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mTORC2 Caught in a SINful Akt

The target of rapamycin (TOR), a central controller of cell growth, is found in two distinct, highly conserved multiprotein complexes. Three recent papers in *Cell* (Jacinto et al., 2006), *Developmental Cell* (Shiota et al., 2006; this issue), and *Current Biology* (Frias et al., 2006) shed light on mTOR complex 2 (mTORC2) composition and in vivo function. An important new finding is that mTORC2 determines Akt/PKB substrate specificity rather than absolute activity.

Cell growth, along with cell division and cell death, is one of the most fundamental aspects of cell behavior. Cell growth is controlled by the two structurally and functionally distinct TOR complexes TORC1 and TORC2 (Wullschleger et al., 2006). These protein kinase complexes, first discovered in yeast, are highly conserved in eukaryotes. The mammalian TORCs (mTORCs) activate cell growth in response to nutrients, growth factors, and cellular energy. Both mTORC1 and mTORC2 contain the ser/thr kinase mTOR and the small Gβ-like protein mLST8. In addition, mTORC1 specifically contains raptor, while mTORC2 specifically contains rictor and mSIN1 (also known as SIN1, hSIN1, or MIP1). mSIN1, an ortholog of the TORC2 member AVO1 in yeast and Rip3 in *Dictyostelium*, is a newly described component of mTORC2 (Frias et al., 2006; Jacinto et al., 2006). mTORC1, the target of the immunosuppressive and anticancer drug rapamycin, controls protein synthesis via its two well-characterized effectors S6 kinase (S6K) and the translational repressor 4E-BP1. mTORC2, on the other hand, has no known specific inhibitor and was discovered only recently (Jacinto et al., 2004; Sarbassov et al., 2004). RNAi-mediated knockdown of rictor in cultured cells has shown that mTORC2 controls actin cytoskeleton organization (Jacinto et al., 2004; Sarbassov et al., 2004) and also directly phosphorylates and activates Akt/PKB (Sarbassov et al., 2005).

Akt/PKB, a particularly famous member of the AGC protein kinase family, controls metabolism, proliferation, cell survival, and other readouts. Full activation of Akt/PKB involves its recruitment to the plasma membrane, where it is phosphorylated on Ser473 in the hydrophobic motif by mTORC2, and on Thr308 in the activation loop by PDK1. There is longstanding disagreement on whether phosphorylation of Ser473 is a prerequisite for phosphorylation of Thr308 or Thr308 phosphorylation is independent of Ser473.

Jacinto, Su, and coworkers (Jacinto et al., 2006) and Magnuson and coworkers (Shiota et al., 2006) knocked out *mSIN1* and *rictor*, respectively, to further elucidate mTORC2 function and in particular the role of mTORC2 in the regulation of Akt/PKB. Shiota et al. (2006) first replaced part of the wild-type *rictor* gene with a *lacZ* cassette, which enabled them to visualize rictor expression in whole animals. They found high and ubiquitous expression of LacZ (rictor) in mouse embryos. LacZ was also widespread in adult mice, with strongest expression in testis and neurons. They then observed that *rictor*$^{-/-}$ embryos at day E9.5 were smaller than wild-type embryos and died by day E11.5. Although no major structural defect in any organ system was found, cellular degeneration, including nuclear swelling and formation of vacuoles, was observed. The embryos also showed placental defects; however, expression of rictor in the placenta did not restore viability, indicating that placental defects alone were not the cause of the embryonic lethality. Knockout of *mTOR*, as reported previously, results in significantly earlier embryonic lethality (Gangloff et al., 2004; Murakami et al., 2004), suggesting that mTORC1 also has an essential role early in development. An mTORC1-specific knockout has yet to be reported.

Jacinto et al. (2006) and Frias et al. (2006), in agreement with earlier findings in yeast (Wullschleger et al., 2006), demonstrated that mSIN1 is a member of mTORC2 in mammals. Frias et al. (2006) also showed that three of five known mSIN1 isoforms can assemble into mTORC2. Jacinto et al. (2006) then disrupted the *mSIN1* gene in mice. Knockout of *mSIN1*, like knockout of *rictor*, resulted in embryonic lethality; however, further details on the embryonic lethality of the *mSIN1* knockout remain to be reported. Thus, like their counterparts in yeast, both *rictor* and *mSIN1* are essential.

To further examine mTORC2 function, Jacinto et al. (2006) and Shiota et al. (2006) isolated MEFs from *rictor* or *mSIN1* null embryos. As expected, both found that knockout of mTORC2 inhibited phosphorylation of Ser473 in Akt/PKB, suggesting that mTORC2 is the Ser473 kinase. Although the two studies are in remarkable agreement, there are some minor differences. The most noteworthy is that Shiota et al. (2006) observed residual Akt/PKB Ser473 phosphorylation in *rictor* null MEFs, whereas Jacinto et al. (2006) found that Ser473 phosphorylation was completely abolished in *mSIN1* null MEFs. This difference could be due to different growth conditions, different genetic backgrounds, or the fact that different mTORC2 subunits were knocked out. However, residual Ser473 phosphorylation in the absence of intact mTORC2 suggests that another kinase(s), such as DNA-PK (Feng et al., 2004), is able to phosphorylate Ser473.

