KII in the heart may be an appealing therapeutic approach for preventing sudden death following a heart attack. Moreover, ROS-dependent pathology is important in many neurological conditions including Alzheimer's disease (Infanger et al., 2006) and the vascular dysfunction that follows subarachnoid hemorrhage (Zemke et al., 2007). The ability of CaMKII to be directly stimulated by the generation of ROS may connect this versatile enzyme to a variety of other diseases.

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# Substrate Selectivity APPLies to Akt

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**The protein kinase Akt occupies a central position in multiple signaling pathways. Although numerous Akt substrates have been identified, less is known about the factors that regulate specific cellular responses to Akt signaling. In this issue, Schenck et al. (2008) demonstrate that the endosomal protein Appl1 modulates Akt's substrate selectivity to promote cell survival during zebrafish development.**

The serine/threonine protein kinase Akt is activated by a wide range of stimuli, such as growth factors and cytokines (Manning and Cantley, 2007), and has been implicated in many aspects of cellular regulation, including cell survival, growth, proliferation, migration, and metabolism. Likewise, numerous Akt substrates have been identified that mediate these effects (Figure 1). Because phosphorylation of specific Akt substrates determines the cellular response, a major question in Akt signaling is how substrate selectivity is achieved. In this issue, Schenck et al. (2008) provide evidence for the role of spatial regulation in Akt signaling. They show that the binding of Akt to the endosomal protein Appl1 contributes to Akt's substrate selectivity and promotes cell survival in zebrafish development.

Human APPL1 (*a*daptor protein containing *P*H domain, *P*TB domain, and *l*eucine zipper) was originally identified as an Akt-interacting protein (Mitsuuchi et al., 1999). Subsequent studies have established that APPL1 is a direct downstream effector of Rab5 (Miaczynska et al., 2004), a GTPase involved in endocytosis. In new work using a zebrafish model, Schenck et al. now show that downregulation of Appl1 or Appl2 by antisense morpholinos markedly increases apoptosis in tissues that normally have high levels of expression of Appl1 and Appl2 (Schenck et al., 2008). A role for Appl2 in cell survival during development is further supported by genetic mutation analyses.

Previous work has shown that activity of mammalian Akt is enhanced by binding to APPL1 in response to various signals. Similarly, Schenck et al. show that activation of Akt by growth factor stimulation is diminished by morpholino knockdown of Appl1 in zebrafish. Based on these and other findings the authors conclude that Appl1 is essential for the prosurvival function of Akt. They then examined the effect of depleting Appl1 on the phosphorylation of two Akt substrates, glycogen synthase kinase 3 (Gsk3) and tuberous sclerosis protein 2 (Tsc2). Surprisingly, inactivation of Appl1 only decreases the phosphorylation of Gsk3 but not Tsc2 (Figure 1), suggesting that Appl1 may affect the substrate selectivity of Akt in vivo.

The colocalization of APPL1 with Akt and GSK3, but not TSC2, further supports the notion that Appl1 regulates Akt substrate selectivity (Schenck et al., 2008). Human APPL1 is localized in multiple cellular compartments, including endosomes (Miaczynska et al., 2004). Schenck et al. show that stimulation of HeLa cells by insulin-like growth factor 1 (IGF-1) results in a transient colocalization of Akt and APPL1 in endosomes. Importantly, a fraction of APPL1-positive endosomes also display positive staining for GSK3, and IGF-1 treatment

results in rapid dissociation of GSK3 from the APPL1-positive endosomes. Furthermore, coexpression of constitutively active Rab5 induces formation of large endosomes and a striking recruitment of GSK3 to endosomes with APPL1, whereas TSC2 was not recruited. These results establish a correlation between GSK3 phosphorylation and its colocalization with APPL1 and Akt.

