Oncogene (2008) 27, 5431–5442

www.nature.com/onc

REVIEW

Assessment of PTEN tumor suppressor activity in nonmammalian models: the year of the yeast

VJ Cid[1], I Rodríguez-Escudero[1], A Andrés-Pons[2], C Romá-Mateo[2], A Gil[2], J den Hertog[3], M Molina[1] and R Pulido[2]

[1]*Departamento de Microbiología II, Universidad Complutense de Madrid, Madrid, Spain;* [2]*Centro de Investigación Príncipe Felipe, Valencia, Spain and* [3]*Hubrecht Institute, Utrecht, The Netherlands*

Model organisms have emerged as suitable and reliable biological tools to study the properties of proteins whose function is altered in human disease. In the case of the PI3K and PTEN human cancer-related proteins, several vertebrate and invertebrate models, including mouse, fly, worm and amoeba, have been exploited to obtain relevant functional information that has been conserved from these organisms to humans along evolution. The yeast *Saccharomyces cerevisiae* is an eukaryotic unicellular organism that lacks a canonical mammalian-like PI3K/PTEN pathway and PIP3 as a physiological second messenger, PIP2 being essential for its life. The mammalian PI3K/PTEN pathway can be reconstituted in *S. cerevisiae*, generating growth alteration phenotypes that can be easily monitored to perform *in vivo* functional analysis of the molecular constituents of this pathway. Here, we review the current nonmammalian model systems to study PTEN function, summarize our knowledge of PTEN orthologs in yeast species and propose the yeast *S. cerevisiae* as a sensitive biological sensor of PI3K oncogenicity and PTEN tumor suppressor activity.
Oncogene (2008) **27,** 5431–5442; doi:10.1038/onc.2008.240

Keywords: PTEN; PI3K; tumor suppression; oncogenesis; yeast

PTEN orthologs in eukaryotic model organisms

The PTEN protein, encoded by a single gene in mammalian species, is the major enzyme that counteracts signaling by the distinct proto-oncogenic PI3Ks, converting the PI3K product, PtdIns(3,4,5)P_3 (PIP3), to PtdIns(4,5)P_2 (PIP2). The conservation of PTEN amino-acid sequence in vertebrates is very high, ranging from nearly 100% identity between primates and rodents to 80% identity between primates and teleost fish (Table 1; Figure 1a; see Figure 2 for a depiction of PTEN domains). Many mice strains have been generated, including *PTEN*-knockout, -conditional knockout and -transgenic mice, that constitute excellent mammalian animal models to study the role of PTEN in human cancer and to validate PTEN-related anticancer therapies (Kishimoto *et al*., 2003; Chow and Baker, 2006; Tamguney and Stokoe, 2007; Pulido and van Huijsduijnen, 2008; Suzuki *et al*., 2008). PTEN studies have also been performed in other vertebrates, including amphibia and fish. In frogs (*Xenopus laevis*), PTEN activity is required for cell cycle elongation associated with morphogenesis during embryo gastrulation, and changes in plasma membrane and nuclear localization of PTEN, likely reflecting changes in PTEN activity, were found during early embryogenesis of this organism (Ueno *et al*., 2006). Zebrafish (*Danio rerio*) contains two highly related *PTEN* genes, *zPTENa* and *zPTENb*, each of them producing a short and a long splice variant. Compared with mammalian PTEN, zPTENa contains an extra amino-acid sequence at the C2 domain. In addition, the long splice variants from both *zPTEN* genes possess a further exon encoding for another extra sequence, also at the C2 domain (Croushore *et al*., 2005). The functional properties of these isoform- and fish-specific regions are unknown, although the *in vitro* activity against PIP3 seems to be lower for zPTENb than for zPTENa (Faucherre *et al*., 2008). Antisense knockdown of *zPTENa* or *zPTENb* has been reported to produce distinct, nonredundant developmental abnormalities during zebrafish embryogenesis (Croushore *et al*., 2005). However, $zPTENa^{-/-}$ or $zPTENb^{-/-}$ single mutants did not display embryonic phenotypes, whereas double $zPTENa^{-/-}$ and $zPTENb^{-/-}$ mutants displayed early embryonic lethality associated with hyperplasia, which could be ameliorated by embryo exposure to the PI3K inhibitor LY294002. In addition, $zPTENb^{-/-}$ animals developed spontaneous eye tumors late in life, suggesting that zPTENb plays tissue-specific-, nonredundant tumor suppressor functions in zebrafish (Faucherre *et al*., 2008).

PTEN orthologs in invertebrates possess both a PTP and a C2 domain with high similarity (especially at the PTP domain) to mammalian PTEN. However, the conservation of the regulatory PTEN C-terminal region in invertebrates with respect to vertebrates is weak. Remarkably, in the fruit fly *Drosophila melanogaster*, the *PTEN* gene generates three alternatively spliced isoforms, which differ in their C-terminus (dPTEN1,

Correspondence: Dr R Pulido, Centro de Investigación Príncipe Felipe, Valencia, Spain.
E-mail: rpulido@cipf.es

Table 1 PTEN orthologs in eukaryotic model organisms

Organism[a]	*Name*	*PIP3 phosphatase activity*[b]	*Identity to human PTEN (%)*[c]	*C2 domain*[d]	*Physiological function*[e]	*PIP3 phosphatase activity in S. cerevisiae*[f]
M. musculus	PTEN	Yes	99	Yes	-essential for early embryogenesis -tumor suppressor activity	Yes
X. laevis	PTEN	ND	88	Yes	-cell cycle elongation at early embryogenesis	Yes
D. rerio	zPTENa/b	Yes	82/86	Yes	-essential for early embryogenesis -tumor suppressor activity	Yes
D. melanogaster	dPTEN	Yes	44	Yes	-essential for early embryogenesis -cell- and organ-size control	Yes
D. discoideum	PTEN	Yes	43	Yes	-control of chemotaxis and motility	ND
A. thaliana	AtPTEN1	Yes	48	Yes	-polen maturation	No
C. elegans	DAF-18	ND	46	No	-dauer formation during larvae development and lifespan control	ND
S. pombe	Ptn1	Yes	37	No	-membrane trafficking (?)	No
S. cerevisiae	Tep1	No	27	No	-spore formation (?)	No

[a]Entries: *Mus musculus* (NP_032986); *Xenopus laevis* (NP_001083831); *Danio rerio* (AAR04345, AAR04346); *Drosophila melanogaster* (NP_477423); *Dictyostelium discoideum* (XM_632484); *Arabidopsis thaliana* (NP_198756); *Caenorhabditis elegans* (NP_499926); *Schizosaccharomyces pombe* (NP_596312); *Saccharomyces cerevisiae* (NP_014271).
[b]Determined *in vitro* or in cell-based systems (see references in the text).
[c]Amino-acid identity determined using BLASTP alignment (www.ncbi.nlm.nih.gov/blast/Blast.cgi), with respect to: human PTEN PTP catalytic domain (*S. cerevisiae*, *Sch. pombe*, *C. elegans* and *A. thaliana*); human PTP domain and a portion of the C2-like domain (*D. discoideum*); human PTP domain and C2 domain (*D. melanogaster*); human full-length PTEN (*D. rerio* –short spliced variants-, *X. laevis* and *M. musculus*). *H. sapiens* PTEN entry: NP_000305.
[d]As annotated in the PROSITE database (www.expasy.org/prosite).
[e]On the basis of PTEN-deficient or PTEN-reconstituted organisms (see references in the text).
[f]As determined by their ability to counteract *S. cerevisiae* growth inhibition by mammalian p110α-CAAX. pYES2 vectors containing the distinct PTEN orthologs used for this analysis will be described elsewhere. Expression of PTEN orthologs which did not display activity in yeast was verified by immunoblot or by immunofluorescence with anti-HA or anti-Myc antibodies on epitope-tagged versions, except for AtPTEN1. ND, not determined.

dPTEN2 and dPTEN3) (Smith *et al*., 1999). Two of these isoforms only differentiate by the presence (dPTEN2) or the absence (dPTEN3) of a C-terminus PDZ domain-binding motif. Alternatively spliced products of human PTEN that affect its C-terminus have also been documented, although they are expected to be destabilized, resulting in lack of function (Sharrard and Maitland, 2000). The three spliced forms of dPTEN dephosphorylate PIP3 *in vitro*. However, dPTEN2, that interacts with the PDZ3 domain of the scaffolding protein Bazooka/PAR-3 (von Stein *et al*., 2005; Pinal *et al*., 2006), was unable to downregulate dAkt activation in cells (Maehama *et al*., 2004). This is in contrast with mammalian PTEN, whose PDZ domain-binding motif favors PTEN activity in cells by interaction with stabilizing and/or plasma membrane-targeting proteins (Wu *et al*., 2000a, b; Valiente *et al*., 2005). Whether this difference is due to different patterns of PDZ domains interactions or to distinct intrinsic functional requirements for mammalian and *Drosophila* PTEN remains unexplored. Noticeably, the PDZ2 domain of Bazooka/PAR-3 binds to phosphoinositides, which may account for specific outputs in the signaling mediated by Bazooka/PAR-3-PTEN complexes (Wu *et al*., 2007). Genetic study in *Drosophila* has largely contributed to the studies of PI3K signaling and oncogenesis (Hariharan and Bilder, 2006). As for vertebrates, homozygous mutation of *dPTEN* results in early embryonic lethality as a consequence of the overactivation of the *Drosophila* insulin/PI3K/Akt pathway, producing hyperplastic growth and increased cell and organ size. Overexpression of dPTEN also causes lethality in embryos, likely by cell cycle and cell growth inhibition, as well as by an excess of apoptosis. In addition, upon eye-specific overexpression, dPTEN caused a small eye phenotype (Goberdhan *et al*., 1999; Huang *et al*., 1999; Gao *et al*., 2000; Scanga *et al*., 2000; Stocker *et al*., 2002). Whether mammalian PTEN is also able to induce this small eye phenotype upon targeted overexpression in flies remains controversial (Huang *et al*., 1999; Song *et al*., 2007). It is likely that mammalian and *Drosophila* PTEN have distinct requirements for tissue-specific physiologic activities.

