Cell

AKT/PKB Signaling: Navigating Downstream

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DOI 10.1016/j.cell.2007.06.009

The serine/threonine kinase Akt, also known as protein kinase B (PKB), is a central node in cell signaling downstream of growth factors, cytokines, and other cellular stimuli. Aberrant loss or gain of Akt activation underlies the pathophysiological properties of a variety of complex diseases, including type-2 diabetes and cancer. Here, we review the molecular properties of Akt and the approaches used to characterize its true cellular targets. In addition, we discuss those Akt substrates that are most likely to contribute to the diverse cellular roles of Akt, which include cell survival, growth, proliferation, angiogenesis, metabolism, and migration.

The serine/threonine kinase Akt/PKB has emerged as a critical signaling node within all cells of higher eukaryotes and as one of the most important and versatile protein kinases at the core of human physiology and disease. Since its discovery as an oncogene within the mouse leukemia virus AKT8 (Bellacosa et al., 1991; Staal, 1987) and as a homolog of protein kinase C (Jones et al., 1991), there have been many exciting breakthroughs elucidating the mechanism of upstream regulation of Akt (summarized in Figure 1). Several excellent recent reviews have covered the molecular details of Akt regulation and its role in human disease (such as Bellacosa et al., 2005; Engelman et al., 2006). Here, we focus on signaling downstream of Akt, with an emphasis on direct phosphorylation targets of the kinase and its bona fide cellular functions.

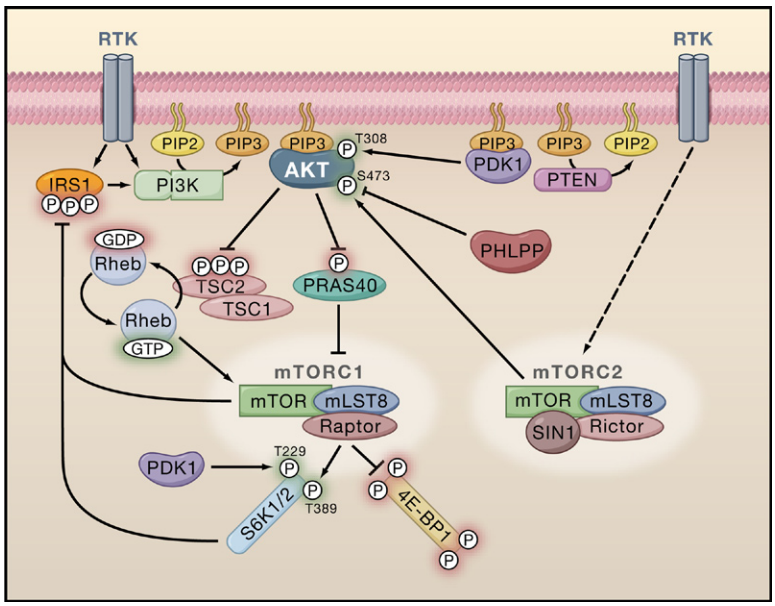

Figure 1. Upstream Activation of Akt by Growth Factors

Also depicted is the complex relationship between Akt signaling and mTOR. Activated receptor tyrosine kinases (RTKs) activate class I phosphatidylinositol 3-kinase (PI3K) through direct binding or through tyrosine phosphorylation of scaffolding adaptors, such as IRS1, which then bind and activate PI3K. PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) to generate phosphatidylinositol-3,4,5-trisphosphate (PIP3), in a reaction that can be reversed by the PIP3 phosphatase PTEN. AKT and PDK1 bind to PIP3 at the plasma membrane, and PDK1 phosphorylates the activation loop of AKT at T308. RTK signaling also activates mTOR complex 2 (mTORC2) through a currently unknown mechanism, and mTORC2 phosphorylates Akt on the hydrophobic motif S473, which can be dephosphorylated by the S473 phosphatase PHLPP. Akt activates mTOR complex 1 (mTORC1) through multisite phosphorylation of TSC2 within the TSC1-TSC2 complex, and this blocks the ability of TSC2 to act as a GTPase-activating protein (GAP) for Rheb, thereby allowing Rheb-GTP to accumulate. Rheb-GTP activates mTORC1, which phosphorylates downstream targets such as 4E-BP1 and the hydrophobic motif on the S6 kinases (S6Ks; T389 on S6K1). PDK1 phosphorylates the activation loop on the S6Ks (T229 on S6K1) in a reaction independent of PDK1 binding to PIP3. Akt can also activate mTORC1 by phosphorylating PRAS40, thereby relieving the PRAS40-mediated inhibition of mTORC1. Once active, both mTORC1 and S6K can phosphorylate serine residues on IRS1, which targets IRS1 for degradation, and this serves as a negative feedback mechanism to attenuate PI3K-Akt signaling. See text for references to recent reviews detailing Akt regulation and mTOR signaling.

Substrate Specificity

The three Akt isoforms (Akt1/PKBα, Akt2/PKBβ, and Akt3/PKBγ) have extensive homology to protein kinases A, G, and C within their kinase domains and are, therefore, members of the AGC kinase family. Following the identification of a residue on the kinase GSK3 as the first direct target of Akt in cells (Cross et al., 1995), groundbreaking experiments with peptides containing variants of this sequence defined the minimal recognition motif of Akt as R-X-R-X-X-S/T-B (Alessi et al., 1996b), where X represents any amino acid and B represents bulky hydrophobic residues. The critical requirement for R residues at both the −5 and −3 positions (that is, 5 and 3 residues, respectively, N-terminal to the phospho-acceptor site) on peptides efficiently phosphorylated by Akt distinguishes the substrate specificity of Akt from that of two other mitogen-stimulated AGC kinases, RSK (MAPKAP-K1) and S6K1 (p70S6K), which can better tolerate K at these positions. In these "peptide-bashing" experiments, more subtle Akt preferences were also uncovered for other residues surrounding the phosphorylation site (such as a preference for T at −2). Structural insights into the molecular interactions dictating the substrate selectivity of Akt have been provided by a high-resolution crystal structure of Akt bound to this GSK3 peptide substrate (Yang et al., 2002).

Further details of the preferred substrate specificity of Akt have been obtained using peptide library screening (Hutti et al., 2004; Obata et al., 2000), which provides an unbiased, systematic, and quantitative score for all 20 amino acids at each site within seven residues N-terminal and C-terminal to the phospho-acceptor site. This approach allows the identification of residues selected both for and against by the kinase of interest at each position surrounding the phosphorylation site. A bioinformatics program called Scansite (http://scansite.mit.edu; Yaffe et al., 2001) has provided the ability to search protein databases with the matrix of data obtained from such screens, rather than a single consensus sequence, and has been instrumental in identifying new Akt substrates and narrowing down target residues on suspected substrates. However, this approach needs to be used with caution, as it is only useful for identifying candidate phosphorylation sites. The sheer number of high-quality Akt sites within the proteome, in addition to the overlap in substrate specificity with other AGC kinases, illustrates the need for a rigorous demonstration of the direct in vivo phosphorylation of a given candidate target by Akt.