Knockout of mTORC2 resulted in loss of Ser473 phosphorylation, but no difference in the phosphorylation of Akt/PKB Thr308. Importantly, this suggests that Thr308 phosphorylation is independent of Ser473 phosphorylation. But, is Akt/PKB active when phosphorylated only on Thr308? Both groups examined several well-known Akt/PKB-dependent phosphorylation targets, including GSK3, TSC2, mTOR, and S6K, and found that mTORC2 knockout had no effect. The only targets affected were the Forkhead transcription factors FoxO1 and FoxO3, whose phosphorylation was strongly reduced in *mSIN1* null MEFs. This finding is in agreement with a previous report that FoxO4 phosphorylation is reduced upon knockdown of rictor (Sarbassov et al., 2005). These

observations have several notable implications. First, the fact that most but not all Akt/PKB targets are still phosphorylated in mTORC2 knockout MEFs suggests that Ser473 phosphorylation determines Akt/PKB specificity rather than absolute activity. Second, since reduction in FoxO phosphorylation is the only observed Akt/PKB signaling defect, does this defect alone account for the embryonic lethality? Akt/PKB negatively regulates FoxO. Reduced FoxO phosphorylation should therefore lead to FoxO hyperactivation. FoxO transcription factors control various target genes in a tissue-dependent manner. It would be of interest to determine which FoxO target genes are affected in the mTORC2 knockout MEFs, and to test whether disruption of FoxO rescues the mTORC2-disrupted mice. Obviously, it would also be of interest to look for other abnormalities in the mTORC2-disrupted mice that might account for the embryonic lethality. Third, the finding that Akt/PKB Ser473 phosphorylation is not necessary for phosphorylation of TSC2, mTOR, and S6K solves a puzzle. mTORC2 was assumed to regulate Akt/PKB activity toward all its substrates, including TSC2, which negatively regulates mTORC1. Thus, mTORC2 should activate mTORC1. However, contrary to this assumption, mTORC2 knockdown was previously shown not to affect the mTORC1 target S6K (Jacinto et al., 2004; Sarbassov et al., 2004). We now know that Ser473 phosphorylation is not required for Akt/PKB to signal to mTORC1. Thus, mTORC2 is not upstream of mTORC1.

mTORC2, like yeast and *Dictyostelium* TORC2, has been shown to regulate actin cytoskeleton organization. However, Shiota et al. (2006) failed to observe an actin defect in the *rictor* knockout MEFs. Shiota et al. (2006) also point out that the *rictor* knockout mice seemed to develop normally until day E9.5, including gastrulation, neurulation, and formation of the cardiovascular system—all of which require an intact actin cytoskeleton. This could indicate that mTORC2 is not necessary for regulation of actin cytoskeleton organization in vivo. Still, actin defects following TORC2 disruption have been detected in all organisms examined. It is possible that mTORC2 becomes active in actin organization only late in development, or that this mTORC2 function is required only under specific conditions.

Although Jacinto et al. (2006) focus on the role of mTORC2 in the regulation of Akt/PKB and Shiota et al. (2006) focus on the role of mTORC2 in embryogenesis, the two papers complement and support each other. Together with the study from Frias et al. (2006), they provide a major advance in our understanding of the regulation of cell growth by mTORC2.

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Found in Translation: A New Player in EMT

Epithelial mesenchymal transition (EMT) is a complex process that involves changes in gene expression, cytoskeleton organization, cell adhesion, and extracellular matrix composition. Screening for genes mediating EMT and cancer metastasis, Waerner, Alacakaptan, and colleagues identified ILEI, a cytokine-like protein that plays an essential role in EMT, tumor growth, and late steps of metastasis.

Epithelial and mesenchymal cells exhibit distinct morphological and functional characteristics (reviewed in Thiery and Sleeman, 2006). In addition to a specific gene expression pattern, epithelial cells display apical-basal polarity manifested in the specific distribution of cell-surface molecules, organization of cell-cell junctions, polarized organization of the cytoskeleton and formation of a basal lamina. In contrast to epithelial cells, mesenchymal cells do not form an organized cell layer; they do not polarize and can be highly motile. Epithelial sheets can undergo a transition into a mesenchyme in a process termed epithelial mesenchymal transition (EMT). EMT is characterized by repression of E-cadherin, gain of vimentin expression, and an increase in cell motility. The transition from epithelium to mesenchyme is important for diverse processes involved in tissue formation and organogenesis during embryonic development (Shook and Keller, 2003).