The *PTEN* gene product in the nematode *Caenorhabditis elegans*, DAF-18, is also directly linked to the negative regulation of signaling through the insulin/PI3K/Akt pathway, which controls metabolism, dauer formation during larvae development and longevity in this organism. *DAF-18* mutation is not lethal but causes secondary phenotypes, including reproductive defects and shorter lifespan. *DAF-18* mutants fail to form dauers under overgrowth or starvation conditions, or on genetic backgrounds (insulin receptor-like or PI3K-like mutants) that cause constitutive dauer formation (Ogg and Ruvkun, 1998; Gil *et al*., 1999; Mihaylova *et al*., 1999; Rouault *et al*., 1999; Masse *et al*., 2005; Solari *et al*., 2005). Also, *DAF-18* mutants are defective in the prefeeding growth arrest at the first stage of *C. elegans* larvae development, resulting in inappropriate germline growth (Fukuyama *et al*., 2006). These genetic findings

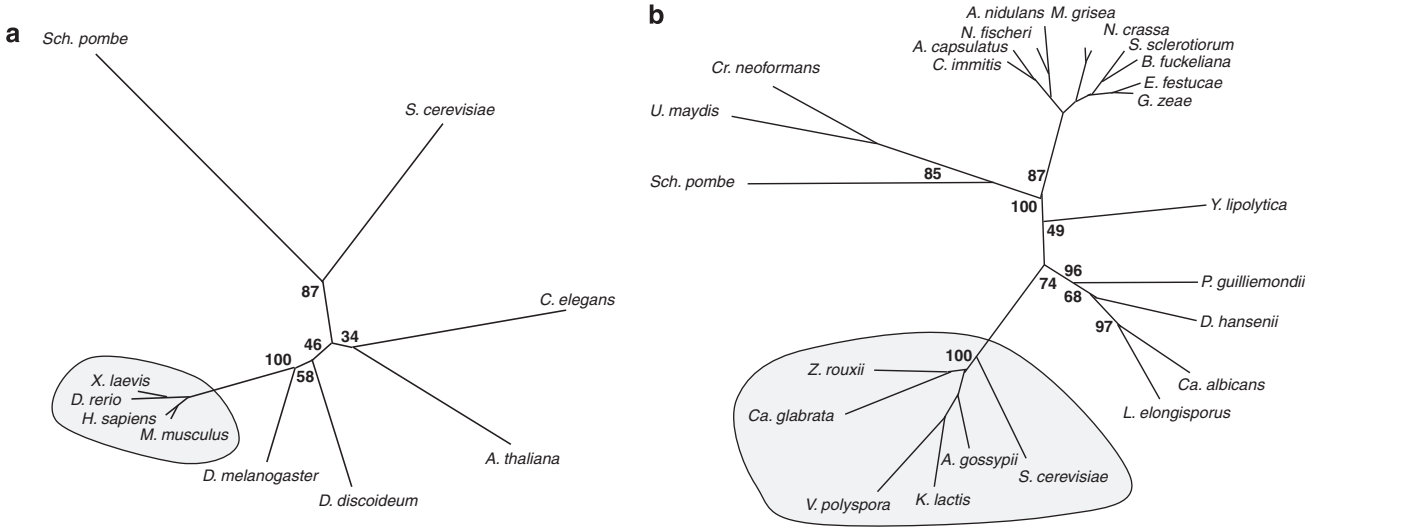

Figure 1 Phylogenetic dendrograms of PTEN orthologs in model organisms (**a**) and in representative fungal species (**b**). Full-length PTEN orthologs were aligned using ClustalW (www.ebi.ac.uk/clustalw) and neighbour-joining trees (100 bootstrap replicates) were made. The clusters of highly conserved PTEN sequences from vertebrates (**a**) and of PTEN/Tep1 sequences from the *Saccharomycetales* yeast species most related to *S. cerevisiae* that display a divergent catalytic P-loop (**b**) are indicated with gray shaded areas. Accession numbers for sequences in (**a**) are given in Table 1. Accession numbers for sequences in (**b**) are: *Schizosaccharomyces pombe* (NP_596312); *S. cerevisiae* (NP_014271); *Zygosaccharomyces rouxii* (AAR88367); *Candida glabrata* (XP_448956); *Vanderwaltozyma polyspora* (XP_001644103); *Kluyveromyces lactis* (XP_456162); *Ashbya gossypii* (NP_987007); *Lodderomyces elongisporus* (XP_001523695); *Candida albicans* (XP_710767); *Debaryomyces hansenii* (XP_457227); *Pichia guilliermondii* (XP_001487413); *Yarrowia lipolytica* (XP_501705); *Gibberella zeae* (XP_385158); *Epichloe festucae* (ABK27197); *Botryotinia fuckeliana* (XP_001554863); *Sclerotinia sclerotiorum* (XP_001585725); *Neurospora crassa* (XP_962609); *Magnaporthe grisea* (XP_362422); *Aspergillus nidulans* (XP_663816); *Neosartorya fischeri* (XP_001260658); *Ajellomyces capsulatus* (XP_001541812); *Coccidioides immitis* (XP_001245965); *Cryptococcus neoformans* (XP_567548); *Ustilago maydis* (XP_759907).

suggest that DAF-18 counteracts the action of *C. elegans* PI3K by dephosphorylating PIP3. In addition to the conserved catalytic and C2 domains, DAF-18 possesses a large C-terminal domain of unknown function, not present in other organisms. Despite phylogenetic distance, conservation of PTEN function in worms is suggested by the observation that dauer and longevity phenotypes in worms devoid of DAF-18 can be restored by expression of human PTEN (Solari *et al*., 2005). In the social amoeba *Dictyostelium discoideum*, PTEN is involved in the regulation of chemotaxis and motility to cAMP gradients. *PTEN*-mutated *Dictyostelium* cells show defects in the chemotactic response, involving speed, cell polarity and pseudopod formation, as a result of the disorganization of PIP3 local gradients in the plasma membrane of the anterior and posterior regions of the cell (Funamoto *et al*., 2002; Iijima and Devreotes, 2002; Chen *et al*., 2007; Hoeller and Kay, 2007; Wessels *et al*., 2007). Noticeably, in mammalian cells responsive to chemotactic stimuli, both positive and negative roles for PTEN in chemotaxis have been described, depending on the cell type (Fox *et al*., 2002; Lacalle *et al*., 2004; Gao *et al*., 2005; Subramanian *et al*., 2007; Wan *et al*., 2007). Furthermore, in mouse neutrophils, the SHIP1 5-phosphatase (that also has PIP3 as a substrate), but not PTEN, has been found to be the major regulator of polarization and motility (Nishio *et al*., 2007). Thus, mammalian cells may follow cell type- and/or chemoattractant-specific patterns of chemotaxis regulation, including PTEN-independent chemotactic responses. As for DAF-18, PTEN from *D. discoideum* contains an unique C-terminal region of unknown function, whereas the regulatory N-terminal PIP2-binding motif is functionally conserved (Maehama *et al*., 2001; Iijima *et al*., 2004).

The *A. thaliana* genome contains three putative *PTEN*-like genes, *AtPTEN1*, *-2* and *-3*. AtPTEN2 and AtPTEN3 are large highly related proteins, whose activity and function have not been tested yet. All PTEN from metazoans listed in Table 1 contain an identical P-loop core signature (IHCKAGKGRT), whereas AtPTEN1 contains a slightly divergent P-loop (VHCMAGKGRT) and has been shown to have phosphatase activity *in vitro* against P-Tyr and PIP3 (Gupta *et al*., 2002). AtPTEN1 is expressed specifically in pollen, and silencing of *AtPTEN1* caused abnormal pollen development in the form of collapsed, shrunken and small pollen grains. Remarkably, no plant class I PI3Ks have been identified, and there is no evidence of the existence of PIP3 in plants, suggesting that the physiologic substrates for PTEN-like enzymes in plants could be different.