It remains possible that there are unknown sequence contexts or macromolecular interactions within cells that might allow Akt to phosphorylate motifs other than the minimally required R-X-R-X-X-S/T. Currently, however, there are no rigorously demonstrated and independently confirmed Akt substrates that fall into this category. Therefore, in the discussion of substrate characterization and the role of specific substrates below, we consider the presence of an R-X-R-X-X-S/T motif to be essential.

Defining Criteria for Bona Fide Akt Substrates

Here we review criteria that, in our view, should be met to define an in vivo Akt substrate. We recognize the limits of such standards, as it is possible that the same site phosphorylated by Akt in one cell type under one condition might be phosphorylated by additional kinases in other settings. However, given the increasing complexity of Akt signaling and its importance in human physiology and disease, it is worth evaluating the experimental paradigms currently used to define a direct downstream target of Akt. Our discussion is specific to Akt, but the criteria are similar to that put forward for other protein kinases (Frame and Cohen, 2001). Many approaches have been used to demonstrate phosphorylation of sites on target proteins within cells. However, no single method is sufficient; rather, both Akt loss- and gain-of-function approaches are needed. In the end, it is essential to demonstrate that phosphorylation of a candidate site on an endogenous substrate is induced by specific physiological stimuli that activate endogenous Akt and that the phosphorylation is lost upon Akt inactivation.

In Vitro Phosphorylation

Demonstration of direct in vitro phosphorylation of a candidate R-X-R-X-X-S/T site by Akt is a necessary step in defining an Akt substrate. Phosphorylation of the full-length protein of interest should be demonstrated, rather than fragments or motifs fused to other proteins (e.g., GST). Phosphate incorporation at the relevant site must be demonstrated. However, Akt is a somewhat promiscuous kinase in vitro and will phosphorylate most R-X-R-X-X-S/T sites and some R-X-X-S/T sites if given enough time and substrate. Therefore, in vitro phosphorylation, although necessary, is not sufficient for defining a new phosphorylation site for Akt.

Phosphorylation-Site Readout

In order to confirm candidate phosphorylation sites detected using bioinformatics and/or in vitro approaches, one first needs a reliable and specific readout of the phosphorylation event in question. This can occasionally be detected as a shift to a slower migrating form on denaturing polyacrylamide gels. However, it is rare that phosphorylation of an Akt site causes such a shift, which is usually indicative of a proline-directed site (i.e., S/T-P), and Akt poorly phosphorylates such sites (Alessi et al., 1996b; Hutti et al., 2004). Mass spectrometry techniques and in vivo [^{32}P]-orthophosphate labeling followed by two-dimensional phospho-peptide mapping are indispensable tools for identifying in vivo phosphorylation sites. However, due to their time-consuming and low-throughput nature, these approaches are somewhat less useful as an experimental readout when characterizing cellular conditions and kinases responsible for the phosphorylation. Antibodies specific for a phosphorylated motif (i.e., phospho-substrate antibodies) have been extremely valuable for identifying and characterizing new Akt substrates over the past five years (e.g., Kovacina et al., 2003; Manning et al., 2002; Sano et al., 2003). These antibodies are raised against degenerate

phospho-peptides containing the phosphorylated minimal Akt substrate motif (R-X-R-X-X-pS/pT; Zhang et al., 2002). Although these antibodies are helpful in identifying candidate Akt sites, mutation analysis must be used to confirm that the antibody is recognizing the predicted site(s). As a cautionary note, at least some of the currently available Akt phospho-substrate antibodies can recognize R-X-X-pS/pT motifs in sequence contexts lacking an R at the −5 position (Zhang et al., 2002), and this is especially true when such a phospho-protein is overexpressed (B.D.M., unpublished data). Finally, once data are obtained suggesting the regulated phosphorylation of a specific site, the gold standard is the use of a phospho-specific antibody unique to the site of interest. Regardless of the readout for a given phosphorylation event, this approach must be followed by experiments that demonstrate a requirement for Akt.

Pharmacological

The growth-factor-stimulated phosphorylation of an Akt site should be sensitive to pretreatment with phosphatidylinositol 3-kinase (PI3K) inhibitors, such as wortmannin (≤100 nM) or LY294002 (≤20 μM), as class I PI3Ks are upstream activators of Akt (Figure 1). Although a low dose of wortmannin is specific to PI3K, it inhibits all classes of PI3Ks, and there are likely to be PI3K-regulated kinases, other than Akt, that are affected by such inhibition. Resistance to highly specific inhibitors of the kinases RSK (e.g., BI-D1870; Sapkota et al., 2007) and mTORC1 (e.g., rapamycin) is also important for ruling out RSK and S6K, respectively, as candidate kinases for the in vivo phosphorylation event observed. Specificity issues exist with currently available Akt inhibitors, thereby limiting their use for defining Akt as the relevant kinase for a given phosphorylation event or cellular function. However, inhibitors that target regions of Akt outside of its highly conserved ATP-binding pocket are promising and should show increased specificity for Akt over other AGC kinases. As the specificity of AGC kinase inhibitors improves, they will certainly be valuable tools in identifying and verifying Akt substrates.

Loss of Function

The current limitations of pharmacological approaches dictate that Akt-specific loss-of-function approaches are needed. Although recently developed cell lines lacking Akt function provide the most stringent setting for confirming a substrate (see below), several types of dominant-negative Akt constructs are available, with variable success at inhibiting endogenous Akt. In our experience, Akt lacking its two activating phosphorylation sites (Akt-T308A/S473A) works more reproducibly as a dominant-negative than the kinase-dead (K179D) version. However, the molecular mechanisms underlying the dominant-negative effects of these various constructs are unknown, and potential effects on upstream kinases such as phosphoinositide-dependent kinase-1 (PDK1) and mTORC2 make this approach less desirable. Knockdown of Akt isoforms through small interfering (si)RNAs is becoming used more often to define Akt substrates and functions. Knowledge of the expression patterns of different Akt isoforms and the use of isoform-specific antibodies are important considerations here. It is likely that, in any given setting, knockdown of more than one isoform will be required. However, recent studies suggest that isoform-specific substrates and functions of Akt are more common than previously suspected (see below).

