelzle, T., Hall, M. N. and Riezman, H. (2001) Sphingoid base signaling via Pkh kinases is required for endocytosis in yeast. EMBO J. **20**, 6783–6792

105 Roelants, F. M., Torrance, P. D. and Thorner, J. (2004) Differential roles of PDK1- and PDK2-phosphorylation sites in the yeast AGC kinases Ypk1, Pkc1 and Sch9. Microbiology **150**, 3289–3304

106 Toda, T., Cameron, S., Sass, P. and Wigler, M. (1988) SCH9, a gene of *Saccharomyces cerevisiae* that encodes a protein distinct from, but functionally and structurally related to, cAMP-dependent protein kinase catalytic subunits. Genes Dev. **2**, 517–527

107 Fabrizio, P., Pozza, F., Pletcher, S. D., Gendron, C. M. and Longo, V. D. (2001) Regulation of longevity and stress resistance by Sch9 in yeast. Science **292**, 288–290

108 Jorgensen, P., Nishikawa, J. L., Breitkreutz, B. J. and Tyers, M. (2002) Systematic identification of pathways that couple cell growth and division in yeast. Science **297**, 395–400

109 Jorgensen, P., Rupes, I., Sharom, J. R., Schneper, L., Broach, J. R. and Tyers, M. (2004) A dynamic transcriptional network communicates growth potential to ribosome synthesis and critical cell size. Genes Dev. **18**, 2491–2505

110 Pascual-Ahuir, A. and Proft, M. (2007) The Sch9 kinase is a chromatin-associated transcriptional activator of osmostress-responsive genes. EMBO J. **26**, 3098–3108

111 Mellor, H. and Parker, P. J. (1998) The extended protein kinase C superfamily. Biochem. J. **332**, 281–292

112 Kamada, Y., Qadota, H., Python, C. P., Anraku, Y., Ohya, Y. and Levin, D. E. (1996) Activation of yeast protein kinase C by Rho1 GTPase. J. Biol. Chem. **271**, 9193–9196

113 Nomoto, S., Watanabe, Y., Ninomiya-Tsuji, J., Yang, L. X., Nagai, Y., Kiuchi, K., Hagiwara, M., Hidaka, H., Matsumoto, K. and Irie, K. (1997) Functional analyses of mammalian protein kinase C isozymes in budding yeast and mammalian fibroblasts. Genes Cells **2**, 601–614

114 Levin, D. E. (2005) Cell wall integrity signaling in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. **69**, 262–291

115 Schmidt, A., Bickle, M., Beck, T. and Hall, M. N. (1997) The yeast phosphatidylinositol kinase homolog TOR2 activates RHO1 and RHO2 via the exchange factor ROM2. Cell **88**, 531–542

116 Inagaki, M., Schmelzle, T., Yamaguchi, K., Irie, K., Hall, M. N. and Matsumoto, K. (1999) PDK1 homologs activate the Pkc1-mitogen-activated protein kinase pathway in yeast. Mol. Cell. Biol. **19**, 8344–8352

117 Audhya, A. and Emr, S. D. (2002) Stt4 PI 4-kinase localizes to the plasma membrane and functions in the Pkc1-mediated MAP kinase cascade. Dev. Cell **2**, 593–605

118 Gao, T. and Newton, A. C. (2002) The turn motif is a phosphorylation switch that regulates the binding of Hsp70 to protein kinase C. J. Biol. Chem. **277**, 31585–31592

119 Bornancin, F. and Parker, P. J. (1996) Phosphorylation of threonine 638 critically controls the dephosphorylation and inactivation of protein kinase Cα. Curr. Biol. **6**, 1114–1123

120 Wright, C. M., Fewell, S. W., Sullivan, M. L., Pipas, J. M., Watkins, S. C. and Brodsky, J. L. (2007) The Hsp40 molecular chaperone Ydj1p, along with the protein kinase C pathway, affects cell-wall integrity in the yeast *Saccharomyces cerevisiae*. Genetics **175**, 1649–1664

121 Martin, D. E., Soulard, A. and Hall, M. N. (2004) TOR regulates ribosomal protein gene expression via PKA and the Forkhead transcription factor FHL1. Cell **119**, 969–979

