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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Developmental Cell *11*, October, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.devcel.2006.09.010

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).

Table 1. ILEI Effect on EMT and Cancer Formation

Cell Line	Ras Activity	ILEI Expression	EMT[a]	Tumorigenesis	Metastasis
EpH4, Spontaneously immortalized mammary epithelial cells	-/+	-/+	–	–	–
	+	*over-expressed*	+	+	+
	inhibited by L739749	*over-expressed*	–	–	–
EpRasXT, Ras-transformed EpH4, treated with TGFβ to induce EMT	++	+	+	+	+
	++	*inhibited by siRNA*	–	–	–

Experimental manipulations are highlighted in italics.
[a] As assayed by decreased cadherin and increased vimentin expression.

As opposed to the constructive role EMT plays during normal development, deregulation of components that control this process can promote tumorigenesis. A prominent example for such a molecule is Twist, a transcription factor that plays a critical role in tissue reorganization during development (Castanon and Baylies, 2002) and has been implicated in promoting cancer cell metastasis (Yang et al., 2004).

In the September issue of *Cancer Cell*, Waerner, Alacakaptan, et al. report of a screen for molecules involved in EMT and cancer metastasis (Waerner et al., 2006). This effort led to the identification of a novel interleukin-related protein (ILEI) whose function is necessary and sufficient for EMT, tumorigenesis, and metastasis of normal epithelial cells (Table 1). The identification of ILEI relied on an expression-profiling assay that employs polysome bound mRNAs. Unlike other expression profiling techniques, this method allows the identification of RNAs whose translation rather than their transcriptional profile is correlated with the relevant process—EMT in this case (Jechlinger et al., 2003; Pradet-Balade et al., 2001).

The level of translated and secreted ILEI was found to be elevated in murine mammary epithelial cells that express oncogenic Ras protein and undergo EMT in response to TGFβ (termed "EpRasXT cells"). To determine whether ILEI alone is capable of inducing EMT, immortalized epithelial cell (termed "EpH4") derivates were engineered to overexpress the cytokine or were exposed to exogenous ILEI (Table 1). Remarkably, treated cells exhibited EMT as judged by loss of epithelial morphology and gain of mesenchymal gene expression profile (e.g., reduction in E-cadherin and elevation in vimentin expression). The opposite effect could be observed when cells that performed EMT in response to TGFβ were treated with siRNA directed against ILEI as the cells reacquired characteristic epithelial morphology and gene expression (Table 1). To determine the signaling events associated with ILEI-induced EMT, the authors focused on the Ras pathway, which had been previously implicated in EMT induced by TGFβ (Janda et al., 2002). Indeed, EpH4 cell lines overexpressing ILEI exhibited a dramatic increase in the level of Erk/MAPK activation. Conversely, when the cells expressing ILEI were treated with a Ras farnesylation inhibitor, EMT was reverted lending further support to the notion that ILEI-induced EMT requires activation of the Ras-signaling pathway (Table 1). The biochemical cascade in which ILEI participates appears to play a central role in EMT as depletion of ILEI abrogates EMT induced by other means, such as TGFβ expression. Importantly, the biological function of ILEI was found to be of relevance for tumor formation and metastasis. Specifically, whereas EpH4 cells are not tumorigenic, ILEI overexpression enabled the cells to form tumors when injected into nude mice. Interestingly, cells derived from these tumors showed the characteristic low E-cadherin and high vimentin expression profile indicating that they underwent EMT in vivo. Introduction of ILEI overexpressing cells directly into the blood by tail vein injection allowed late stages of metastasis (e.g., extravasation, invasion, and proliferation at distant sites) to be examined. Significantly, it was demonstrated that most of the mice injected with an originally nonmetastatic EpH4-derived cell line that was engineered to overexpress ILEI developed metastasis. In this context, the finding that ILEI overexpression results in upregulation of multiple chemokines, cytokines, and growth factor receptors is significant. These other molecules could facilitate homing of the metastatic cells to their targets and promote cell growth at these locations (e.g., Muller et al., 2001; Orimo et al., 2005).