Gain of Function

Overexpression of exogenous Akt constructs invariably increases the basal and stimulated phosphorylation of its substrates. However, given the strict temporal and spatial regulation that accompany kinase-substrate interactions in cells, exogenously overexpressed constructs should never be used as the sole basis for defining an Akt substrate. Membrane-targeting versions of Akt, such as myristoylated PH domain-deleted Akt (myr-ΔPH-Akt)—which are brought to the plasma membrane independent of PI3K activity—have been the most widely used for determining the cellular effects of constitutive activation of Akt (Andjelkovic et al., 1997; Kohn et al., 1996). However, depending on the membrane-targeting construct used, differential effects on downstream signaling can be detected. An alternative is the phospho-mimetic mutant of Akt (Akt-T308D/S473D), which has lower activity than the myr-Akt derivatives but is constitutively active and PI3K independent (Alessi et al., 1996a). Overall, the general concerns regarding potential overexpression artifacts definitely apply to kinase-substrate interactions and phosphorylation events, and results obtained with such constructs should be interpreted with caution.

Genetics

Genetic systems have been instrumental in identifying and confirming kinase substrates. *C. elegans* and *Drosophila* genetics have been powerful tools for elucidating signaling pathways. An important example of using a genetic approach to elucidate Akt signaling was the finding that the forkhead box O (FOXO) transcription factor Daf-16 is downstream of the PI3K and Akt orthologs in *C. elegans* for control of the developmental and metabolic state referred to as dauer (Paradis and Ruvkun, 1998). Although genetic epistasis analysis is useful for positioning new candidate substrates downstream of a specific kinase and for functional insights into the effects of a kinase on the given target, more rigorous approaches are needed to demonstrate a direct phosphorylation. However, loss-of-function or deletion alleles of a kinase can be valuable for confirming the kinase specificity of an in vivo phosphorylation event. To this end, mouse genetic models have been increasingly important for defining bona fide targets of Akt. A variety of isoform-specific Akt knockout mice and combinations have now been made (reviewed in Dummler and Hemmings, 2007), and these can be used to demonstrate that phosphorylation of a given substrate requires a specific Akt isoform in a particular cell type of interest. Other pow-

Table 1. Characteristics and Experimental Evidence for a Subset of Akt Substrates[a]

Target	Human Site(s)[b]	In Vitro[c]	In Vivo[d]			Genetic Evidence[e]	Regulatory Effect?[f]
			W/L	LOF	GOF		
FOXO1	T24, S256, S319	+	+	+	+	M, F, W	Inhibit
FOXO3A	T32, S253, S315	+	+	+	+	M, F, W	Inhibit
FOXO4	T32, S197, S262	+	+	+	+	M, F, W	Inhibit
TSC2	S939, T1462	+	+	+	+	M, F	Inhibit
GSK3α/β	S21/S9	+	+	+	+	M	Inhibit
RAF1	S259	+	+	+	+	–	Inhibit
PRAS40	T246	+	+	–	+	M	Inhibit
AS160	S588, T642	+	+	–	–	–	Inhibit
BAD	S99	+	+	+	+	–	Inhibit
WNK1	T60	+	+	+	–	M	?
MDM2	S166, S186	+	+	+	+	–	Activate
Chk1	S280	+	+	+	+	M	Inhibit
eNOS	S1177	+	+	+	+	M	Activate
ASK1	S83	+	+	+	+	–	Inhibit
IKKα	T23	+	–	–	–	–	Activate
p21CIP1	T145	+	+	+	+	–	Inhibit
p27KIP1	T157	+	+	+	+	–	Inhibit
Casp9	S196	+	–	+	+	–	Inhibit

[a]For an expanded version of this table see Table S1.
[b]Human numbering of sites with strongest evidence of being phosphorylated by Akt in vivo.
[c]Direct phosphorylation of the given site(s) with purified Akt and full-length substrate in vitro.
[d]Evidence of Akt-dependent phosphorylation of the given site(s) within cells (– means no published evidence), including: W/L, sensitivity to PI3K inhibition using ≤ 100 nM wortmannin (W) and/or ≤ 20 μM LY294002 (L); LOF, loss-of-AKT-function evidence using dominant-negative mutants and/or RNAi approaches; GOF, gain-of-Akt-function evidence using overexpression and/or constitutively active mutants.
[e]Genetic evidence in model organisms, including epistasis analyses in *Drosophila* (F) or *C. elegans* (W) or loss-of-phosphorylation in mouse mutants (M) lacking Akt function.
[f]Functional consequence of Akt-mediated phosphorylation.

erful mouse models for the confirmation of in vivo Akt substrates are a pair of PDK1 knockin mutants generated by Alessi and colleagues. The first is a knockin of a PDK1-PH domain mutant (PKD1-RRR472-474LLL or PDK1-PH_{KI}), which cannot bind to PI3K lipid products and is defective in the phosphorylation and activation of Akt isoforms, while maintaining the ability to phosphorylate the other AGC kinases (McManus et al., 2004). The phosphorylation of several proposed Akt substrates has been confirmed using PDK1-PH_{KI} embryonic stem cells, including sites on FOXO1 and 3, TSC2, GSK3, PRAS40, and WNK1 (McManus et al., 2004). The other PDK1 knockin mutant is the PDK1-PIF pocket mutant (PDK1-L155E), which can activate Akt but not the other AGC kinases (Collins et al., 2003). Embryonic stem cells expressing this mutant are useful in parallel to critically test in vivo phosphorylation sites for Akt specificity. Ultimately, the dependence on Akt activity for the phosphorylation of all candidate substrates and sites should be verified in such cell and mouse models.

Akt Substrates and Functions

A careful search of the literature finds over 100 reported nonredundant Akt substrates, of which approximately 25% do not contain the minimal requirements for an Akt site (e.g., R-X-R-X-X-S/T). Furthermore, many of those that do contain this motif have not been characterized further than in vitro kinase assays. Although we have not determined how all of the remaining published candidate Akt substrates measure up to the criteria outlined above, we have assessed the literature on 18 substrates for which there have been multiple independent published reports (Tables 1 and S1). Within this survey, we have included an analysis of evolutionary conservation (Table S1). The degree of conservation of an identified Akt phosphorylation site can be indicative of the relevance of the substrate toward the defined cellular functions of Akt. Most thoroughly studied and independently confirmed Akt phosphorylation sites are conserved amongst the orthologs from all mammals, and a handful are conserved down to invertebrate models such as *Drosophila* or *C. elegans* (Table S1, Metazoan). Conserva-