In sum, multiple nonmammalian model organisms are available to study PTEN function, based on the general conservation of the signaling PI3K-dependent module. Nonetheless, technical caveats emerge if large-scale genetic analyses are planned in most of the systems described above. These problems have led researchers in

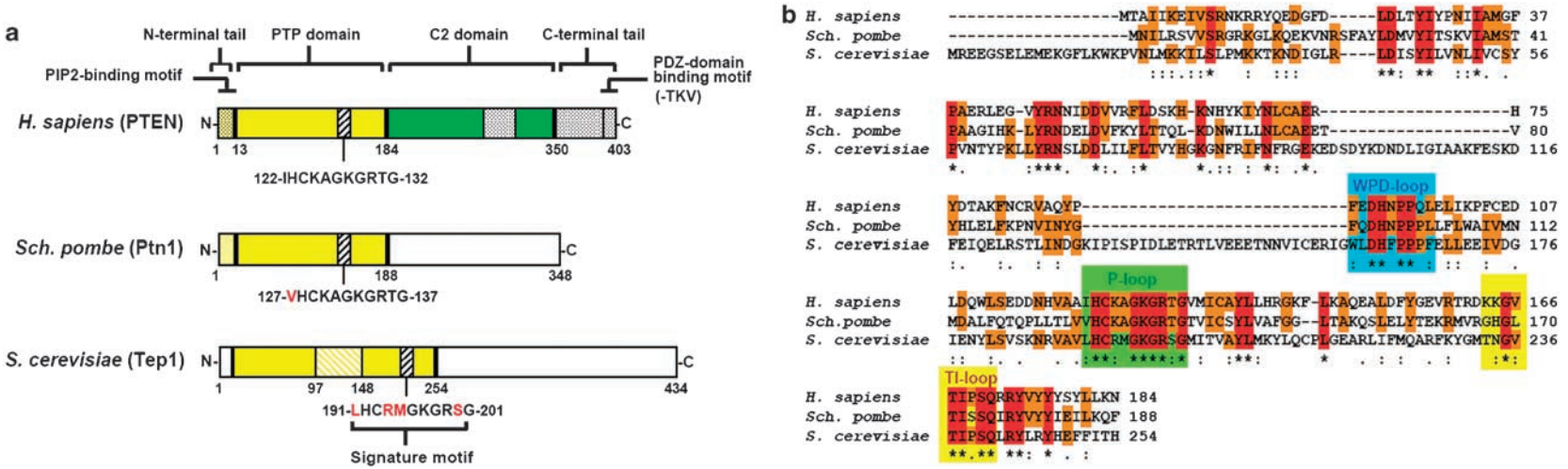

Figure 2 (**a**) Schematic depiction of functional domains on PTEN (*H. sapiens*), Ptn1 (*Sch. pombe*), and Tep1 (*S. cerevisiae*). Amino-acid numbering of domains is indicated, according to entries given in Table 1. Dotted squares in PTEN indicate unstructured regions, which are absent in the resolved crystal structure (Lee *et al*., 1999). The amino-acid sequences of the catalytic signature motifs are shown. Ptn1, but not Tep1, possesses a consensus PIP2-binding motif (L/R-X_4-L/R-X-L/R-L/R) at its N-terminus. The C2 domain and the PDZ domain-binding motif are absent from both Ptn1 and Tep1. The PTP domain of Tep1 possesses an unique region (residues 97–148) and a catalytic signature motif with divergent amino-acid sequence (residues in red). (**b**) Alignment of the amino-acid sequences of the PTP domains from PTEN, Ptn1 and Tep1. Alignment was made by ClustalW. Identical residues are highlighted, and the catalytic WPD-, P- and TI-loops are indicated. '*' means that the residues or nucleotides in that column are identical in all sequences in the alignment. ':' means that conserved substitutions have been observed. '.' means that semiconserved substitutions are observed. Amino-acid identity by BLASTP alignment (PTP domains): human/*Sch. pombe*, 37%; human/*S. cerevisiae*, 27%; *Sch. pombe*/*S. cerevisiae*, 29%).

the last decades to explore the feasibility and ease of handling of unicellular eukaryotic organisms, such as yeasts.

The PI3K/PTEN pathway in fission and budding yeasts: just a missing link?

Both the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe* have been widely exploited as single eukaryotic cell models. Most basic signaling pathways, such as those involving CDK-dependent cell cycle regulation, G protein-coupled receptors and MAPK pathways, for instance, have been thoroughly studied in yeasts. Conclusions derived from signaling studies in these model organisms have proved, in general, relevant for our understanding of their counterparts in mammalian cells. However, lipid signaling through PIP3 may be an exception to this rule. In contrast to the key role of PIP3 as a second messenger in higher eukaryotes, neither class I PI3K activity nor genes coding for putative orthologs have been found in yeasts. Nevertheless, the role of other phosphoinositides is well documented in these organisms, especially in the budding yeast, where many studies have contributed to elucidate the involvement of PIP2 in actin cytoskeleton remodeling, or monophosphorylated PtdIns(3)P and PtdIns(4)P in trafficking and secretion (Strahl and Thorner, 2007). In this context, it was surprising to find that both *S. cerevisiae* and *Sch. pombe* encode in their respective genomes a putative PTEN ortholog, respectively the *TEP1* and *ptn1* gene products (Li *et al*., 1997; Mitra *et al*., 2004) (Figure 2), as well as orthologs to classic mammalian PIP3 targets such as the AGC kinases PDK1 and PKB/Akt (Figure 3). Furthermore, mTor orthologs, now considered a key downstream effector of PI3K-dependent signaling in higher eukaryotes, were actually first uncovered in yeast (Kunz *et al*., 1993; Lorenz and Heitman, 1995).

Very little is known about the function of PTEN yeast orthologs, whose similarity with mammalian PTEN is restricted to the PTP catalytic domain (Table 1; Figure 2). In fission yeast, Mitra *et al*. (2004) reported a

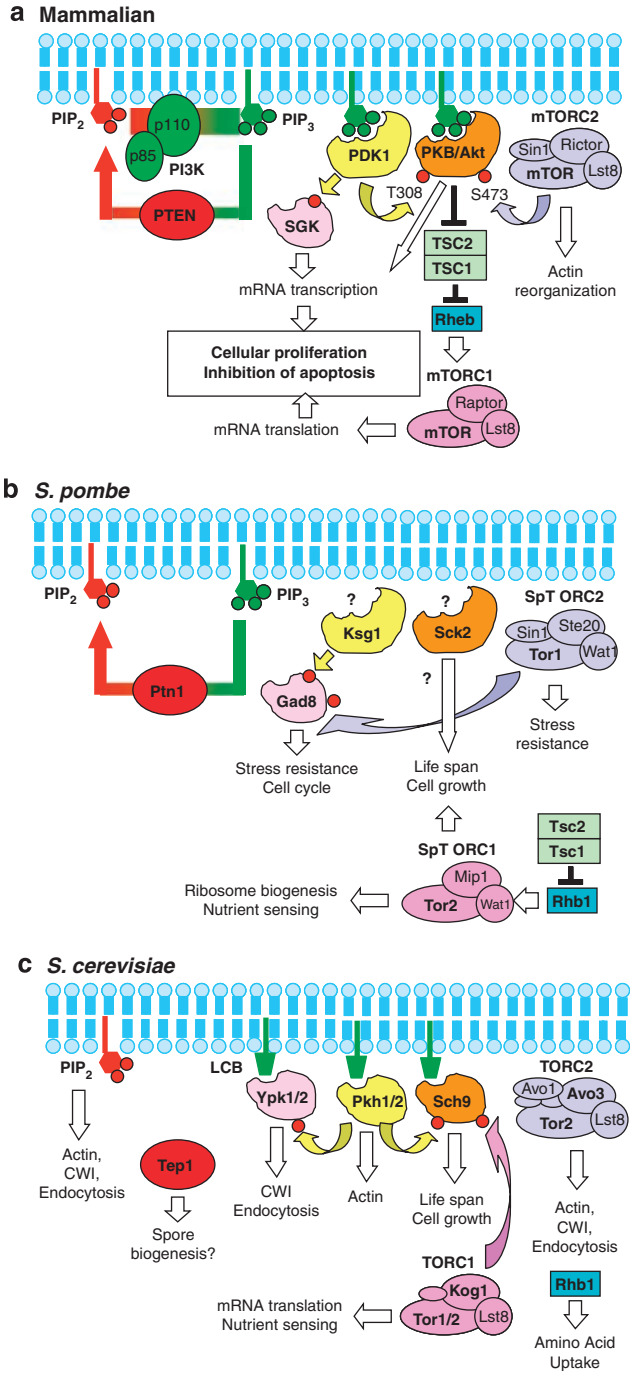

Figure 3 Conservation of the components of mammalian PIP3-dependent pathways in yeasts. (**a**) In mammalian cells, PIP3 generated by class I PI3K locally recruits the PH domain-containing protein kinases PDK1 and PKB/Akt. PDK1 targets and activates Akt and SGK kinase. Full Akt activation requires phosphorylation of its C-terminal domains by the TORC2 kinase complex. Among its multiple targets, Akt phosphorylates the TSC1–TSC2 complex, allowing the small GTPase RheB to activate the TORC1 kinase, a key regulator of protein synthesis. By this means, PIP3 generation is a key step for cellular proliferation and tumor progression. PTEN, by counteracting the effect of PI3K, constitutes the major downregulator of this pathway. (**b**) *Sch. pombe* lacks class I PI3K, but expresses putative orthologs of mammalian PTEN (Ptn1), PDK1 (Ksg1), Akt (Sck2), SGK (Gad8) and both TORC complexes. PIP3, although existing, has not been to date involved as a second messenger signaling towards any of these kinases. The Gad8 kinase is phosphorylated in conserved activation sites by both Ksg1 and SpTORC2. TORC1 function in the control of protein translation, as well as its regulation by the TSC pathway, are conserved from fission yeast to mammals, but no evidence that AGC kinases act upstream SpTORC1 has been provided so far. (**c**) In *S. cerevisiae* no PIP3 has been yet detected. PIP2, by interaction with PH domain-containing proteins, works in a complex signaling network that also involves the TORC2 complex, sphingolipid long-chain bases (LCB)-dependent PDK1 orthologs Pkh1 and Pkh2 and SGK orthologs Ypk1 and Ypk2. This network regulates actin cytoskeleton assembly in response to stress, as well as cell wall integrity (CWI) and endocytosis. Sch9, considered a putative Akt or S6K ortholog, performs multiple functions, including the regulation of protein synthesis in response to stress through TORC1. Thus, TORC1 function seems conserved in budding yeast, but not its regulation. The PTEN ortholog, Tep1, has not been related to any of these functions.

