mTOR is a protein kinase (although it is closely related to lipid kinases) and functions by phosphorylating specific substrate proteins. This specificity appears to be determined by the partner proteins with which mTOR interacts. It forms two distinct complexes, mTORC1 and mTORC2 (Figure 1), which possess different substrate specificities and are regulated in distinct ways.

mTORC1 contains the protein Raptor, and mTORC2 contains Rictor. Each complex also includes other proteins (Wullschleger et al., 2006). Signaling through mTORC1 is inhibited by short-term (minutes) treatment of cells with rapamycin and related compounds, although it is now clear that not all the functions of mTORC1 are inhibited by such drugs. In the short term, mTORC2 is insensitive to rapamycin. mTORC1 positively regulates a number of processes including protein synthesis and ribosome biogenesis, both of which play key roles in cell growth. Indeed, mTORC1 signaling drives cell growth in disease states such as cardiac hypertrophy and benign tumors called hamartomas which arise in 'tuberous sclerosis complex' (TSC). TSC is caused by hyperactivation of mTORC1 due to the loss of the inhibitory constraints provided by proteins TSC1 and TSC2. mTORC1 also regulates the translation of specific mRNAs and this contributes to its ability to promote cell cycle progression. This, along with effects on cell death (apoptosis), probably contributes to the key role that mTORC1 plays in certain cancers (Guertin and Sabatini, 2007), e.g. where phosphatidylinositol 3-kinase (PI3K) signaling becomes constitutively activated due to loss of the tumor suppressor, PTEN, a lipid phosphatase (Figure 1). Indeed, mutations in PTEN (or other events that activate PI3K signaling) occur in many cancers—but not all: this feature is important for the conclusions of this report. mTORC1 phosphorylates several proteins linked to protein synthesis including the 70 kDa protein kinases that phosphorylate ribosomal protein S6, a component of the small ribosomal subunit (hence they are termed S6ks; Figure 1). Although the role of S6 phosphorylation remains enigmatic, S6K signaling provides a 'feedback loop' which inhibits the activation of PI3K by certain receptors.

Protein kinase B (PKB; also termed Akt) is a well-studied target downstream of mTORC2 (Figure 1), which phosphorylates the corresponding site in PKB equivalent to that phosphorylated by mTORC1 in S6ks. Several substrates are known for PKB and it is strongly implicated in suppressing apoptosis. By inhibiting PI3K activation, mTORC1/S6K signaling impairs the activation of PKB by agents that stimulate PI3K. This feedback inhibition is evaded in cells with dysregulated PI3K signaling, e.g. PTEN null cells. By inhibiting mTORC1/S6K activation, rapamycin can actually stimulate PKB signaling; this effect may contribute to the limited success achieved by rapamycin (and its analogs) as anti-cancer drugs. Recent data indicate that mTOR also activates a close relative of PKB, SGK1 (serum/glucocorticoid-regulated kinase 1) (Figure 1), although it was controversial whether mTORC1 or mTORC2 is responsible for this (Garcia-Martinez and Alessi, 2008; Hong et al., 2008).

In their recent Cell paper, Peterson et al. (2009) describe the identification of a novel binding partner for mTOR, a protein they term DEPTOR to reflect the presence in its sequence of two DEP (dishevelled, egl-10, pleckstrin) domains. The functions of DEP domains are uncertain. DEPTOR also contains a PDZ domain, a motif that is often involved in protein–protein interactions; indeed, DEPTOR binds to mTOR via its PDZ domain. Importantly, DEPTOR binds to either mTORC1 or mTORC2, as revealed by its co-immunoprecipitation with raptor or rictor.

Since it associates with mTORC1 and mTORC2, DEPTOR seemed unlikely to mediate their differing effects: what then is the role of DEPTOR? Its precise function is still unclear, but Peterson et al. nicely show that knocking down DEPTOR actually activates signaling through mTORC1 and mTORC2. This is demonstrated both by the observation that phosphorylation of S6K1 and PKB within cells is elevated when DEPTOR levels are decreased (by RNA-based interference) and by the increased in vitro activity against these substrates of mTOR complexes from cells where DEPTOR levels have been decreased. DEPTOR-depleted cells show increased size, which is reversed by treatment with rapamycin, and reduced susceptibility to apoptosis in response to serum starvation. These findings are consistent with enhanced signaling through mTORC1 and mTORC2, respectively.