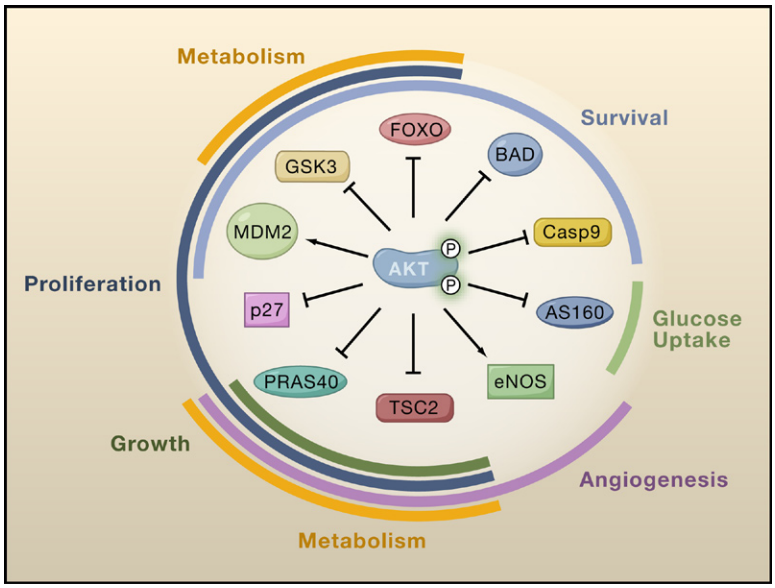

Figure 2. Cellular Functions of Ten Akt Substrates

Akt-mediated phosphorylation of these proteins leads to their activation (arrows) or inhibition (blocking arrows). Regulation of these substrates by Akt contributes to activation of the various cellular processes shown (i.e., survival, growth, proliferation, glucose uptake, metabolism, and angiogenesis). As illustrated by these ten targets, a high degree of functional versatility and overlap exists amongst Akt substrates. See text for detailed descriptions of substrates and functions.

tion analysis is particularly important when using animal models to determine the role of Akt substrates in human disease. For instance, a study using the rat version of the protein Par-4/PAWR concluded that this protein is a direct target of Akt essential for Akt's role in cancer cell survival (Goswami et al., 2005), but the proposed Akt site is not conserved in human, or even mouse, Par-4/PAWR. Finally, it should be stated that exclusion from the list in Table 1 or the discussion below does not rule out a published substrate as being a bona fide Akt target. We anxiously await further study on these other candidate substrates.

In this section, we focus on the known cellular functions of Akt and the direct downstream targets of Akt that are most likely to mediate these functions. This discussion illustrates the most challenging aspect of characterizing any kinase-substrate pair: defining the functional consequences of phosphorylation of a specific site or sites on a new substrate. To this end, phosphorylation-site mutants (S/T to A) are particularly useful if the substrate has a known function. Such a mutant should block the positive or negative effects of Akt on the activity of the substrate or a cellular process involving the substrate. Although it has been done for very few substrates, the generation of phosphorylation-site mutant knockin mice is an especially powerful approach for determining the physiological importance of phosphorylation of a given target by Akt (e.g., McManus et al., 2005). Phosphomimetic mutants (S/T to D/E) have a more limited usefulness. Changing the phospho-acceptor site to an acidic residue might mimic phosphorylation, but not in all cases. This is especially true when the Akt phosphorylation site creates a 14-3-3 binding motif, which is a common mode of regulation (see below), as D and E residues are poor substitutes for phospho-S/T in mediating 14-3-3 binding. One theme that is obvious from the discussion below is that the diverse cellular roles of Akt do not fall under a one-substrate-one-function paradigm; rather, each physiological response downstream of Akt appears to be mediated by multiple targets (Figure 2). Furthermore, some Akt substrates control more than one cellular function, and these functions may vary in a cell- and signal-context-dependent manner.

Cell Survival

Even before the relevant substrates were identified, several groups independently demonstrated a critical role for Akt in promoting cell survival downstream of growth factors, oncogenes, and cell stress (reviewed in Marte and Downward, 1997). Akt enhances the survival of cells by blocking the function of proapoptotic proteins and processes. Akt negatively regulates the function or expression of several Bcl-2 homology domain 3 (BH3)-only proteins, which exert their proapoptotic effects by binding to and inactivating prosurvival Bcl-2 family members. For instance, Akt directly phosphorylates and inhibits the BH3-only protein BAD (Datta et al., 1997; delPeso et al., 1997). Survival factors stimulate Akt-mediated phosphorylation of BAD on S136, and this creates a binding site for 14-3-3 proteins, which triggers release of BAD from its target proteins (Datta et al., 2000). The ability to phosphorylate S136 on BAD is important for the survival effects of Akt on neurons and other cell types (Datta et al., 1997, 2002). Akt also inhibits the expression of BH3-only proteins through effects on transcription factors, such as FOXO and p53. Akt phosphorylates FOXO1 on T24, S256, and S319, and it phosphorylates FOXO3a and FOXO4 on three equivalent sites (reviewed in Tran et al., 2003). Akt's phosphorylation of FOXO proteins occurs in the nucleus, and, as with BAD, phosphorylated T24 and S256 are bound by 14-3-3 proteins, which displace FOXO transcription factors from target genes and trigger their export from the nucleus. Through this mechanism, Akt blocks FOXO-mediated transcription of target genes that promote apoptosis, cell-cycle arrest, and metabolic processes (see below). An important proapoptotic target of FOXO proteins is the BH3-only protein BIM, which

stimulates cell death in hematopoietic lineages upon cytokine withdrawal (Dijkers et al., 2002). In addition, when unphosphorylated and active, FOXO factors can induce the expression of the proapoptotic cytokine Fas ligand (FasL; Brunet et al., 1999). A third target of Akt that promotes survival by inhibiting BH3-only proteins is MDM2 (or HDM2 in humans), an E3 ubiquitin ligase that triggers p53 degradation. Akt phosphorylates MDM2 on S166 and S186, and this promotes translocation of MDM2 to the nucleus, where it negatively regulates p53 function (Mayo and Donner, 2001; Zhou et al., 2001b). Two transcriptional targets of p53 are the BH3-only proteins Puma and Noxa, which appear to be the essential targets in p53-induced apoptosis (Villunger et al., 2003). However, the relative importance of downregulation of these p53 targets to Akt-mediated cell survival has not been thoroughly examined.

The importance of other direct targets of Akt toward inhibiting apoptosis is less well understood. Akt phosphorylates GSK3 isoforms on a highly conserved N-terminal regulatory site (GSK3α-S21, GSK3β-S9), and this phosphorylation inactivates the kinase (Cross et al., 1995). Although GSK3 has many substrates that could explain a proapoptotic role for this Ser/Thr kinase (reviewed in Frame and Cohen, 2001), the prosurvival Bcl-2 family member MCL-1 has recently been found to be a direct target inhibited by GSK3 (Maurer et al., 2006). Downstream of the Bcl-2 family members within the mitochondrial pathway of apoptosis are a cascade of caspase-family proteases initiated by caspase-9. Caspase-9 is proteolytically processed and activated from its procaspase form upon cytochrome *c* release from the mitochondria. Therefore, Akt activation prevents the processing of procaspase-9 through the effects on Bcl-2 family members described above. Akt has also been found to directly phosphorylate S196 on human procaspase-9, and this phosphorylation correlates with a decrease in the protease activity of caspase-9 in vitro (Cardone et al., 1998). A procaspase-9-S196A mutant is more efficient than wild-type caspase-9 at inducing apoptosis when co-overexpressed with activated Akt, suggesting a role for caspase-9 phosphorylation in the antiapoptotic effects of Akt. However, the sequence surrounding caspase-9-S196 is unusual for an Akt substrate in that the +1 position is a P, and such proline-directed sites are strongly selected against by Akt in peptide phosphorylation experiments (Alessi et al., 1996b; Hutti et al., 2004). Furthermore, despite the highly conserved role of Akt in blocking apoptosis, this Akt phosphorylation site is not found in all mammals and appears to be restricted to caspase-9 in primates. Additional studies are required to determine the physiological relevance of caspase-9 relative to other proapoptotic proteins inhibited by Akt.