122 Zurita-Martinez, S. A. and Cardenas, M. E. (2005) Tor and cyclic AMP-protein kinase A: two parallel pathways regulating expression of genes required for cell growth. Eukaryot. Cell **4**, 63–71

123 Schmelzle, T., Beck, T., Martin, D. E. and Hall, M. N. (2004) Activation of the RAS/cyclic AMP pathway suppresses a TOR deficiency in yeast. Mol. Cell. Biol. **24**, 338–351

124 Portela, P. and Moreno, S. (2006) Glucose-dependent activation of protein kinase A activity in *Saccharomyces cerevisiae* and phosphorylation of its TPK1 catalytic subunit. Cell. Signalling **18**, 1072–1086

125 Tamaskovic, R., Bichsel, S. J. and Hemmings, B. A. (2003) NDR family of AGC kinases–essential regulators of the cell cycle and morphogenesis. FEBS Lett. **546**, 73–80

126 Xie, M. W., Jin, F., Hwang, H., Hwang, S., Anand, V., Duncan, M. C. and Huang, J. (2005) Insights into TOR function and rapamycin response: chemical genomic profiling by using a high-density cell array method. Proc. Natl. Acad. Sci. U.S.A. **102**, 7215–7220

127 Wilson, W. A., Wang, Z. and Roach, P. J. (2002) Systematic identification of the genes affecting glycogen storage in the yeast *Saccharomyces cerevisiae*: implication of the vacuole as a determinant of glycogen level. Mol. Cell Proteomics **1**, 232–242

128 Mah, A. S., Jang, J. and Deshaies, R. J. (2001) Protein kinase Cdc15 activates the Dbf2–Mob1 kinase complex. Proc. Natl. Acad. Sci. U.S.A. **98**, 7325–7330

129 Pedruzzi, I., Dubouloz, F., Cameroni, E., Wanke, V., Roosen, J., Winderickx, J. and De Virgilio, C. (2003) TOR and PKA signaling pathways converge on the protein kinase Rim15 to control entry into G0. Mol. Cell **12**, 1607–1613

130 Wanke, V., Pedruzzi, I., Cameroni, E., Dubouloz, F. and De Virgilio, C. (2005) Regulation of G0 entry by the Pho80–Pho85 cyclin–CDK complex. EMBO J. **24**, 4271–4278

131 De Camilli, P., Emr, S. D., McPherson, P. S. and Novick, P. (1996) Phosphoinositides as regulators in membrane traffic. Science **271**, 1533–1539

132 Bimbo, A., Jia, Y., Poh, S. L., Karuturi, R. K., den Elzen, N., Peng, X., Zheng, L., O'Connell, M., Liu, E. T., Balasubramanian, M. K. and Liu, J. (2005) Systematic deletion analysis of fission yeast protein kinases. Eukaryot. Cell **4**, 799–813

133 Jin, M., Fujita, M., Culley, B. M., Apolinario, E., Yamamoto, M., Maundrell, K. and Hoffman, C. S. (1995) sck1, a high copy number suppressor of defects in the cAMP-dependent protein kinase pathway in fission yeast, encodes a protein homologous to the *Saccharomyces cerevisiae* SCH9 kinase. Genetics **140**, 457–467

134 Fujita, M. and Yamamoto, M. (1998) *S. pombe*. sck2+, a second homologue of *S. cerevisiae* SCH9 in fission yeast, encodes a putative protein kinase closely related to PKA in function. Curr. Genet. **33**, 248–254

135 Niederberger, C. and Schweingruber, M. E. (1999) A *Schizosaccharomyces pombe* gene, ksg1, that shows structural homology to the human phosphoinositide-dependent protein kinase PDK1, is essential for growth, mating and sporulation. Mol. Gen. Genet. **261**, 177–183

136 Mitra, P., Zhang, Y., Rameh, L. E., Ivshina, M. P., McCollum, D., Nunnari, J. J., Hendricks, G. M., Kerr, M. L., Field, S. J. et al. (2004) A novel phosphatidylinositol(3,4,5)P3 pathway in fission yeast. J. Cell Biol. **166**, 205–211