Given the role of mTORC2 in activating PKB, which is, in turn, implicated in suppressing apoptosis, it was therefore unexpected...
that depleting DEPTOR did not protect PKB from dephosphorylation following serum starvation. This implies that the protective effect of DEPTOR depletion is not mediated by PKB, but by another target of mTORC1 or mTORC2. SGK1 is one candidate based on its reported pro-survival effects (Tessier and Woodgett, 2006) and its ability to be phosphorylated by mTOR complexes. Peterson et al. go on to show that DEPTOR depletion does protect SGK1 against inactivation due to serum-starvation (as assessed from the phosphorylation state of its substrate NDRG1) and that depleting cells of SGK1 resensitized DEPTOR-depleted cells to induction of apoptosis when starved of serum. Thus, the data are consistent with (i) SGK1 protecting cells against apoptosis and (ii) SGK1 being negatively controlled by DEPTOR, presumably through DEPTOR’s inhibitory effect on mTOR signaling. Treatment of cells with Torin 1, an ATP-competitive inhibitor of mTORC1 and mTORC2, restored the induction of apoptosis, and suppressed NDRG1 phosphorylation, in DEPTOR-depleted cells. Interestingly, rapamycin and depletion of raptor did not have this effect, pointing to mTORC2, not mTORC1, as the mTOR complex responsible for regulating SGK1. As summarized in Figure 1, these findings indicate that mTORC2 suppresses apoptosis via SGK1 and that DEPTOR impairs this by inhibiting mTORC2. A key question is: how does SGK1 suppress apoptosis? That is, what are the relevant SGK1 substrates? Possible candidates include NDRG1 and FoxO3a (Brunet et al., 2001).

Further twists to this complex regulatory network are provided by the observations that mTOR signaling (through mTORC1 and mTORC2) negatively regulates DEPTOR protein expression, and that mTOR promotes the phosphorylation of DEPTOR on many residues. Intriguingly, the phosphorylation of DEPTOR impairs its binding to mTOR and impairs DEPTOR’s ability to inhibit mTORC2 signaling.

One would anticipate that artificially overexpressing DEPTOR would inhibit mTORC1 signaling and indeed it does. However, in contrast to what is expected from the DEPTOR depletion experiments, higher levels of DEPTOR did not inhibit, but rather activated, mTORC2 signaling (e.g. increased PKB phosphorylation). Why? Inhibiting mTORC1 signaling (e.g. using rapamycin) alleviates the feedback inhibition of PI3K signaling by S6Ks, and the authors argue that DEPTOR exerts a similar effect. In other words, higher levels of DEPTOR inhibit mTORC1 and S6Ks, thereby, ‘reactivating’ PI3K/mTORC2/PKB signaling. Similar effects are seen at relatively low levels of Torin 1 (whereas higher levels block both mTORC1 and mTORC2 signaling). Thus, impairment of mTOR signaling (by DEPTOR or low levels of Torin 1) causes ‘asymmetrical’ effects on mTOR signaling, such that the mTORC1 arm is inhibited, whereas mTORC2 signaling is stimulated (Figure 1). It is not clear why DEPTOR exerts this ‘selective’ inhibitory effect on mTORC1 signaling relative to mTORC2.

As we have seen, both mTORC1 and mTORC2/PKB (and likely SGK1) signaling are implicated in the network of events that drives tumorigenesis and cancer cell survival (Lang et al., 2006; Tessier and Woodgett, 2006; Duronio, 2008). Dysregulation of PI3K/PKB signaling (e.g. due to loss of PTEN) can drive the activation of mTORC2 and (through PKB-mediated inhibition of TSC1/2) the activation of mTORC1. It is thus highly relevant to ask whether DEPTOR expression is altered in cancer cells. A survey revealed that DEPTOR levels are low in various types of cancer (perhaps because mTORC1/2 signaling is enhanced, thus repressing expression of DEPTOR). However, many multiple myelomas (MMs) provide a striking exception to this overall picture, as DEPTOR protein expression is actually increased, often very substantially, in many MMs. As expected from the experiments described above, depleting MM cells of DEPTOR activated mTORC1 and inhibited PKB phosphorylation (i.e. mTORC2 signaling). In addition, DEPTOR depletion inhibited the proliferation of MM cells and led to increased apoptosis in them.
The enhanced expression of DEPTOR in MM cells may provide an alternative mechanism for promoting PI3K/mTORC2 signaling, playing a similar role to those of the loss of PTEN or activation of PI3K seen in many other tumors. But why should DEPTOR assume this role in MMs rather than more generally in other tumors? MMs are derived from B-lymphocytes and generally synthesize large quantities of immunoglobulin proteins, which may elicit endoplasmic reticulum (ER) stress. This can, in turn, lead to cell death. The authors speculate that, by inhibiting mTORC1 and thus negatively affecting the protein synthesis machinery, the high levels of DEPTOR in MMs may protect the cells against ER stress while also suppressing apoptosis through a mechanism involving mTORC2 and its downstream targets.

These are important findings: first, they uncover new complexities in the network of components that modulate mTOR signaling; secondly, they help explain why PTEN or PI3K mutations are rare in MM; and, thirdly, they prompt the idea that inhibiting the DEPTOR/mTOR interaction may provide a new avenue for treating MM.

References