similar *in vitro* substrate specificity for Ptn1 as compared with mammalian PTEN, suggesting that it should work *in vivo* as a phosphoinositide phosphatase. Indeed, deletion of *Sch. pombe ptn1* yielded detectable PIP3 levels, an important finding that led the authors to conclude that alternative PIP3 biosynthetic pathways exist in fungi that involve the concourse of class III PI3K (Vps34) before PI 5-kinases. Such alternative PIP3 biosynthetic route has been also described in mammalian cells (Zhang *et al*., 1997). To date, both the biological meaning of PIP3 pools and the biological function of Ptn1 in *Sch. pombe* remain unknown, although several lines of evidence point towards a role in membrane dynamics and trafficking: first, cells deleted for *ptn1* have an abnormal vacuolar morphology; and second, a Ptn1-GFP fusion localized to cytoplasmic membranous compartments and septa (Mitra *et al*., 2004). In *S. cerevisiae* there is no direct evidence, either *in vivo* or *in vitro*, that the PTEN ortholog Tep1 acts as a phosphoinositide phosphatase. The facts that *TEP1* gene expression is enhanced during sporulation and that diploid *tep1*/*tep1* mutants develop spores with a defective cell wall, entail a role in spore formation. Interestingly, a mutation mimicking PTEN G129E, which implies a loss of catalytic activity on lipid substrates (Tep1-G199E) caused the same sporulation defects as a deletion of the gene, suggesting that dephosphorylation of phosphoinositides is crucial for the function of Tep1 (Heymont *et al*., 2000). Localization of Tep1-GFP is reminiscent of that of Ptn1 in cytoplasmic spots, but Tep1 does not decorate the septum area in *S. cerevisiae* (Rodríguez-Escudero *et al*., 2005). Thus, PTEN orthologs may play common as well as specialized roles in divergent fungal species. A search for PTEN orthologs in fungal species that have their sequence deposited in genomic databases reveals that *PTEN* is an ubiquitous gene in fungi, supporting an important role for its protein product. Figure 1b provides a phylogenetic tree based on the alignment of the PTEN/Tep1/Ptn1 predicted proteins from representative species of yeast, dimorphic and filamentous fungi. The essential residues in the catalytic P-loop signature motif are conserved in these organisms with respect to metazoans, as well as essential residues in the WPD- and TI-loops. However, a cluster of yeast species within the *Saccharomycetales* order, including *S. cerevisiae*, possess a distinctive catalytic P-loop ((I/V/L)HC(R/K) MGKGR) and a variable insertion in the vicinity of the WPD-loop region (Figures 1b and 2), suggesting specific substrate specificity and/or physiological substrates.

Many of the major effectors downstream PIP3 in mammals are present in yeast cells. In higher cells, the transducers of PIP3 signaling are protein kinases that recognize this lipid by a specific PH domain, namely PDK1, and its activation target, PKB/Akt. PIP3 generation by PI3K upon the proper stimulus at cellular membranes brings into proximity Akt and PDK1, triggering phosphorylation events that lead to the downstream activation of the mTor kinase complex 1 (TORC1), a key regulator of protein synthesis. For full activation, in addition to phosphorylation of the activation loop by PDK1, Akt requires a second phosphorylation in its C-terminal hydrophobic motif, which is performed by mTor itself as part of a different complex (TORC2) (Figure 3a) (Hanada *et al*., 2004; Bhaskar and Hay, 2007). The existence of *bona fide* PDK1 homologs in both budding and fission yeast has been reported (Casamayor *et al*., 1999; Niederberger and Schweingruber, 1999). The redundant Pkh1 and Pkh2 proteins from *S. cerevisiae* play important roles in the control of endocytosis, actin cytoskeleton organization and activation of protein kinase C (Pkc1) signaling (Inagaki *et al*., 1999; Sun *et al*., 2000; Friant *et al*., 2001; Liu *et al*., 2005a; Walther *et al*., 2007). Similar to their mammalian ortholog, PDK1, Pkh1/2 phosphorylate and activate a second pair of redundant kinases, Ypk1 and Ypk2, that are AGC kinases related to mammalian SGK (50–52% identity) (Casamayor *et al*., 1999; Roelants *et al*., 2002) and S6K (47–48% identity). Instead of responding to PIP3, the lipid messengers involved in the modulation of the budding yeast AGC kinases are sphingoid long-chain bases, such as phytosphingosine (Friant *et al*., 2001; Liu *et al*., 2005b). In fission yeast, however, the PDK1 ortholog Ksg1, that also phosphorylates the SGK/S6K *Sch. pombe* ortholog Gad8 (Matsuo *et al*., 2003), has a PH domain in its primary sequence, so it might respond to phosphoinositides. PH domains, especially those that target PIP2 are common in both budding and fission yeast proteins related to cytoskeletal function. Some of them have been shown to bind PIP3 as well *in vitro*, although none has shown high specificity for binding to this lipid compared with PIP2 (Mitra *et al*., 2004; Yu *et al*., 2004).

A putative Akt ortholog in the budding yeast is the Sch9 protein kinase, involved in multiple functions, including aging, nutrient sensing and stress response (Fabrizio *et al*., 2001; Pedruzzi *et al*., 2003; Kaeberlein *et al*., 2005; Roosen *et al*., 2005; Pascual-Ahuir and Proft, 2007). Sch9 has been reported to be an *in vitro* substrate for Pkh1, consistently with a conservation of a PDK1-Akt signaling module in yeast. However, such module would rely again on long-chain bases instead of phosphoinositides, as Sch9, like Pkh1/2, responds to sphingolipid levels (Liu *et al*., 2005b). In addition, Sch9 has been recently discovered as a phosphorylation target for the TORC1 complex, instead of the TORC2 complex as it would be expected for an Akt ortholog. This would functionally relate Sch9 to the S6K AGC kinase rather than to Akt. In fact, the yeast Tor1-Sch9 pathway regulates ribosome biogenesis and translation initiation in a conserved manner, as compared with mammalian TORC1-S6K (Urban *et al*., 2007). Thus, Sch9 may play multiple roles that are reminiscent of those of different AGC kinases in mammalian cells. The Akt-dependent activation of TORC1 by inhibitory phosphorylation of TSC2 (tuberous sclerosis complex), a negative regulator of the small GTPase Rheb, does not seem to be conserved in yeast. In fact, there are no discernable TSC-coding genes in the budding yeast genome. Although *Sch. pombe* possesses orthologs of

this pathway, there is no evidence to date that TORC1 and TSC orthologs may act downstream of any of the fission yeast AGC kinases, including Sgk2, a putative functional ortholog to Sch9 (Roux *et al*., 2006). In summary, as illustrated in Figure 3, the yeast AGC kinase-related modules contribute to signaling networks that balance growth and survival signals in response to nutrients and environmental conditions. Despite of the lack of significant signaling through PIP3 in the yeast cell, many components of the PIP3-dependent higher eukaryotic pathways are conserved in lower eukaryotes. Nevertheless, unlike in higher cells, they do not seem to be linked in a linear PI3K-Akt-TORC1-like pathway and, remarkably, different lipidic second messengers are involved.