137 Tang, Y. and McLeod, M. (2004) *In vivo* activation of protein kinase A in *Schizosaccharomyces pombe* requires threonine phosphorylation at its activation loop and is dependent on PDK1. Genetics **168**, 1843–1853

138 Graub, R., Hilti, N., Niederberger, C. and Schweingruber, M. E. (2003) Ksg1, a homologue of the phosphoinositide-dependent protein kinase 1, controls cell wall integrity in *Schizosaccharomyces pombe*. J. Basic Microbiol. **43**, 473–482

139 Price, D. J., Grove, J. R., Calvo, V., Avruch, J. and Bierer, B. E. (1992) Rapamycin-induced inhibition of the 70-kilodalton S6 protein kinase. Science **257**, 973–977

140 Chung, J., Kuo, C. J., Crabtree, G. R. and Blenis, J. (1992) Rapamycin-FKBP specifically blocks growth-dependent activation of and signaling by the 70 kd S6 protein kinases. Cell **69**, 1227–1236

© The Authors Journal compilation © 2008 Biochemical Society

141 Shima, H., Pende, M., Chen, Y., Fumagalli, S., Thomas, G. and Kozma, S. C. (1998) Disruption of the p70(s6k)/p85(s6k) gene reveals a small mouse phenotype and a new functional S6 kinase. EMBO J. **17**, 6649–6659

142 Pullen, N. and Thomas, G. (1997) The modular phosphorylation and activation of p70s6k. FEBS Lett. **410**, 78–82

143 Nojima, H., Tokunaga, C., Eguchi, S., Oshiro, N., Hidayat, S., Yoshino, K., Hara, K., Tanaka, N., Avruch, J. and Yonezawa, K. (2003) The mammalian target of rapamycin (mTOR) partner, raptor, binds the mTOR substrates p70 S6 kinase and 4E-BP1 through their TOR signaling (TOS) motif. J. Biol. Chem. **278**, 15461–15464

144 Weng, Q. P., Andrabi, K., Kozlowski, M. T., Grove, J. R. and Avruch, J. (1995) Multiple independent inputs are required for activation of the p70 S6 kinase. Mol. Cell. Biol. **15**, 2333–2340

145 Cheatham, L., Monfar, M., Chou, M. M. and Blenis, J. (1995) Structural and functional analysis of p70S6k. Proc. Natl. Acad. Sci. U.S.A. **92**, 11696–11700

146 Williams, M. R., Arthur, J. S., Balendran, A., van der Kaay, J., Poli, V., Cohen, P. and Alessi, D. R. (2000) The role of 3-phosphoinositide-dependent protein kinase 1 in activating AGC kinases defined in embryonic stem cells. Curr. Biol. **10**, 439–448

147 Collins, B. J., Deak, M., Arthur, J. S., Armit, L. J. and Alessi, D. R. (2003) *In vivo* role of the PIF-binding docking site of PDK1 defined by knock-in mutation. EMBO J. **22**, 4202–4211

148 McManus, E. J., Collins, B. J., Ashby, P. R., Prescott, A. R., Murray-Tait, V., Armit, L. J., Arthur, J. S. and Alessi, D. R. (2004) The *in vivo* role of PtdIns(3,4,5)P3 binding to PDK1 PH domain defined by knockin mutation. EMBO J. **23**, 2071–2082

149 Burnett, P. E., Barrow, R. K., Cohen, N. A., Snyder, S. H. and Sabatini, D. M. (1998) RAFT1 phosphorylation of the translational regulators p70 S6 kinase and 4E-BP1. Proc. Natl. Acad. Sci. U.S.A. **95**, 1432–1437

150 Isotani, S., Hara, K., Tokunaga, C., Inoue, H., Avruch, J. and Yonezawa, K. (1999) Immunopurified mammalian target of rapamycin phosphorylates and activates p70 S6 kinase α *in vitro*. J. Biol. Chem. **274**, 34493–34498

151 Ali, S. M. and Sabatini, D. M. (2005) Structure of S6 kinase 1 determines whether raptor–mTOR or rictor–mTOR phosphorylates its hydrophobic motif site. J. Biol. Chem. **280**, 19445–19448