It is also possible that Akt exerts some of its cell-survival effects through crosstalk with other pathways or through effects on nutrient uptake and metabolism. For instance, it has been reported that, under some conditions, the PI3K-Akt pathway activates NFκB survival signaling or inhibits JNK/p38 apoptotic signaling (e.g., Kim et al., 2001; Ozes et al., 1999; Romashkova and Makarov, 1999). An important, but more indirect, effect of Akt signaling on cell survival is through its roles in nutrient uptake, metabolism, and the maintenance of mitochondrial membrane potential (reviewed in Plas and Thompson, 2005; Robey and Hay, 2006). Both signaling crosstalk and effects of Akt on cellular metabolism are discussed in detail below.

Cell Growth

One of the best-conserved functions of Akt is its role in promoting cell growth (i.e., an increase in cell mass). The predominant mechanism appears to be through activation of mTOR complex 1 (mTORC1 or the mTOR-raptor complex), which is regulated by both nutrients and growth factor signaling. MTORC1 is a critical regulator of translation initiation and ribosome biogenesis and plays an evolutionarily conserved role in cell growth control (reviewed in Wullschleger et al., 2006). The enhanced sensitivity of cancer cells and mouse tumor models exhibiting oncogenic activation of the PI3K-Akt pathway to mTORC1 inhibitors, such as rapamycin, illustrates the importance of mTORC1 activation downstream of Akt (reviewed in Sabatini, 2006). Although Akt activation has been known for some time to effect downstream substrates of mTORC1, such as S6K1 and eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1), the mechanism by which Akt regulates mTORC1 signaling to these targets was more difficult to ascertain. Akt has been suggested to directly phosphorylate mTOR on S2448, which often correlates with mTORC1 activation, but mutational analysis has failed to demonstrate any functional significance for this phosphorylation (Scott et al., 1998; Sekulic et al., 2000). Moreover, S6K1 rather than Akt has recently been shown to be responsible for phosphorylation of this site (Chiang and Abraham, 2005; Holz and Blenis, 2005).

Complementary approaches by several independent laboratories using *Drosophila* genetics and mammalian cell biology found that the tuberous sclerosis complex 2 (TSC2; also known as tuberin) tumor suppressor is a critical negative regulator of mTORC1 signaling (Gao and Pan, 2001; Goncharova et al., 2002; Tapon et al., 2001) and that Akt-mediated phosphorylation inhibits TSC2 function (Inoki et al., 2002; Manning et al., 2002; Potter et al., 2002). Akt has been shown to directly phosphorylate two sites on TSC2 (S939 and T1462 on the full-length human protein), which are conserved and phosphorylated in *Drosophila* TSC2, and is likely to phosphorylate two or three additional sites (S981 and S1130/S1132). The relative importance of these distinct sites for Akt's regulation of TSC2 downstream of different stimuli has yet to be determined. However, various phosphorylation-site mutants (S/T to A) of TSC2 can dominantly block Akt-mediated activation of mTORC1 signaling (Cai et al., 2006; Inoki et al., 2002; Manning et al., 2002). TSC2, in a complex with its binding partner

TSC1 (also known as hamartin), acts as a GTPase-activating protein (GAP) for the Ras-related small G protein Rheb, which strongly activates mTORC1 when in its GTP-bound active form (Figure 1; reviewed in Manning and Cantley, 2003). Therefore, Akt activates mTORC1 indirectly by inhibiting TSC2, thereby allowing Rheb-GTP to activate mTORC1 signaling.

More recently, a second Akt substrate has been found to be involved in mTORC1 regulation. Like TSC2 (Manning et al., 2002), the proline-rich Akt substrate of 40 kDa (PRAS40) was identified using the Akt phospho-substrate antibody and was found to be a major protein bound to 14-3-3 in response to insulin (Kovacina et al., 2003). PRAS40 was independently identified (dubbed p39) for its ability to bind to 14-3-3 proteins in a growth-factor- and nutrient-regulated manner (Harthill et al., 2002). Although the function of the protein was unknown, Akt was shown to directly phosphorylate PRAS40 on T246 (Kovacina et al., 2003), and this phosphorylation was later shown to be important for 14-3-3 binding (Vander Haar et al., 2007). It should be noted that T246 has the minimal requirements for an Akt phosphorylation site, yet it scores significantly low relative to other Akt substrates (Table S1), suggesting that additional kinases might also phosphorylate this site. Importantly, PRAS40 has been found to associate with mTORC1 and appears to negatively regulate mTORC1 signaling (Sancak et al., 2007; Vander Haar et al., 2007). Overexpression of a PRAS40-T246A mutant can block Akt activation of S6K1, suggesting that Akt-mediated phoshorylation of PRAS40 at T246 stimulates mTORC1 signaling. In addition, there is a weak homolog of PRAS40 in *Drosophila* called Lobe, which contains a site with similarity to T246, and Lobe appears to behave like PRAS40 in *Drosophila* S2 cells (Sancak et al., 2007). A requirement for Lobe phosphorylation in parallel to TSC2 phosphorylation might explain the lack of an obvious developmental phenotype in *Drosophila* expressing an Akt phosphorylation-site mutant of TSC2 (Dong and Pan, 2004). Therefore, there appears to be two parallel and, perhaps, functionally redundant mechanisms by which Akt activates mTORC1 (Figure 1). It will be interesting to determine the relative importance of TSC2 and PRAS40 for Akt-mediated mTORC1 activation under different physiological conditions.

Aside from the ability of mTORC1 to enhance protein synthesis through its downstream targets, additional anabolic processes through which Akt stimulates an increase in cell size are poorly defined. Both mTORC1-dependent and -independent functions of Akt in nutrient uptake and metabolism are likely to contribute to increases in cell growth (e.g., Edinger and Thompson, 2002; Plas and Thompson, 2005). In addition to increasing protein content, in order for a cell to gain size it must increase membrane biosynthesis. One suggested target of Akt that would contribute to the synthesis on new lipids is ATP citrate lyase (ACL; Berwick et al., 2002), which converts citrate to cytosolic acetyl CoA thereby providing the essential building block for lipid biosynthesis. However, the proposed Akt phosphorylation site on ACL (S454; P-S-R-T-A-S-F) lacks the critical −5 R. It will be important to examine the phosphorylation of this site in the Akt loss-of-function mouse models described above.