S. cerevisiae as a biological sensor of PI3K oncogenicity and PTEN tumor suppressor function

In addition to the widespread use of the yeast model for the elucidation of conserved eukaryotic functions, including key molecular mechanisms related to oncogenesis, the expression in yeast of mammalian genes related to disease can be a rewarding complementary strategy for their study (Mager and Winderickx, 2005). Expression of the catalytic subunit of mammalian class I PI3K (p110α) in *S. cerevisiae* was first used to identify PIP3-specific mammalian PH domains, where no toxicity of the heterologous kinase for the yeast system was reported (Isakoff *et al*., 1998). Overexpression of human p110α from the strong galactose-inducible promoter *GAL1* was reported to be toxic in a systematic study on the expression of human cDNAs in yeast (Tugendreich *et al*., 2001). Toxicity of p110α in yeast is dramatic when the protein is targeted to membranes by means of a C-terminal fusion to a CAAX prenylation box and expressed from the *GAL1* promoter (Rodríguez-Escudero *et al*., 2005). The protein lacking the CAAX signal in the same expression system is not toxic in yeast, but growth is impaired when gain-of-function oncogenic mutants, such as p110α-H1047R, are expressed. This proves that the yeast cell is sensitive to changes in the catalytic activity of p110α, which can reflect clinically relevant pathogenic mutations in the *PIK3CA* gene. Toxicity in yeast by hyperactive p110α is concomitant to conversion of its essential PtdIns(4,5)P_2 plasma membrane pools to PtdIns(3,4,5)P_3 leading to actin and septin cytoskeleton disassembly. The p110β PI3K catalytic subunit, when expressed in yeast, generates less amount of PIP3 and is less toxic than p110α, even when fused to a -CAAX box (Andrés-Pons *et al*., 2007). Both *PIK3CA* and *PIK3CB* genes are amplified in tumors, but only the *PIK3CA* gene has been found to be targeted by gain-of-function mutations (Karakas *et al*., 2006; Vogt *et al*., 2007). It seems that p110α is a more robust enzyme in yeast than p110β, which may resemble its functional intrinsic activity in mammals (Funaki *et al*., 1999; Beeton *et al*., 2000). Coexpression of GFP-tagged mammalian Akt1 in yeast cells expressing PI3K catalytic subunits leads to efficient relocalization of GFP-Akt1 to the plasma membrane, as a consequence of the production of PIP3, and microscopic examination of this phenomenon provides a visual report of the production of PIP3 in individual cells. Remarkably, coexpression of wild-type PTEN in p110-expressing yeast cells efficiently neutralizes all PI3K-induced effects, including PIP3 accumulation and GFP-Akt1 relocalization, as well as cell toxicity. Quantification of the relocalization of GFP-Akt1 on populations expressing mutant versions of p110 and/or PTEN serves as a readout for the oncogenicity of p110 and the tumor suppressor activity of PTEN (Andrés-Pons *et al*., 2007). Activation of Akt1 by p110 in the yeast cell also triggers Akt kinase-dependent toxicity and alterations of the gene expression profile (our unpublished results). Thus, the use of transcriptional reporters could provide additional yeast-based genetic strategies to measure the pathogenicity of mutations in these proteins.

Thanks to the ease of genetic manipulation of the yeast model, the effects of p110 and PTEN can be exploited for several purposes (Figure 4). First, small molecules can be readily tested as candidate PI3K inhibitors or PTEN modulators, an approach that is easily scalable for high throughput screening. Drugs that are able to efficiently inhibit p110 *in vivo* but do not display inhibitory effects on other essential cellular functions should allow yeast cells expressing hyperactive p110α to grow, selecting for nontoxic but specific PI3K inhibitors as potential antitumoral compounds. The impermeability of yeast cell wall and the existence of efficient detoxification transporters in yeast may constitute the main caveats for the development of this application (Melese and Hieter, 2002). The classic PI3K inhibitor wortmannin, for instance, is not able to rescue growth of p110α-CAAX-expressing yeast cells, whereas LY294002 and other commercial inhibitors counteract the effects of p110α-CAAX at sublethal concentrations (our unpublished results). Also, genetic screens based on positive selection of clones that allow yeast growth in the presence of hyperactive p110, could be devised: mutational analyses by *in vitro* mutagenesis on the sequence of *PIK3CA* or *PTEN* genes (see below), either randomly or directed to residues of structural or functional interest, might help mapping essential domains that could define novel targets for the design of inhibitors. Analogously, screen of mammalian cDNA expression libraries in this yeast system could reveal genes that, such as *PTEN*, act directly on the down-regulation of p110 activity or genes that affect PTEN activity *in vivo*.

The effect of PTEN in rescuing the lack-of-growth phenotype caused by hyperactive p110 is highly specific, as other PIP3 phosphoinositide phosphatases, such as the PIP3 5-phosphatase SHIP1, did not counteract PIP3 production (our unpublished results), in agreement with the notion that yeast is specifically sensitive to PtdIns(4,5)P_2-PtdIns(3,4,5)P_3 balance. Peculiarly, the *S. cerevisiae* PTEN ortholog Tep1 did not behave like human PTEN in the yeast reconstitution system (Table 1) (Rodríguez-Escudero *et al*., 2005), in accor-

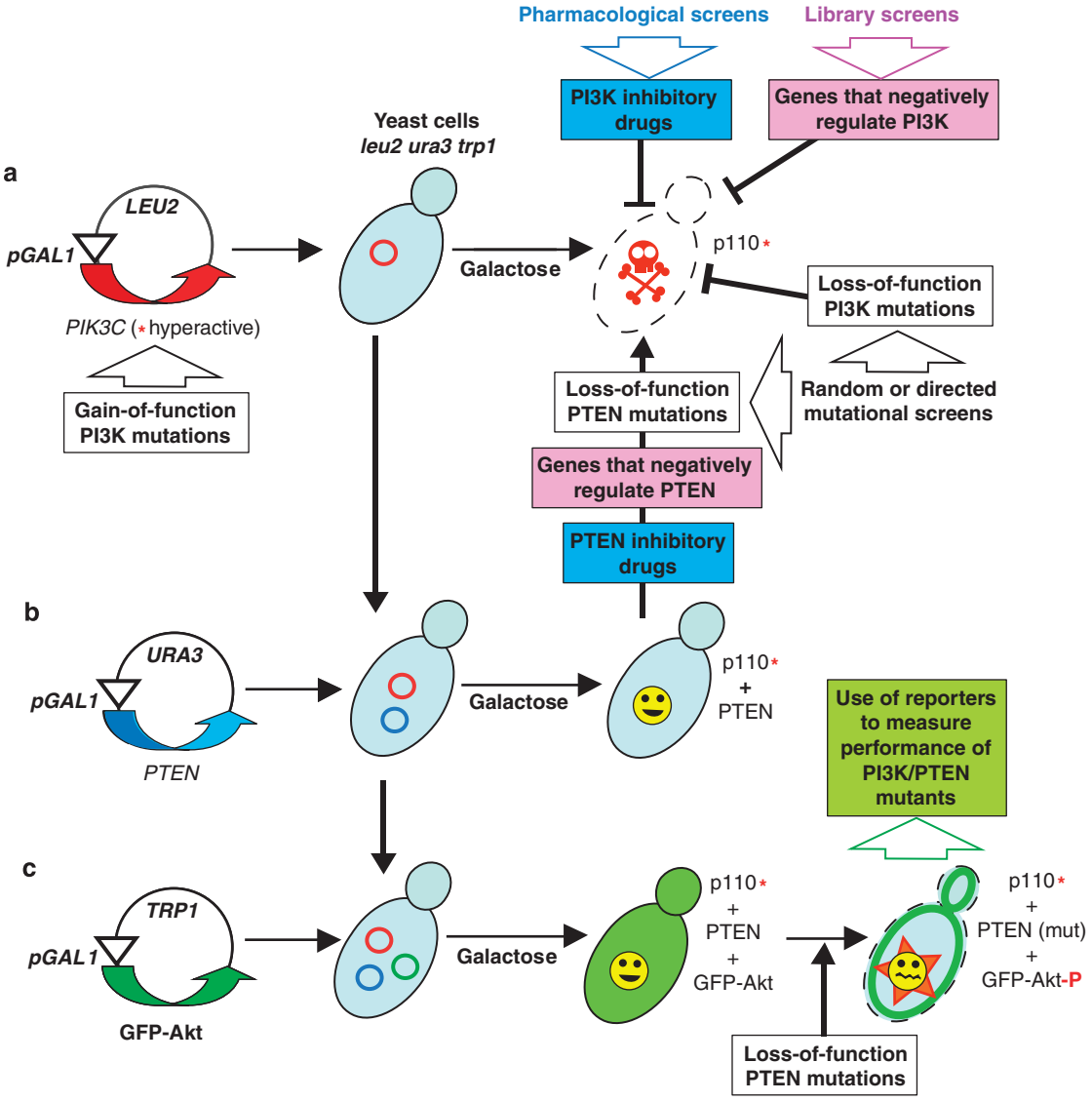

Figure 4 Potential of the yeast model to support studies on the PI3K pathway. (**a**) Expression of hyperactive mammalian p110 from the regulatable promoter *GAL1* (galactose inducible) leads to loss of viability. This can be exploited to screen for PI3K inhibitors, such as small molecules of potential pharmacological interest, or genes whose products block, sequester or impinge a negative regulation on p110 when coexpressed from genomic expression libraries. Also, random *in vitro* mutagenesis can be performed on the *PIK3C* genes in search for loss-of-function or gain-of-function mutants. The suppressor and loss-of-function genetic screenings have the technical advantage that selection of clones would be positive. (**b**) Coexpression of PTEN fully rescues yeast cells from hyperactive p110-imposed toxicity, and the ability of PTEN to counteract PI3K function is related to its tumor suppressor activity. Mutations of clinical origin or artificial mutations generated either randomly or by directly targeting PTEN functional domains can be tested for activity *in vivo*. Also, screens for PTEN inhibitors can be implemented. In these cases, screening approaches are technically restrained by the need for a negative selection. (**c**) The binding to membranes of GFP-tagged PKB/Akt produced in p110-expressing cells serves as an *in vivo* marker of PIP3 production leading to phosphorylation and activation of the Akt kinase. These effects are fully reverted by the presence of wild-type PTEN, but not by PTEN loss-of-function mutants. Thus, monitoring relocation of Akt or using reporters of *in vivo* Akt activity in this heterologous system is a feasible platform to measure the oncogenicity or tumor suppressor activity of mutations in components of the pathway.