152 Balendran, A., Casamayor, A., Deak, M., Paterson, A., Gaffney, P., Currie, R., Downes, C. P. and Alessi, D. R. (1999) PDK1 acquires PDK2 activity in the presence of a synthetic peptide derived from the carboxyl terminus of PRK2. Curr. Biol. **9**, 393–404

153 Belham, C., Comb, M. J. and Avruch, J. (2001) Identification of the NIMA family kinases NEK6/7 as regulators of the p70 ribosomal S6 kinase. Curr. Biol. **11**, 1155–1167

154 Romanelli, A., Dreisbach, V. C. and Blenis, J. (2002) Characterization of phosphatidylinositol 3-kinase-dependent phosphorylation of the hydrophobic motif site Thr(389) in p70 S6 kinase 1. J. Biol. Chem. **277**, 40281–40289

155 Moser, B. A., Dennis, P. B., Pullen, N., Pearson, R. B., Williamson, N. A., Wettenhall, R. E., Kozma, S. C. and Thomas, G. (1997) Dual requirement for a newly identified phosphorylation site in p70s6k. Mol. Cell. Biol. **17**, 5648–5655

156 Schalm, S. S., Tee, A. R. and Blenis, J. (2005) Characterization of a conserved C-terminal motif (RSPRR) in ribosomal protein S6 kinase 1 required for its mammalian target of rapamycin-dependent regulation. J. Biol. Chem. **280**, 11101–11106

157 Dennis, P. B., Pullen, N., Kozma, S. C. and Thomas, G. (1996) The principal rapamycin-sensitive p70(s6k) phosphorylation sites, T-229 and T-389, are differentially regulated by rapamycin-insensitive kinase kinases. Mol. Cell. Biol. **16**, 6242–6251

158 Holz, M. K., Ballif, B. A., Gygi, S. P. and Blenis, J. (2005) mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. Cell **123**, 569–580

159 Byfield, M. P., Murray, J. T. and Backer, J. M. (2005) hVps34 is a nutrient-regulated lipid kinase required for activation of p70 S6 kinase. J. Biol. Chem. **280**, 33076–33082

160 Nobukuni, T., Joaquin, M., Roccio, M., Dann, S. G., Kim, S. Y., Gulati, P., Byfield, M. P., Backer, J. M., Natt, F., Bos, J. L. et al. (2005) Amino acids mediate mTOR/raptor signaling through activation of class 3 phosphatidylinositol 3OH-kinase. Proc. Natl. Acad. Sci. U.S.A. **102**, 14238–14243

161 Chan, T. O., Rittenhouse, S. E. and Tsichlis, P. N. (1999) AKT/PKB and other D3 phosphoinositide-regulated kinases: kinase activation by phosphoinositide-dependent phosphorylation. Annu. Rev. Biochem. **68**, 965–1014

162 Stokoe, D., Stephens, L. R., Copeland, T., Gaffney, P. R., Reese, C. B., Painter, G. F., Holmes, A. B., McCormick, F. and Hawkins, P. T. (1997) Dual role of phosphatidylinositol-3,4,5-trisphosphate in the activation of protein kinase B. Science **277**, 567–570

163 Alessi, D. R., James, S. R., Downes, C. P., Holmes, A. B., Gaffney, P. R., Reese, C. B. and Cohen, P. (1997) Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Bα. Curr. Biol. **7**, 261–269

164 Calleja, V., Alcor, D., Laguerre, M., Park, J., Vojnovic, B., Hemmings, B. A., Downward, J., Parker, P. J. and Larijani, B. (2007) Intramolecular and intermolecular interactions of protein kinase B define its activation *in vivo*. PLoS Biol. **5**, e95

165 Stephens, L., Anderson, K., Stokoe, D., Erdjument-Bromage, H., Painter, G. F., Holmes, A. B., Gaffney, P. R., Reese, C. B., McCormick, F., Tempst, P. et al. (1998) Protein kinase B kinases that mediate phosphatidylinositol 3,4,5-trisphosphate-dependent activation of protein kinase B. Science **279**, 710–714