Cell Proliferation

Although Akt is best known for promoting cell survival and growth through pathways parallel to Erk's control over cell proliferation, Akt activation can also stimulate proliferation through multiple downstream targets impinging on cell-cycle regulation. Several groups have independently found that Akt phosphorylates the $p27^{Kip1}$ cyclin-dependent kinase inhibitor on T157 (Liang et al., 2002; Shin et al., 2002; Viglietto et al., 2002), and this leads to cytosolic sequestration via 14-3-3 binding (Sekimoto et al., 2004). Preventing p27 localization to the nucleus attenuates its cell-cycle inhibitory effects. Expression of a phosphorylation-site mutant of p27 (p27-T157A) blocks the proliferative effects of various constitutively active Akt derivatives (Liang et al., 2002; Shin et al., 2002; Viglietto et al., 2002). However, Akt-mediated phosphorylation of p27 is not required for its effects on proliferation in all systems, as T157 is not conserved in rodent versions of p27. Akt also inhibits p27 expression through phosphorylation and inhibition of the FOXO transcription factors (Medema et al., 2000). Akt has also been found to phosphorylate the cyclin-dependent kinase inhibitor $p21^{Cip1/WAF1}$ on T145, which is a low-scoring Akt site on Scansite, and, like p27, this phosphorylation leads to p21 cytosolic localization (Zhou et al., 2001a). Interestingly, it has been suggested that Akt1, but not Akt2, phosphorylates and inhibits p21, whereas Akt2 appears to bind and stabilize p21, thereby blocking cell-cycle progression (Heron-Milhavet et al., 2006). Akt might also inhibit p21 expression through its phosphorylation and activation of MDM2 and subsequent down-regulation of p53-mediated transcription of p21 (Mayo and Donner, 2001; Zhou et al., 2001b).

Akt-dependent phosphorylation of targets already discussed, such as GSK3, TSC2, and PRAS40, is also likely to drive cell proliferation through regulation of the stability and synthesis of proteins involved in cell-cycle entry. GSK3-mediated phosphorylation of the G1 cyclins cyclin D and cyclin E and the transcripton factors c-*jun* and c-*myc*, which all play a central role in the G1-to-S-phase cell-cycle transition, targets them for proteasomal degradation (Diehl et al., 1998; Wei et al., 2005; Welcker et al., 2003; Yeh et al., 2004). Therefore, phosphorylation and inhibition of GSK3 by Akt should enhance the stability of these proteins. Akt signaling also controls the translation of proteins important for cell-cycle progression, predominantly through phosphorylation of TSC2 and PRAS40 and subsequent activation of mTORC1. Although best known for its role in promoting cell growth, mTORC1 is a critical regulator of cell proliferation. The predominant cellular effect of mTORC1 inhibitors, such as rapamycin, is to cause a G1 cell-cycle arrest, and Akt-mediated cell proliferation and oncogenic transformation

has recently been shown to be dependent on mTORC1 activation (Skeen et al., 2006). Akt signaling leads to activation of eIF4E through mTORC1's inhibition of 4E-BP1, and eIF4E promotes the cap-dependent translation of many target mRNAs, including those encoding cyclin D1 and c-Myc (reviewed in Mamane et al., 2004). Therefore, as with cell survival and growth, Akt controls cell proliferation through multiple complementary downstream pathways.

Whether PI3K-Akt signaling is important in the transition through other phases of the cell cycle is less clear. In some cell lines, Akt activity has been shown to be elevated during the G2/M phase of the cell cycle, and Akt activation promotes progression through mitosis, even in the presence of DNA damage (Kandel et al., 2002; Shtivelman et al., 2002). One mechanism explaining this observation is that Akt directly phosphorylates the DNA damage checkpoint kinase Chk1 on S280 (King et al., 2004; Puc et al., 2005). S280 phosphorylation blocks checkpoint function by stimulating Chk1 translocation to the cytosol, where it is sequestered from the DNA damage-sensing kinases ATM and ATR (Puc et al., 2005). Therefore, loss of the PTEN tumor suppressor causes a checkpoint defect due to constitutive Akt activation and phosphorylation of Chk1, and this might contribute to genomic instability in this setting.

Angiogenesis

Akt plays important roles in both physiological and pathological angiogenesis through effects in both endothelial cells and cells producing angiogenic signals, such as tumor cells. In endothelial cells, the PI3K-Akt pathway is robustly activated by vascular endothelial growth factor (VEGF; reviewed in Olsson et al., 2006), and phosphorylation of the Akt targets discussed above is likely to contribute to endothelial cell survival, growth, and proliferation. In addition, Akt activates endothelial nitric oxide (NO) synthase (eNOS) through direct phosphorylation of S1177 (Dimmeler et al., 1999; Fulton et al., 1999). The release of NO produced by activated eNOS can stimulate vasodilation, vascular remodeling, and angiogenesis (reviewed in Morbidelli et al., 2003). Akt signaling also leads to an increased production of the hypoxia-inducible factor α (HIF1α and HIF2α) transcription factors, at least in part, through mTORC1-dependent translation (reviewed in Gordan and Simon, 2007; Semenza, 2003). HIFα activation in endothelial cells and other cells leads to expression and subsequent secretion of VEGF and other angiogenic factors, thereby stimulating angiogenesis through both autocrine and paracrine signaling. Finally, Akt1, the primary isoform in endothelial cells, is required for proper endothelial cell migration (Ackah et al., 2005), although the relevant downstream targets have not been defined.

Cellular Metabolism

In response to growth factors, Akt signaling regulates nutrient uptake and metabolism in a cell-intrinsic and cell-type-specfic manner through a variety of downstream targets. One of the most important physiological functions of Akt is to acutely stimulate glucose uptake in response to insulin. Akt2, the primary isoform in insulin-responsive tissues, has been found to associate with glucose transporter 4 (Glut4)-containing vesicles upon insulin stimulation of adipocytes (Calera et al., 1998), and Akt activation leads to Glut4 translocation to the plasma membrane (Kohn et al., 1996). Although the precise molecular mechanisms by which Akt stimulates Glut4 translocation are still being intensely studied, the Rab-GAP AS160 (also known as TBC1 domain family member 4; TBC1D4) has emerged as an important direct target of Akt involved in this process (Eguez et al., 2005; Sano et al., 2003). Five putative Akt sites are phosphorylated on AS160 in response to insulin, of which S588 and T642 score the highest using the Scansite program. Importantly, mutation of these two sites to alanine significantly blocks insulin-stimulated Glut4 translocation (Sano et al., 2003). The current model is that Akt-mediated phosphorylation of some combination of the sites on AS160 inhibits its GAP activity, thereby allowing a Rab-family GTPase to become GTP loaded and stimulate Glut4 vesicle translocation. However, recent studies have suggested AS160-independent mechanisms of regulation of this process (Bai et al., 2007), and other candidate Akt substrates involved in various steps of Glut4 translocation have been identified, including PIKfyve (Berwick et al., 2004). Glut1 is the main glucose transporter in most cell types, and unlike Glut4, it appears to be regulated primarily through alterations in expression levels. Activation of mTORC1, through Akt-mediated phosphorylation of TSC2 and PRAS40, can contribute to both HIFα-dependent transcription of the Glut1 gene and cap-dependent translation of Glut1 mRNA (e.g., Taha et al., 1999; Zelzer et al., 1998). The frequent occurrence of PI3K-Akt pathway activation and HIFα accumulation in human cancers is likely to explain the high levels of Glut1 and enhanced glucose uptake observed in tumors (reviewed in Majumder and Sellers, 2005; Semenza, 2003). In addition to glucose uptake, Akt has also been suggested to regulate the cell surface expression of transporters for other nutrients, such as amino acids, in an mTORC1-dependent manner (Edinger and Thompson, 2002). However, the mechanism and broader applicability of this effect of Akt and mTORC1 are currently unclear.