dance with its reported undetectable catalytic activity (Heymont *et al*., 2000; Maehama *et al*., 2001). Furthermore, a saturated screen for suppressors of p110α-CAAX-induced toxicity with a yeast cDNA expression library did not yield any clone (our unpublished observations), suggesting that *S. cerevisiae* does not have a PIP3 3-phosphatase activity as efficient as that of human PTEN. Ptn1, the *Sch. pombe* PTEN ortholog, was also unable to rescue the growth in p110α-CAAX-expressing *S. cerevisiae*, whereas PTEN from vertebrates (including mouse, frog and zebrafish PTEN) and from insects (fruit fly) were effective in suppressing the p110α-CAAX-induced yeast toxicity (Table 1). We have taken advantage of this model to perform an extensive mutational analysis on the human *PTEN* gene, and we have related the pathogenicity of particular *PTEN* point

Table 2 Functional categories of PTEN amino-acid substitution mutations as defined by analysis of PTEN activity *in vitro* and in yeast

Mutation effect	*Activity in vitro*	*Activity in yeast*	*Expression in yeast*	*Potential therapy*[a]
No effect (wt)	+	+	+	
Defect in intrinsic catalysis[b] (C124S)	−	−	+	Inhibition of PI3K/Akt/mTOR signaling
Defect in intrinsic catalysis and protein stability (L345Q)	−	−	−	
Defect in protein stability	+	−	−	Inhibition of PTEN degradation Increasing of PTEN expression
Defect in catalysis in cells (S10N)	+	−	+	Increasing of PTEN catalytic activity in cells

Examples of tumor-found mutations that belong to the distinct functional categories are indicated in brackets.
[a]Potential therapy to overcome the effect of the mutation.
[b]This category may display a dominant negative effect.

mutations described in tumors or hereditary PHTS oncogenic disorders to the activity of the mutant proteins *in vivo* (Andrés-Pons *et al*., 2007). The systematic functional analysis in yeast of *PTEN* missense mutations may help in the unequivocal definition of pathogenic mutations. Similar approaches have been exploited in yeast to monitor the pathogenicity of mutations in some other tumor suppressor genes, such as *p53*, *hMLH1*, *BRCA1* and *APC* (Ishioka *et al*., 1993; Humphrey *et al*., 1997; Shimodaira *et al*., 1998; Suzuki *et al*., 1998). The fact that the PI3K/PTEN yeast reconstitution system allows the measurement of the reaction products of PI3K and PTEN *in vivo* makes such system highly sensitive and reliable. Furthermore, random mutagenesis of *PTEN* in a small-scale screen for loss-of-function mutants allowed the isolation of mutations that mostly coincided to oncogenic mutations described in patients. Working at a larger scale in both directed and random mutagenesis strategies on *PTEN* would probably contribute to a greater extent to our understanding of the structure–function relation of this tumor suppressor. Nevertheless, important aspects of PTEN regulation, such as those involving their regulatory C2-flexible loop and C-terminal tail, may not be sensed by the yeast system, as PTEN truncated forms lacking these regions behave in yeast similar to the wild-type protein. The appropriate folding and stabilization of PTEN is critical for its tumor suppressor function, as illustrated by the finding that PTEN mutations found in tumors target not only the catalytic activity of the enzyme, but also its stability. Importantly, the stability of PTEN mutants in yeast correlates with its stability in mammalian cells, reinforcing the versatility of the PI3K/PTEN yeast reconstitution system to predict the pathogenicity of PTEN mutations found in patients. The study of the functional activity of PTEN mutations *in vitro* and in mammalian cell systems together with the analysis of PTEN PIP3 catalytic activity and stability in yeast (Georgescu *et al*., 1999, 2000; Han *et al*., 2000; Kato *et al*., 2000; Andrés-Pons *et al*., 2007) allows the definition of several categories of PTEN pathogenic mutations with distinctive altered functional properties (Table 2). These mutation-specific differences may be relevant for the design of therapies aimed to decrease the enhanced PI3K/Akt signaling that takes place in tumor cells. The combination of PI3K or mTOR inhibitors with proteasome inhibitors or other anticancer drugs (currently in preclinical or clinical use) (Grunwald *et al*., 2002; Lopiccolo *et al*., 2008; Marone *et al*., 2008; Steelman *et al*., 2008), or with compounds that could increase moderatelly and/or locally PTEN expression or activity, may cooperate, in some cases, to ameliorate the hyperactivation of the PI3K/PTEN/Akt pathway. In this regard, it is remarkable that several anticancer radiotherapy and chemotherapy treatments (including anti-erbB2, anti-EGFR and anti-γ-secretase therapies) rely on PTEN expression and function for efficacy (Nagata *et al*., 2004; Berns *et al*., 2007; Frattini *et al*., 2007; Jiang *et al*., 2007; Palomero *et al*., 2007). The adaptation of versatile and reliable eukaryotic cellular models to the study of the oncogenesis-related PI3K and PTEN pathways should accelerate basic research and provide a platform for the development of diagnostic, prognostic and therapeutic strategies in cancer in the near future.

Acknowledgements

We thank T Maehama (National Institute of Infectious Diseases, Tokyo, Japan) and E Hidalgo (Universitat Pompeu Fabra, Barcelona, Spain) for providing DNA samples. Work at the authors' labs is supported by Grants SAF2006-083139 from Ministerio de Educación y Ciencia, and Grants ISCIII-RETIC RD06/0020 (to R Pulido) and CP04/00318 (to A Gil) from Instituto de Salud Carlos III, Spain-FEDER; European Research Training Network Grant MRTN-CT-2006-035830 (to R Pulido and J den Hertog); and Grants BIO2004-02019 from Ministerio de Educación y Ciencia, and S-SAL-0246-2006 from Comunidad Autónoma de Madrid, Spain (to M Molina). A Andrés-Pons and C Romá-Mateo have been the recipients of predoctoral fellowships from Ministerio de Educación y Ciencia and Ayuntamiento de Valencia, Spain.

References

Andrés-Pons A, Rodríguez-Escudero I, Gil A, Blanco A, Vega A, Molina M *et al*. (2007). *In vivo* functional analysis of the counterbalance of hyperactive phosphatidylinositol 3-kinase p110 catalytic oncoproteins by the tumor suppressor PTEN. *Cancer Res* **67**: 9731–9739.

Beeton CA, Chance EM, Foukas LC, Shepherd PR. (2000). Comparison of the kinetic properties of the lipid- and protein-kinase activities of the p110alpha and p110beta catalytic subunits of class-Ia phosphoinositide 3-kinases. *Biochem J* **350**: 353–359.

Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K *et al*. (2007). A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell* **12**: 395–402.

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

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

Chen L, Iijima M, Tang M, Landree MA, Huang YE, Xiong Y *et al*. (2007). PLA2 and PI3K/PTEN pathways act in parallel to mediate chemotaxis. *Dev Cell* **12**: 603–614.

Chow LM, Baker SJ. (2006). PTEN function in normal and neoplastic growth. *Cancer Lett* **241**: 184–196.

Croushore J, Blasiole B, Riddle RC, Thisse C, Thisse B, Canfield VA *et al*. (2005). *ptena* and *ptenb* genes play distinct roles in zebrafish embryogenesis. *Dev Dyn* **234**: 911–921.

Fabrizio P, Pozza F, Pletcher SD, Gendron CM, Longo VD. (2001). Regulation of longevity and stress resistance by Sch9 in yeast. *Science* **292**: 288–290.

Faucherre A, Taylor GS, Overvoorde J, Dixon JE, Hertog J. (2008). Zebrafish *pten* genes have overlapping and non-redundant functions in tumorigenesis and embryonic development. *Oncogene* **27**: 1079–1086.

Fox JA, Ung K, Tanlimco SG, Jirik FR. (2002). Disruption of a single Pten allele augments the chemotactic response of B lymphocytes to stromal cell-derived factor-1. *J Immunol* **169**: 49–54.

Frattini M, Saletti P, Romagnani E, Martin V, Molinari F, Ghisletta M *et al*. (2007). PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. *Br J Cancer* **97**: 1139–1145.

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

Fukuyama M, Rougvie AE, Rothman JH. (2006). C. elegans DAF-18/PTEN mediates nutrient-dependent arrest of cell cycle and growth in the germline. *Curr Biol* **16**: 773–779.

Funaki M, Katagiri H, Kanda A, Anai M, Nawano M, Ogihara T *et al*. (1999). p85/p110-type phosphatidylinositol kinase phosphorylates not only the D-3, but also the D-4 position of the inositol ring. *J Biol Chem* **274**: 22019–22024.

Funamoto S, Meili R, Lee S, Parry L, Firtel RA. (2002). Spatial and temporal regulation of 3-phosphoinositides by PI 3-kinase and PTEN mediates chemotaxis. *Cell* **109**: 611–623.