166 Bellacosa, A., Chan, T. O., Ahmed, N. N., Datta, K., Malstrom, S., Stokoe, D., McCormick, F., Feng, J. and Tsichlis, P. (1998) Akt activation by growth factors is a multiple-step process: the role of the PH domain. Oncogene **17**, 313–325

167 Kohn, A. D., Barthel, A., Kovacina, K. S., Boge, A., Wallach, B., Summers, S. A., Birnbaum, M. J., Scott, P. H., Lawrence, Jr, J. C. and Roth, R. A. (1998) Construction and characterization of a conditionally active version of the serine/threonine kinase Akt. J. Biol. Chem. **273**, 11937–11943

168 Scheid, M. P., Marignani, P. A. and Woodgett, J. R. (2002) Multiple phosphoinositide 3-kinase-dependent steps in activation of protein kinase B. Mol. Cell. Biol. **22**, 6247–6260

169 Woodgett, J. R. (2005) Recent advances in the protein kinase B signaling pathway. Curr. Opin. Cell Biol. **17**, 150–157

170 Feng, J., Park, J., Cron, P., Hess, D. and Hemmings, B. A. (2004) Identification of a PKB/Akt hydrophobic motif Ser-473 kinase as DNA-dependent protein kinase. J. Biol. Chem. **279**, 41189–41196

171 Troussard, A. A., Mawji, N. M., Ong, C., Mui, A., St -Arnaud, R. and Dedhar, S. (2003) Conditional knock-out of integrin-linked kinase demonstrates an essential role in protein kinase B/Akt activation. J. Biol. Chem. **278**, 22374–22378

172 Kawakami, Y., Nishimoto, H., Kitaura, J., Maeda-Yamamoto, M., Kato, R. M., Littman, D. R., Leitges, M., Rawlings, D. J. and Kawakami, T. (2004) Protein kinase C βII regulates Akt phosphorylation on Ser-473 in a cell type- and stimulus-specific fashion. J. Biol. Chem. **279**, 47720–47725

173 Toker, A. and Newton, A. C. (2000) Akt/protein kinase B is regulated by autophosphorylation at the hypothetical PDK-2 site. J. Biol. Chem. **275**, 8271–8274

174 Meier, R., Alessi, D. R., Cron, P., Andjelkovic, M. and Hemmings, B. A. (1997) Mitogenic activation, phosphorylation, and nuclear translocation of protein kinase Bβ. J. Biol. Chem. **272**, 30491–30497

175 Andjelkovic, M., Maira, S. M., Cron, P., Parker, P. J. and Hemmings, B. A. (1999) Domain swapping used to investigate the mechanism of protein kinase B regulation by 3-phosphoinositide-dependent protein kinase 1 and Ser473 kinase. Mol. Cell. Biol. **19**, 5061–5072

176 Alessi, D. R., Andjelkovic, M., Caudwell, B., Cron, P., Morrice, N., Cohen, P. and Hemmings, B. A. (1996) Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J. **15**, 6541–6551

177 Yang, J., Cron, P., Thompson, V., Good, V. M., Hess, D., Hemmings, B. A. and Barford, D. (2002) Molecular mechanism for the regulation of protein kinase B/Akt by hydrophobic motif phosphorylation. Mol. Cell **9**, 1227–1240

178 Andjelkovic, M., Jakubowicz, T., Cron, P., Ming, X. F., Han, J. W. and Hemmings, B. A. (1996) Activation and phosphorylation of a pleckstrin homology domain containing protein kinase (RAC-PK/PKB) promoted by serum and protein phosphatase inhibitors. Proc. Natl. Acad. Sci. U.S.A. **93**, 5699–5704

179 Gao, T., Furnari, F. and Newton, A. C. (2005) PHLPP: a phosphatase that directly dephosphorylates Akt, promotes apoptosis, and suppresses tumor growth. Mol. Cell **18**, 13–24

180 Yan, G., Shen, X. and Jiang, Y. (2006) Rapamycin activates Tap42-associated phosphatases by abrogating their association with Tor complex 1. EMBO J. **25**, 3546–3555