Akt activation can also alter glucose and lipid metabolism within cells. Upon entry into the cell, glucose is converted to its active form glucose 6-phosphate through the action of hexokinases. Akt has been demonstrated to stimulate the association of hexokinase isoforms with the mitochondria, where they more readily phosphorylate glucose, but the direct target of Akt responsible is currently unknown (reviewed in Robey and Hay, 2006). Glucose 6-phosphate can be stored by conversion to glycogen or catabolized to produce cellular energy through glycolysis, and

Akt signaling can regulate both of these processes. Particularly important in muscle and liver, Akt-mediated phosphorylation and inhibition of GSK3 prevents GSK3 from phosphorylating and inhibiting its namesake substrate glycogen synthase, thereby stimulating glycogen synthesis. Akt activation also increases the rate of glycolysis (Elstrom et al., 2004), and this is probably a major factor contributing to the highly glycolytic nature of tumor cells. Akt's ability to enhance the rate of glycolysis is due, at least in part, to its ability to promote the expression of glycolytic enzymes through HIFα (e.g., Lum et al., 2007; Majumder et al., 2004; Semenza et al., 1994). In a cell-context-dependent manner, Akt-mediated phosphorylation and inhibition of FOXO1 also contribute to glucose homeostasis, as FOXO1 promotes hepatic glucose production and regulates the differentiation of cells involved in metabolic control (reviewed in Accili and Arden, 2004). In hepatocytes, Akt can also inhibit gluconeogenesis and fatty acid oxidation through direct phosphorylation of S570 on PGC-1α (Li et al., 2007), which is a coactivator that can coregulate genes with FOXO1 and other transcription factors. Akt signaling regulates lipid metabolism through phosphorylation and inhibition of GSK3. As described above, phosphorylation of substrates by GSK3 often targets them for proteasomal degradation, and GSK3 has been shown to promote degradation of the sterol regulatory element-binding proteins (SREBPs), which are transcription factors that turn on the expression of genes involved in cholesterol and fatty acid biosynthesis (Sundqvist et al., 2005). Therefore, Akt-mediated inhibition of GSK3 promotes SREBP stability and enhances lipid production. As discussed above, Akt has also been proposed to directly activate ACL (Berwick et al., 2002), with the caveat that the relevant phosphorylation site on this enzyme does not meet the minimal consensus for an Akt site. As intense interest in the role of the PI3K-Akt pathway in metabolic diseases and tumor metabolism continues, it is likely that Akt signaling will be found to impinge on many other areas of central metabolism.

Cell Migration and Invasion

Until recently, a role for Akt in the control of cell migration, invasion of the extracellular matrix, and ultimately metastasis has been difficult to ascertain. Strikingly, activation of Akt1 has been found to decrease mammary epithelial cell migration, and Akt1 prevents an epithelial-to-mesenchymal transition that resembles events required for metastasis (Irie et al., 2005; Yoeli-Lerner et al., 2005). Two independent mechanisms for this surprising Akt function have been explored. The first found that the inhibitory effect of Akt1 on the in vitro migration and invasion properties of breast cancer cell lines involved a pathway leading to degradation of the nuclear factor of activated T cells (NFAT) transcription factors (Yoeli-Lerner et al., 2005). However, the molecular mechanism of Akt1-mediated degradation of NFAT is currently unknown. A second group found that siRNA knockdown of Akt1, but not Akt2, led to an increase in the migration of mammary epithelial cells (Irie et al., 2005). Loss of Akt1, specifically, led to an increase in the activation of Erk1 and Erk2, which was found to be required for the enhanced migration. Again, the mechanism by which Akt1, but not Akt2, inhibits Erk signaling in this system remains unknown. Interestingly, mouse tumor models have also suggested that Akt1 inhibits metastases (Hutchinson et al., 2004), whereas Akt2 promotes metastases (Arboleda et al., 2003). However, these differential effects of Akt1 and Akt2 on epithelial cell migration may not translate to other cell types. In fact, studies on cell migration using mouse embryonic fibroblasts deficient of specific Akt isoforms have suggested opposite effects on fibroblast migration, with Akt1 promoting migration and with Akt2 inhibiting it (Zhou et al., 2006). These studies demonstrate both the importance of crosstalk between the PI3K-Akt pathway and other pathways and the emerging recognition that the three isoforms of Akt can have distinct cellular functions.

Crosstalk with Other Pathways

Extensive research on the wiring of signal transduction pathways over the past ten years has uncovered a level of complexity dictating that no single pathway operates in isolation and that pathways are small parts of fully integrated networks within the cell. This is certainly true of the PI3K-Akt pathway, and we discuss just three examples here. Several studies have demonstrated that Akt signaling can activate the NF-κB transcription factor downstream of a variety of stimuli, such as tumor necrosis factor (TNFα), and platelet-derived growth factor (PDGF; e.g., Ozes et al., 1999; Romashkova and Makarov, 1999). Although there are likely to be multiple levels of crosstalk between the PI3K-Akt and NF-κB pathways, one mechanism has been attributed to direct phosphorylation of the amino acid residue T23 on IκB kinase α (IKKα) by Akt, thereby leading to activation of this kinase upstream of NF-κB (Ozes et al., 1999). However, to date this connection between Akt and IKKα has not been verified conclusively in vivo. Akt also blocks Erk signaling through inhibition of c-Raf (Raf1; Rommel et al., 1999; Zimmermann and Moelling, 1999). Akt can directly phosphorylate c-Raf on T259, and this can lead to an inhibitory effect of Akt on the Erk pathway (Zimmermann and Moelling, 1999). As with NF-κB, this crosstalk between Akt and Erk signaling is not ubiquitous and appears to occur only in specific settings. In addition to Erk, the stress-activated MAPKs JNK and p38 have also been shown to be inhibited by Akt signaling. Akt can directly phosphorylate the amino acid residue S83 on apoptosis signal-regulated kinase 1 (ASK1, also known as MAPKKK5), which is an upstream activating kinase within the JNK and p38 pathways (Kim et al., 2001). Akt blocks the apoptotic stimulus-induced activation of ASK1 in a manner dependent on S83. Therefore, a balance between PI3K-Akt survival signaling and JNK/p38 apoptotic signaling can be established through such crosstalk.