Last, the authors could demonstrate a correlation between formation of carcinomas, which are malignancies originating in epithelial tissues, and ILEI overexpression. This finding is especially important considering that carcinomas are the most common type of cancer in humans. Human carcinomas exhibited elevated levels of the ILEI protein in the cytoplasm. Cytoplasmic ILEI, believed to be the fraction of the protein undergoing secretion, was particularly evident at the invasion front, which is the part of the tumor undergoing EMT. Importantly, strong cytoplasmic ILEI expression in tumors was shown to correlate with increased metastasis and decreased patient survival. Therefore, ILEI expression and its subcellular localization could serve as clinical markers for tumor progression. Significantly, cytoplasmic ILEI staining did not appear to correlate with a previously described set of clinical markers for poor prognosis. ILEI could thus represent a novel tool for diagnostics and treatment of certain carcinomas. These findings highlight the importance of determining the protein expression level and localization in the tumor cells; transcription profiling would be ineffective for "ILEI-like" cases where the main control over the function of gene is exerted posttranscriptionally.

A particularly intriguing question concerns the function of ILEI in normal development and adult life. As it defines a new class of EMT regulators, it would be interesting to examine the expression pattern of the protein and its subcellular distribution during processes such as tissue and single cell migration, when cells leave, invade, or integrate into other tissues. Phenotypic analysis of animals in which the activity of ILEI is compromised would provide important clues regarding its role in these processes. As more developmentally

important proteins whose functions depend on post-transcriptional control are being identified, this study provides a further motivation to apply tools for gene identification that take these modes of regulation into consideration.

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Developmental Cell *11*, October, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.devcel.2006.09.011

DEP Domains: More Than Just Membrane Anchors

The DEP domain is present in a number of signaling molecules, including Regulator of G protein Signaling (RGS) proteins, and has been implicated in membrane targeting. New findings in yeast, however, demonstrate a major role for a DEP domain in mediating the interaction of an RGS protein to the C-terminal tail of a GPCR, thus placing RGS in close proximity with its substrate G protein α subunit.

G protein-coupled receptors (GPCRs) transduce extracellular cues through their cognate G proteins, which consist of three subunits, α, β, and γ, that form a heterotrimeric complex (Hamm, 1998). Given the critical role of GPCRs in mediating divergent functions of cells and their adaptation to the environment, it is not surprising that GPCR signaling is subjected to stringent control. Whereas the binding of ligands to receptors initiates signals that are amplified through G proteins and effectors, it also activates negative feedback mechanisms to desensitize signaling. These negative feedback regulations act at almost every step of signal transmission, starting from the receptor. Key players in these desensitization processes are regulator of G protein signal (RGS) proteins, which decrease the intensity and limit the duration of G protein signaling by stimulating the intrinsic GTPase activities of Gα subunits (Abramow-Newerly et al., 2006).

The core RGS domain confers GTPase-activator protein (GAP) activity. It has been known for some time that many RGS proteins show little GAP selectivity toward Gα subunits in vitro but act in a GPCR-dependent manner in vivo. Recent studies indicate that the answer to these paradoxical findings may lie in additional domains of RGS proteins that bind to other cellular proteins and promote substrate specificity of the RGS GAP domain. In a study published recently in *Cell*, Ballon and colleagues demonstrated that the DEP domain contained in the N-terminal extension of a yeast RGS protein, Sst2, mediates the interaction of Sst2 with the C-terminal tail of its cognate GPCR Ste2, thus placing Sst2 in the vicinity of its substrate Gα subunit Gpa1 (Ballon et al., 2006).

The DEP domain is a stretch of ~90 conserved residues that was first identified in three proteins, Dishevelled, EGL-10, and Pleckstrin (Ponting and Bork, 1996). Highly homologous domains are also found in a number of proteins involved in signal transduction, such as Epac2, yeast RGS protein Sst2, and the R7 subfamily (RGS6, 7, 9, and 11) of mammalian RGS proteins. Earlier studies in several proteins have pointed out a function for DEP domains in mediating membrane localization (Wong et al., 2000). Recent data indicate that the DEP domain of RGS9-2 directs its RGS activity toward D2 dopamine, but not M2 muscarinic receptor-mediated signaling pathways (Kovoor et al., 2005). However, the molecular mechanisms underlying this DEP domain-dependent selectivity are unknown.

Ballon et al. (2006) set out to identify binding partners of the DEP domain in Sst2, a yeast RGS protein. They screened a library of overexpressed genes to identify proteins that can suppress the defect in pheromone responses caused by a point mutation in the DEP domain of Sst2. Surprisingly, they pulled out the α-factor receptor Ste2. Using a combination of biochemical, genetic, and cell imaging approaches, they have confirmed an interaction between Sst2 and Ste2 and demonstrated that this interaction is mediated by the DEP