181 Newton, A. C. (2003) Regulation of the ABC kinases by phosphorylation: protein kinase C as a paradigm. Biochem. J. **370**, 361–371

182 Sonnenburg, E. D., Gao, T. and Newton, A. C. (2001) The phosphoinositide-dependent kinase, PDK-1, phosphorylates conventional protein kinase C isozymes by a mechanism that is independent of phosphoinositide 3-kinase. J. Biol. Chem. **276**, 45289–45297

183 Balendran, A., Hare, G. R., Kieloch, A., Williams, M. R. and Alessi, D. R. (2000) Further evidence that 3-phosphoinositide-dependent protein kinase-1 (PDK1) is required for the stability and phosphorylation of protein kinase C (PKC) isoforms. FEBS Lett. **484**, 217–223

184 Ziegler, W. H., Parekh, D. B., Le Good, J. A., Whelan, R. D., Kelly, J. J., Frech, M., Hemmings, B. A. and Parker, P. J. (1999) Rapamycin-sensitive phosphorylation of PKC on a carboxy-terminal site by an atypical PKC complex. Curr. Biol. **9**, 522–529

185 Parekh, D., Ziegler, W., Yonezawa, K., Hara, K. and Parker, P. J. (1999) Mammalian TOR controls one of two kinase pathways acting upon nPKCδ and nPKCε. J. Biol. Chem. **274**, 34758–34764

186 England, K., Watson, J., Beale, G., Warner, M., Cross, J. and Rumsby, M. (2001) Signalling pathways regulating the dephosphorylation of Ser729 in the hydrophobic domain of protein kinase Cε upon cell passage. J. Biol. Chem. **276**, 10437–10442

187 Freeley, M., Volkov, Y., Kelleher, D. and Long, A. (2005) Stimulus-induced phosphorylation of PKCθ at the C-terminal hydrophobic-motif in human T lymphocytes. Biochem. Biophys. Res. Commun. **334**, 619–630