Substrate Selectivity between Akt Isoforms and Other AGC Kinases

From the discussion above, it should be obvious that Akt phosphorylates lots of different targets involved in a large variety of cellular processes and that it is unlikely that Akt hits all of these targets in every setting. Aside from differences in substrate expression under various conditions, Akt is also likely to have stimulus-specific differences in its substrate utilization. Although there are many possible explanations, including temporal considerations, subcellular localization, and degree of kinase activation, the mechanisms dictating substrate selectivity are poorly understood. Recent observations using mouse embryonic fibroblasts lacking components of the Akt-activating kinase mTORC2 have suggested that phosphorylation of Akt at S473 is required for its ability to properly phosphorylate the N-terminal site on the FOXO proteins, but phosphorylation of Akt-S473 does not appear to be required for Akt-mediated phosphorylation of the other FOXO sites or the sites on TSC2 and GSK3 (Guertin et al., 2006; Jacinto et al., 2006). Therefore, it has been argued that S473 phosphorylation can dictate differential substrate utilization (Jacinto et al., 2006). Another viable explanation is that different substrates, and even different sites on a single substrate, require different levels of Akt activity to reach saturation or to be phosphorylated to an extent that we can detect experimentally (i.e., using available phospho-specific antibodies). To this end, it is has been known for some time that S473 phoshorylation is not essential for growth-factor stimulation of Akt, but it does result in a 5- to 10-fold increase in Akt activity (Alessi et al., 1996a). However, to our knowledge, there are no physiological stimuli that result in T308 phosphorylation in the absence of S473 phosphorylation. Although it is an intriguing possibility, further work is clearly required to support a model in which S473 dictates a substrate-switching mechanism.

An exciting area of Akt research is the increasing number of studies demonstrating isoform-specific functions for Akt. The three Akt isoforms are very similar, especially within their kinase domains, and it is unlikely that they have distinguishable substrate specificities in vitro. Furthermore, Akt1 and Akt2 are expressed ubiquitously, and many cell lines express all three isoforms. However, mouse knockouts have uncovered distinct physiological functions for the three Akt isoforms: Akt1-deficient mice display developmental defects, Akt2-deficient mice have defects in glucose homeostasis, and Akt3-deficient mice show defects in brain development (reviewed in Dummler and Hemmings, 2007). Although combined knockouts have illustrated that functional redundancy exists between the three isoforms, the distinct phenotypes resulting from loss of each individually suggests that there are likely to be functional differences at the cellular level. The first clear example of this was the role of Akt in stimulating Glut4 translocation in adipocytes, where it was found that Akt2 rather than Akt1 was the primary isoform responsible (e.g., Katome et al., 2003). A second example is the one discussed above regarding the differential effects of Akt1 and Akt2 on cell migration (Irie et al., 2005; Zhou et al., 2006). In both instances, there must be an isoform-specific substrate to which the other isoforms have less access or ability to recognize. A recent study on the role of two Akt-S473 phosphatases, PHLPP1 and PHLPP2, which differentially regulate the three Akt isoforms, has emphasized the extent to which Akt substrates are isoform specific (Brognard et al., 2007). A survey of the phosphorylation status of six known Akt substrates following siRNA-mediated knockdown of each of the three Akt isoforms beautifully demonstrated that each isoform has both unique and overlapping substrate specificities. More work is required to determine if the specificities observed pertain to all cell lines and, most importantly, what dictates this specificity.

A further complicating factor in the study of Akt signaling is that other AGC family members can, in certain settings, phosphorylate sites previously characterized to be bona fide Akt targets. This often occurs in situations where a stimulus or genetic lesion strongly activates an AGC kinase in the absence of Akt activation. For instance, S6K1 can phosphorylate GSK3 on its Akt site under conditions of elevated mTORC1-S6K1 signaling, which triggers a negative feedback mechanism blocking growth-factor activation of Akt (Zhang et al., 2006). However, other AGC kinases can also be redundant with Akt under circumstances where Akt remains active and capable of phosphorylating a specific site. This appears to be the case for T32 on FOXO3a, where both Akt and the closely related AGC kinase SGK can phosphorylate this site in response to PI3K-activating stimuli (Brunet et al., 2001). How widespread such compensatory and redundant mechanisms are in Akt signaling is not known, but the potential for alternative regulation should be kept in mind when examining any seemingly well-characterized target in a novel setting.

The Future of Akt Research

Incredible insight into the regulation and function of Akt has been gained over the past ten years. However, the clear recognition that defects in Akt signaling underlie a diverse array of human diseases makes the clarification of downstream targets and functions of central importance to our understanding and treatment of such diseases. As our ability to knockout, knockdown, or pharmacologically inhibit specific Akt isoforms and related AGC kinases has significantly improved, previously identified substrates should be more rigorously confirmed. More advanced animal models, such as knockins encoding catalytically inactive mutants of the three Akt isoforms—which should be less susceptible to functional compensation than complete knockouts—will likely provide a more accurate picture of isoform-specific substrates and functions. Knockin mutants encoding substrates lacking Akt phosphorylation sites will allow a critical determination of the in vivo relevance and

contribution of defined phosphorylation events to specific physiological and pathological states. With respect to determinants of Akt substrate specificity within cells, many difficult questions remain. How do the three Akt isoforms differ in their regulation, localization, and substrate utilization? What is the degree of functional redundancy between the Akt isoforms and other AGC kinases? Which substrates are subjected to such redundancy, and how is this dictated? Do Akt isoforms use docking sites separate from phosphorylation sites to recognize substrates? How do different Akt-activating stimuli impinge on substrate selectivity? It will also be important to further define the role of crosstalk between distinct downstream branches of the PI3K-Akt pathway, as well as with other signaling pathways, in the ultimate physiological outcome of Akt activation. Our ability to grasp the complex circuitry of Akt signaling and to predict the consequences of altering specific components of the larger network will require systems biological approaches as a complement to genetics and biochemistry. Although the past ten years have been spent assembling a parts list, the challenging work of piecing it all together and figuring out how it really works lies ahead.

Supplemental Data

Supplemental Data include one table and can be found with this article online at http://www.cell.com/cgi/content/full/129/7/1261/DC1/.

ACKNOWLEDGMENTS

Work on Akt signaling in the Manning and Cantley laboratories is supported by the American Diabetes Association (B.D.M.) and NIH grants CA122617 (B.D.M.), P01-CA120964 (B.D.M.), GM41890 (L.C.C.), and P01-CA089021 (L.C.C.).

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