Gao P, Wange RL, Zhang N, Oppenheim JJ, Howard OM. (2005). Negative regulation of CXCR4-mediated chemotaxis by the lipid phosphatase activity of tumor suppressor PTEN. *Blood* **106**: 2619–2626.

Gao X, Neufeld TP, Pan D. (2000). Drosophila PTEN regulates cell growth and proliferation through PI3K-dependent and -independent pathways. *Dev Biol* **221**: 404–418.

Georgescu MM, Kirsch KH, Akagi T, Shishido T, Hanafusa H. (1999). The tumor-suppressor activity of PTEN is regulated by its carboxyl-terminal region. *Proc Natl Acad Sci USA* **96**: 10182–10187.

Georgescu MM, Kirsch KH, Kaloudis P, Yang H, Pavletich NP, Hanafusa H. (2000). Stabilization and productive positioning roles of the C2 domain of PTEN tumor suppressor. *Cancer Res* **60**: 7033–7038.

Gil EB, Malone Link E, Liu LX, Johnson CD, Lees JA. (1999). Regulation of the insulin-like developmental pathway of Caenorhabditis elegans by a homolog of the PTEN tumor suppressor gene. *Proc Natl Acad Sci USA* **96**: 2925–2930.

Goberdhan DC, Paricio N, Goodman EC, Mlodzik M, Wilson C. (1999). Drosophila tumor suppressor PTEN controls cell size and number by antagonizing the Chico/PI3-kinase signaling pathway. *Genes Dev* **13**: 3244–3258.

Grunwald V, DeGraffenried L, Russel D, Friedrichs W, Ray RB, Hidalgo M. (2002). Inhibitors of mTOR reverse doxorubicin resistance conferred by PTEN status in prostate cancer cells. *Cancer Res* **62**: 6141–6145.

Gupta R, Ting JT, Sokolov LN, Johnson SA, Luan S. (2002). A tumor suppressor homolog, AtPTEN1, is essential for pollen development in Arabidopsis. *Plant Cell* **14**: 2495–2507.

Han SY, Kato H, Kato S, Suzuki T, Shibata H, Ishii S *et al*. (2000). Functional evaluation of PTEN missense mutations using *in vitro* phosphoinositide phosphatase assay. *Cancer Res* **60**: 3147–3151.

Hanada M, Feng J, Hemmings BA. (2004). Structure, regulation and function of PKB/AKT-a major therapeutic target. *Biochim Biophys Acta* **1697**: 3–16.

Hariharan IK, Bilder D. (2006). Regulation of imaginal disc growth by tumor-suppressor genes in *Drosophila*. *Annu Rev Genet* **40**: 335–361.

Heymont J, Berenfeld L, Collins J, Kaganovich A, Maynes B, Moulin A *et al*. (2000). TEP1, the yeast homolog of the human tumor suppressor gene PTEN/MMAC1/TEP1, is linked to the phosphatidylinositol pathway and plays a role in the developmental process of sporulation. *Proc Natl Acad Sci USA* **97**: 12672–12677.

Hoeller O, Kay RR. (2007). Chemotaxis in the absence of PIP3 gradients. *Curr Biol* **17**: 813–817.

Huang H, Potter CJ, Tao W, Li DM, Brogiolo W, Hafen E *et al*. (1999). PTEN affects cell size, cell proliferation and apoptosis during *Drosophila* eye development. *Development* **126**: 5365–5372.

Humphrey JS, Salim A, Erdos MR, Collins FS, Brody LC, Klausner RD. (1997). Human BRCA1 inhibits growth in yeast: potential use in diagnostic testing. *Proc Natl Acad Sci USA* **94**: 5820–5825.

Iijima M, Devreotes P. (2002). Tumor suppressor PTEN mediates sensing of chemoattractant gradients. *Cell* **109**: 599–610.

Iijima M, Huang YE, Luo HR, Vazquez F, Devreotes PN. (2004). Novel mechanism of PTEN regulation by its phosphatidylinositol 4,5-bisphosphate binding motif is critical for chemotaxis. *J Biol Chem* **279**: 16606–16613.

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

Isakoff SJ, Cardozo T, Andreev J, Li Z, Ferguson KM, Abagyan R *et al*. (1998). Identification and analysis of PH domain-containing targets of phosphatidylinositol 3-kinase using a novel *in vivo* assay in yeast. *EMBO J* **17**: 5374–5387.

Ishioka C, Frebourg T, Yan YX, Vidal M, Friend SH, Schmidt S *et al*. (1993). Screening patients for heterozygous p53 mutations using a functional assay in yeast. *Nat Genet* **5**: 124–129.

Jiang Z, Pore N, Cerniglia GJ, Mick R, Georgescu MM, Bernhard EJ *et al*. (2007). Phosphatase and tensin homologue deficiency in glioblastoma confers resistance to radiation and temozolomide that is reversed by the protease inhibitor nelfinavir. *Cancer Res* **67**: 4467–4473.

Kaeberlein M, Powers III RW, Steffen KK, Westman EA, Hu D, Dang N *et al*. (2005). Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. *Science* **310**: 1193–1196.

Karakas B, Bachman KE, Park BH. (2006). Mutation of the PIK3CA oncogene in human cancers. *Br J Cancer* **94**: 455–459.

Kato H, Kato S, Kumabe T, Sonoda Y, Yoshimoto T, Han S *et al*. (2000). Functional evaluation of p53 and PTEN gene mutations in gliomas. *Clin Cancer Res* **6**: 3937–3943.

Kishimoto H, Hamada K, Saunders M, Backman S, Sasaki T, Nakano T *et al*. (2003). Physiological functions of Pten in mouse tissues. *Cell Struct Funct* **28**: 11–21.

Kunz J, Henriquez R, Schneider U, Deuter-Reinhard M, Movva NR, Hall MN. (1993). Target of rapamycin in yeast, TOR2, is an

essential phosphatidylinositol kinase homolog required for G1 progression. *Cell* **73**: 585–596.

Lacalle RA, Gómez-Moutón C, Barber DF, Jiménez-Baranda S, Mira E, Martínez AC *et al*. (2004). PTEN regulates motility but not directionality during leukocyte chemotaxis. *J Cell Sci* **117**: 6207–6215.

Lee JO, Yang H, Georgescu MM, Di Cristofano A, Maehama T, Shi Y *et al*. (1999). Crystal structure of the PTEN tumor suppressor: implications for its phosphoinositide phosphatase activity and membrane association. *Cell* **99**: 323–334.

Li L, Ernsting BR, Wishart MJ, Lohse DL, Dixon JE. (1997). A family of putative tumor suppressors is structurally and functionally conserved in humans and yeast. *J Biol Chem* **272**: 29403–29406.

Liu K, Zhang X, Lester RL, Dickson RC. (2005a). 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.

Liu K, Zhang X, Sumanasekera C, Lester RL, Dickson RC. (2005b). Signalling functions for sphingolipid long-chain bases in *Saccharomyces cerevisiae*. *Biochem Soc Trans* **33**: 1170–1173.

Lopiccolo J, Blumenthal GM, Bernstein WB, Dennis PA. (2008). Targeting the PI3K/Akt/mTOR pathway: effective combinations and clinical considerations. *Drug Resist Updat* **11**: 32–50.

Lorenz MC, Heitman J. (1995). TOR mutations confer rapamycin resistance by preventing interaction with FKBP12-rapamycin. *J Biol Chem* **270**: 27531–27537.

Maehama T, Kosaka N, Okahara F, Takeuchi K, Umeda M, Dixon JE *et al*. (2004). Suppression of a phosphatidylinositol 3-kinase signal by a specific spliced variant of *Drosophila* PTEN. *FEBS Lett* **565**: 43–47.

Maehama T, Taylor GS, Dixon JE. (2001). PTEN and myotubularin: novel phosphoinositide phosphatases. *Annu Rev Biochem* **70**: 247–279.

Mager WH, Winderickx J. (2005). Yeast as a model for medical and medicinal research. *Trends Pharmacol Sci* **26**: 265–273.

Marone R, Cmiljanovic V, Giese B, Wymann MP. (2008). Targeting phosphoinositide 3-kinase: moving towards therapy. *Biochim Biophys Acta* **1784**: 159–185.

Masse I, Molin L, Billaud M, Solari F. (2005). Lifespan and dauer regulation by tissue-specific activities of *Caenorhabditis elegans* DAF-18. *Dev Biol* **286**: 91–101.

Matsuo T, Kubo Y, Watanabe Y, 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.

Melese T, Hieter P. (2002). From genetics and genomics to drug discovery: yeast rises to the challenge. *Trends Pharmacol Sci* **23**: 544–547.

Mihaylova VT, Borland CZ, Manjarrez L, Stern MJ, Sun H. (1999). The PTEN tumor suppressor homolog in *Caenorhabditis elegans* regulates longevity and dauer formation in an insulin receptor-like signaling pathway. *Proc Natl Acad Sci USA* **96**: 7427–7432.

Mitra P, Zhang Y, Rameh LE, Ivshina MP, McCollum D, Nunnari JJ *et al*. (2004). A novel phosphatidylinositol(3,4,5)P3 pathway in fission yeast. *J Cell Biol* **166**: 205–211.