© The Authors Journal compilation © 2008 Biochemical Society

188 Edwards, A. S., Faux, M. C., Scott, J. D. and Newton, A. C. (1999) Carboxyl-terminal phosphorylation regulates the function and subcellular localization of protein kinase CβII. J. Biol. Chem. **274**, 6461–6468
189 Behn-Krappa, A. and Newton, A. C. (1999) The hydrophobic phosphorylation motif of conventional protein kinase C is regulated by autophosphorylation. Curr. Biol. **9**, 728–737
190 Flint, A. J., Paladini, R. D. and Koshland, Jr, D. E. (1990) Autophosphorylation of protein kinase C at three separated regions of its primary sequence. Science **249**, 408–411
191 Gao, T. and Newton, A. C. (2006) Invariant Leu preceding turn motif phosphorylation site controls the interaction of protein kinase C with Hsp70. J. Biol. Chem. **281**, 32461–32468
192 Messerschmidt, A., Macieira, S., Velarde, M., Badeker, M., Benda, C., Jestel, A., Brandstetter, H., Neuefeind, T. and Blaesse, M. (2005) Crystal structure of the catalytic domain of human atypical protein kinase C-ι reveals interaction mode of phosphorylation site in turn motif. J. Mol. Biol. **352**, 918–931
193 Grodsky, N., Li, Y., Bouzida, D., Love, R., Jensen, J., Nodes, B., Nonomiya, J. and Grant, S. (2006) Structure of the catalytic domain of human protein kinase C βII complexed with a bisindolylmaleimide inhibitor. Biochemistry **45**, 13970–13981
194 Hauge, C., Antal, T. L., Hirschberg, D., Doehn, U., Thorup, K., Idrissova, L., Hansen, K., Jensen, O. N., Jorgensen, T. J., Biondi, R. M. and Frodin, M. (2007) Mechanism for activation of the growth factor-activated AGC kinases by turn motif phosphorylation. EMBO J. **26**, 2251–2261
195 Hauge, C. and Frodin, M. (2006) RSK and MSK in MAP kinase signalling. J. Cell Sci. **119**, 3021–3023
196 Frodin, M., Jensen, C. J., Merienne, K. and Gammeltoft, S. (2000) A phosphoserine-regulated docking site in the protein kinase RSK2 that recruits and activates PDK1. EMBO J. **19**, 2924–2934
197 Dalby, K. N., Morrice, N., Caudwell, F. B., Avruch, J. and Cohen, P. (1998) Identification of regulatory phosphorylation sites in mitogen-activated protein kinase (MAPK)-activated protein kinase-1a/p90rsk that are inducible by MAPK. J. Biol. Chem. **273**, 1496–1505
198 Zaru, R., Ronkina, N., Gaestel, M., Arthur, J. S. and Watts, C. (2007) The MAPK-activated kinase Rsk controls an acute Toll-like receptor signaling response in dendritic cells and is activated through two distinct pathways. Nat. Immunol. **8**, 1227–1235
199 Rane, M. J., Coxon, P. Y., Powell, D. W., Webster, R., Klein, J. B., Pierce, W., Ping, P. and McLeish, K. R. (2001) p38 kinase-dependent MAPKAPK-2 activation functions as 3-phosphoinositide-dependent kinase-2 for Akt in human neutrophils. J. Biol. Chem. **276**, 3517–3523
200 Richards, S. A., Dreisbach, V. C., Murphy, L. O. and Blenis, J. (2001) Characterization of regulatory events associated with membrane targeting of p90 ribosomal S6 kinase 1. Mol. Cell. Biol. **21**, 7470–7480
201 Roux, P. P., Shahbazian, D., Vu, H., Holz, M. K., Cohen, M. S., Taunton, J., Sonenberg, N. and Blenis, J. (2007) RAS/ERK signaling promotes site-specific ribosomal protein S6 phosphorylation via RSK and stimulates cap-dependent translation. J. Biol. Chem. **282**, 14056–14064
202 Shahbazian, D., Roux, P. P., Mieulet, V., Cohen, M. S., Raught, B., Taunton, J., Hershey, J. W., Blenis, J., Pende, M. and Sonenberg, N. (2006) The mTOR/PI3K and MAPK pathways converge on eIF4B to control its phosphorylation and activity. EMBO J. **25**, 2781–2791
203 Pende, M., Um, S. H., Mieulet, V., Sticker, M., Goss, V. L., Mestan, J., Mueller, M., Fumagalli, S., Kozma, S. C. and Thomas, G. (2004) S6K1$^{-/-}$/S6K2$^{-/-}$ mice exhibit perinatal lethality and rapamycin-sensitive 5′-terminal oligopyrimidine mRNA translation and reveal a mitogen-activated protein kinase-dependent S6 kinase pathway. Mol. Cell. Biol. **24**, 3112–3124
204 Tessier, M. and Woodgett, J. R. (2006) Serum and glucocorticoid-regulated protein kinases: variations on a theme. J. Cell Biochem. **98**, 1391–1407
205 Tessier, M. and Woodgett, J. R. (2006) Role of the Phox homology domain and phosphorylation in activation of serum and glucocorticoid-regulated kinase-3. J. Biol. Chem. **281**, 23978–23989
206 Ellson, C. D., Andrews, S., Stephens, L. R. and Hawkins, P. T. (2002) The PX domain: a new phosphoinositide-binding module. J. Cell Sci. **115**, 1099–1105
207 Pao, A. C., McCormick, J. A., Li, H., Siu, J., Govaerts, C., Bhalla, V., Soundararajan, R. and Pearce, D. (2007) NH2 terminus of serum and glucocorticoid-regulated kinase 1 binds to phosphoinositides and is essential for isoform-specific physiological functions. Am. J. Physiol. Renal Physiol. **292**, F1741–F1750
208 Kobayashi, T. and Cohen, P. (1999) Activation of serum- and glucocorticoid-regulated protein kinase by agonists that activate phosphatidylinositide 3-kinase is mediated by 3-phosphoinositide-dependent protein kinase-1 (PDK1) and PDK2. Biochem. J. **339**, 319–328
209 Biondi, R. M., Kieloch, A., Currie, R. A., Deak, M. and Alessi, D. R. (2001) The PIF-binding pocket in PDK1 is essential for activation of S6K and SGK, but not PKB. EMBO J. **20**, 4380–4390
210 Park, J., Leong, M. L., Buse, P., Maiyar, A. C., Firestone, G. L. and Hemmings, B. A. (1999) Serum and glucocorticoid-inducible kinase (SGK) is a target of the PI 3-kinase-stimulated signaling pathway. EMBO J. **18**, 3024–3033
211 Cheng, X., Ma, Y., Moore, M., Hemmings, B. A. and Taylor, S. S. (1998) Phosphorylation and activation of cAMP-dependent protein kinase by phosphoinositide-dependent protein kinase. Proc. Natl. Acad. Sci. U.S.A. **95**, 9849–9854
212 Sapkota, G. P., Kieloch, A., Lizcano, J. M., Lain, S., Arthur, J. S., Williams, M. R., Morrice, N., Deak, M. and Alessi, D. R. (2001) Phosphorylation of the protein kinase mutated in Peutz–Jeghers cancer syndrome, LKB1/STK11, at Ser431 by p90(RSK) and cAMP-dependent protein kinase, but not its farnesylation at Cys(433), is essential for LKB1 to suppress cell growth. J. Biol. Chem. **276**, 19469–19482
213 Kimball, S. R., Siegfried, B. A. and Jefferson, L. S. (2004) Glucagon represses signaling through the mammalian target of rapamycin in rat liver by activating AMP-activated protein kinase. J. Biol. Chem. **279**, 54103–54109
214 King, C. C., Zenke, F. T., Dawson, P. E., Dutil, E. M., Newton, A. C., Hemmings, B. A. and Bokoch, G. M. (2000) Sphingosine is a novel activator of 3-phosphoinositide-dependent kinase 1. J. Biol. Chem. **275**, 18108–18113
215 Casamayor, A., Morrice, N. A. and Alessi, D. R. (1999) Phosphorylation of Ser-241 is essential for the activity of 3-phosphoinositide-dependent protein kinase-1: identification of five sites of phosphorylation *in vivo*. Biochem. J. **342**, 287–292
216 Lawlor, M. A., Mora, A., Ashby, P. R., Williams, M. R., Murray-Tait, V., Malone, L., Prescott, A. R., Lucocq, J. M. and Alessi, D. R. (2002) Essential role of PDK1 in regulating cell size and development in mice. EMBO J. **21**, 3728–3738
217 Rexhepaj, R., Grahammer, F., Volkl, H., Remy, C., Wagner, C. A., Sandulache, D., Artunc, F., Henke, G., Nammi, S., Capasso, G., Alessi, D. R. and Lang, F. (2006) Reduced intestinal and renal amino acid transport in PDK1 hypomorphic mice. FASEB J. **20**, 2214–2222
218 Kelly, A. P., Finlay, D. K., Hinton, H. J., Clarke, R. G., Fiorini, E., Radtke, F. and Cantrell, D. A. (2007) Notch-induced T cell development requires phosphoinositide-dependent kinase 1. EMBO J. **26**, 3441–3450
219 Tominaga, Y., Tamguney, T., Kolesnichenko, M., Bilanges, B. and Stokoe, D. (2005) Translational deregulation in PDK-1$^{-/-}$ embryonic stem cells. Mol. Cell. Biol. **25**, 8465–8475
220 Stock, J. B., Stock, A. M. and Mottonen, J. M. (1990) Signal transduction in bacteria. Nature **344**, 395–400
221 Bogusz, A. M., Brickley, D. R., Pew, T. and Conzen, S. D. (2006) A novel N-terminal hydrophobic motif mediates constitutive degradation of serum- and glucocorticoid-induced kinase-1 by the ubiquitin–proteasome pathway. FEBS J. **273**, 2913–2928
222 Edinger, A. L. and Thompson, C. B. (2004) An activated mTOR mutant supports growth-factor-independent, nutrient-dependent cell survival. Oncogene **23**, 5654–5663

Received 5 November 2007/11 December 2007; accepted 12 December 2007
Published on the Internet 29 January 2008, doi:10.1042/BJ20071518

© The Authors Journal compilation © 2008 Biochemical Society