Nagata Y, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA *et al*. (2004). PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell* **6**: 117–127.

Niederberger C, Schweingruber ME. (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.

Nishio M, Watanabe K, Sasaki J, Taya C, Takasuga S, Iizuka R *et al*. (2007). Control of cell polarity and motility by the PtdIns(3,4,5)P3 phosphatase SHIP1. *Nat Cell Biol* **9**: 36–44.

Ogg S, Ruvkun G. (1998). The C. elegans PTEN homolog, DAF-18, acts in the insulin receptor-like metabolic signaling pathway. *Mol Cell* **2**: 887–893.

Palomero T, Sulis ML, Cortina M, Real PJ, Barnes K, Ciofani M *et al*. (2007). Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia. *Nat Med* **13**: 1203–1210.

Pascual-Ahuir A, Proft M. (2007). Control of stress-regulated gene expression and longevity by the Sch9 protein kinase. *Cell Cycle* **6**: 2445–2447.

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

Pinal N, Goberdhan DC, Collinson L, Fujita Y, Cox IM, Wilson C *et al*. (2006). Regulated and polarized PtdIns(3,4,5)P3 accumulation is essential for apical membrane morphogenesis in photoreceptor epithelial cells. *Curr Biol* **16**: 140–149.

Pulido R, van Huijsduijnen RH. (2008). Protein tyrosine phosphatases: dual-specificity phosphatases in health and disease. *FEBS J* **275**: 848–866.

Rodríguez-Escudero I, Roelants FM, Thorner J, Nombela C, Molina M, Cid VJ. (2005). Reconstitution of the mammalian PI3K/PTEN/Akt pathway in yeast. *Biochem J* **390**: 613–623.

Roelants FM, Torrance PD, Bezman N, 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.

Roosen J, Engelen K, Marchal K, Mathys J, Griffioen G, Cameroni E *et al*. (2005). PKA and Sch9 control a molecular switch important for the proper adaptation to nutrient availability. *Mol Microbiol* **55**: 862–880.

Rouault JP, Kuwabara PE, Sinilnikova OM, Duret L, Thierry-Mieg D, Billaud M. (1999). Regulation of dauer larva development in *Caenorhabditis elegans* by daf-18, a homologue of the tumour suppressor PTEN. *Curr Biol* **9**: 329–332.

Roux AE, Quissac A, Chartrand P, Ferbeyre G, Rokeach LA. (2006). Regulation of chronological aging in *Schizosaccharomyces pombe* by the protein kinases Pka1 and Sck2. *Aging Cell* **5**: 345–357.

Scanga SE, Ruel L, Binari RC, Snow B, Stambolic V, Bouchard D *et al*. (2000). The conserved PI3′K/PTEN/Akt signaling pathway regulates both cell size and survival in *Drosophila*. *Oncogene* **19**: 3971–3977.

Sharrard RM, Maitland NJ. (2000). Alternative splicing of the human PTEN/MMAC1/TEP1 gene. *Biochim Biophys Acta* **1494**: 282–285.

Shimodaira H, Filosi N, Shibata H, Suzuki T, Radice P, Kanamaru R *et al*. (1998). Functional analysis of human MLH1 mutations in *Saccharomyces cerevisiae*. *Nat Genet* **19**: 384–389.

Smith A, Alrubaie S, Coehlo C, Leevers SJ, Ashworth A. (1999). Alternative splicing of the *Drosophila* PTEN gene. *Biochim Biophys Acta* **1447**: 313–317.

Solari F, Bourbon-Piffaut A, Masse I, Payrastre B, Chan AM, Billaud M. (2005). The human tumour suppressor PTEN regulates longevity and dauer formation in *Caenorhabditis elegans*. *Oncogene* **24**: 20–27.

Song Z, Saghafi N, Gokhale V, Brabant M, Meuillet EJ. (2007). Regulation of the activity of the tumor suppressor PTEN by thioredoxin in *Drosophila melanogaster*. *Exp Cell Res* **313**: 1161–1171.

Steelman LS, Navolanic PM, Sokolosky ML, Taylor JR, Lehmann BD, Chappell WH *et al*. (2008). Suppression of PTEN function increases breast cancer chemotherapeutic drug resistance while conferring sensitivity to mTOR inhibitors. *Oncogene* **27**: 4086–4095.

Stocker H, Andjelkovic M, Oldham S, Laffargue M, Wymann MP, Hemmings BA *et al*. (2002). Living with lethal PIP3 levels: viability of flies lacking PTEN restored by a PH domain mutation in Akt/PKB. *Science* **295**: 2088–2091.

Strahl T, Thorner J. (2007). Synthesis and function of membrane phosphoinositides in budding yeast, *Saccharomyces cerevisiae*. *Biochim Biophys Acta* **1771**: 353–404.

Subramanian KK, Jia Y, Zhu D, Simms BT, Jo H, Hattori H *et al*. (2007). Tumor suppressor PTEN is a physiologic suppressor of chemoattractant-mediated neutrophil functions. *Blood* **109**: 4028–4037.

Sun Y, Taniguchi R, Tanoue D, Yamaji T, Takematsu H, Mori K *et al*. (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.

Suzuki A, Nakano T, Mak TW, Sasaki T. (2008). Portrait of PTEN: messages from mutant mice. *Cancer Sci* **99**: 209–213.

Suzuki T, Ishioka C, Kato S, Mitachi Y, Shimodaira H, Sakayori M *et al*. (1998). Detection of APC mutations by a yeast-based protein truncation test (YPTT). *Genes Chromosomes Cancer* **21**: 290–297.

Tamguney T, Stokoe D. (2007). New insights into PTEN. *J Cell Sci* **120**: 4071–4079.

Tugendreich S, Perkins E, Couto J, Barthmaier P, Sun D, Tang S *et al*. (2001). A streamlined process to phenotypically profile heterologous cDNAs in parallel using yeast cell-based assays. *Genome Res* **11**: 1899–1912.

Ueno S, Kono R, Iwao Y. (2006). PTEN is required for the normal progression of gastrulation by repressing cell proliferation after MBT in Xenopus embryos. *Dev Biol* **297**: 274–283.

Urban J, Soulard A, Huber A, Lippman S, Mukhopadhyay D, Deloche O *et al*. (2007). Sch9 is a major target of TORC1 in *Saccharomyces cerevisiae*. *Mol Cell* **26**: 663–674.

Valiente M, Andrés-Pons A, Gomar B, Torres J, Gil A, Tapparel C *et al*. (2005). Binding of PTEN to specific PDZ domains contributes to PTEN protein stability and phosphorylation by microtubule-associated serine/threonine kinases. *J Biol Chem* **280**: 28936–28943.

Vogt PK, Kang S, Elsliger MA, Gymnopoulos M. (2007). Cancer-specific mutations in phosphatidylinositol 3-kinase. *Trends Biochem Sci* **32**: 342–349.

von Stein W, Ramrath A, Grimm A, Muller-Borg M, Wodarz A. (2005). Direct association of Bazooka/PAR-3 with the lipid phosphatase PTEN reveals a link between the PAR/aPKC complex and phosphoinositide signaling. *Development* **132**: 1675–1686.

Walther TC, Aguilar PS, Frohlich F, Chu F, Moreira K, Burlingame AL *et al*. (2007). Pkh-kinases control eisosome assembly and organization. *EMBO J* **26**: 4946–4955.

Wan W, Zou H, Sun R, Liu Y, Wang J, Ma D *et al*. (2007). Investigate the role of PTEN in chemotaxis of human breast cancer cells. *Cell Signal* **19**: 2227–2236.

Wessels D, Lusche DF, Kuhl S, Heid P, Soll DR. (2007). PTEN plays a role in the suppression of lateral pseudopod formation during *Dictyostelium* motility and chemotaxis. *J Cell Sci* **120**: 2517–2531.

Wu H, Feng W, Chen J, Chan LN, Huang S, Zhang M. (2007). PDZ domains of Par-3 as potential phosphoinositide signaling integrators. *Mol Cell* **28**: 86–898.

Wu X, Hepner K, Castelino-Prabhu S, Do D, Kaye MB, Yuan XJ *et al*. (2000a). Evidence for regulation of the PTEN tumor suppressor by a membrane-localized multi-PDZ domain containing scaffold protein MAGI-2. *Proc Natl Acad Sci USA* **97**: 4233–4238.

Wu Y, Dowbenko D, Spencer S, Laura R, Lee J, Gu Q *et al*. (2000b). Interaction of the tumor suppressor PTEN/MMAC with a PDZ domain of MAGI3, a novel membrane-associated guanylate kinase. *J Biol Chem* **275**: 21477–21485.

Yu JW, Mendrola JM, Audhya A, Singh S, Keleti D, DeWald DB *et al*. (2004). Genome-wide analysis of membrane targeting by *S.cerevisiae* pleckstrin homology domains. *Mol Cell* **13**: 677–688.

Zhang X, Loijens JC, Boronenkov IV, Parker GJ, Norris FA, Chen J *et al*. (1997). Phosphatidylinositol-4-phosphate 5-kinase isozymes catalyze the synthesis of 3-phosphate-containing phosphatidylinositol signaling molecules. *J Biol Chem* **272**: 17756–17761.