To further investigate the functional importance of the endosomal localization of Appl1 for Akt activation in zebrafish, the authors tested whether mutant Appl1 proteins that localize to other cellular compartments could rescue the apoptosis induced by knockdown of endogenous Appl1. The authors found that only endosomal Appl1 supports Akt activation and promotes cell survival; neither nuclear nor soluble Appl1 proteins could rescue apoptosis induced by the knockdown of Appl1. These observations reveal an exciting model in which Appl1 regulates Akt substrate selectivity, possibly by recruiting Akt and its substrate Gsk3 to the same subcellular compartment (Figure 1).

In addition to spatial regulation, previous studies have indicated a role for signal strength in Akt substrate selectivity. The mammalian target of rapamycin complex 2 (mTORC2) is responsible for phosphorylation of Akt at serine 473, which increases kinase activity several-fold (Sarbassov et al., 2005). Genetic deletion of Sin1 (an essential subunit of mTORC2) in mice abolishes serine 473 phosphorylation but has little effect on phosphorylation of threonine 308 in Akt's activation loop. Interestingly, cells lacking Sin1 display a severe defect in the phosphorylation of Foxo1, whereas the effect on Gsk3 and Tsc2 phosphorylation is minor (Jacinto et al., 2006). This suggests that full activation of Akt is required for the phosphorylation of some but not all Akt substrates. Together, these studies demonstrate that Akt substrate specificity can be controlled by multiple mechanisms, including signal strength and spatial regulation.

The report by Schenck et al. provides new insights into the substrate selectivity of Akt but also raises many important questions regarding Akt signaling and APPL1. How does APPL1 enhance Akt phosphorylation? Does it recruit mTORC2 to the endosome? APPL1 is known to interact with numerous cell-surface receptors, including receptors for nerve growth factor, follicle-stimulating hormone, and adiponectin. Does APPL1 play a general role in dictating signaling specificity of these interacting proteins? If APPL1 mainly contributes to cell survival, it should promote phosphorylation of Akt substrates such as BAD and FOX1 that regulate apoptosis but not the phosphorylation of substrates that regulate proliferation, such as p27. Future work may also reveal mechanisms other than spatial regulation and signal strength that contribute to the signaling specificity of Akt.

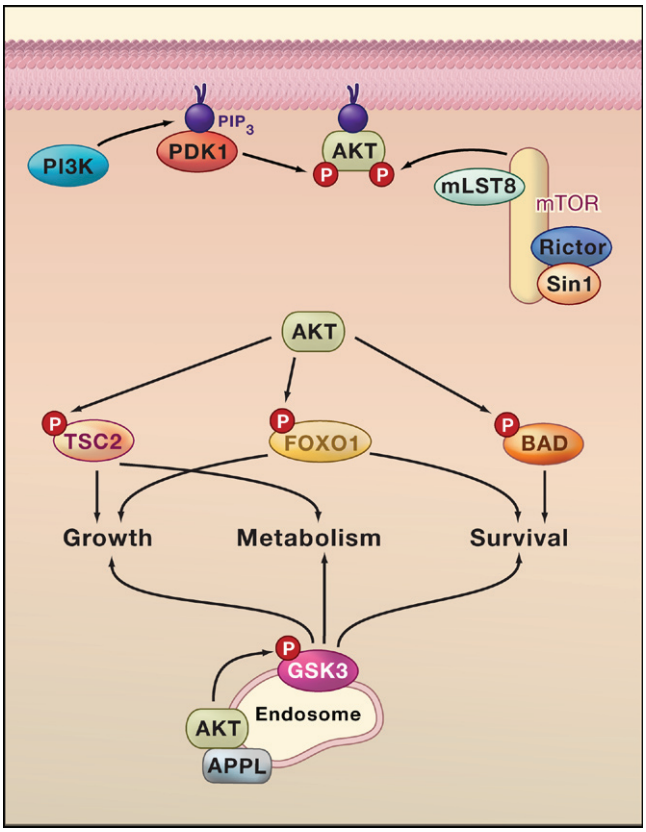


**Figure 1. Akt Activation and Substrate Phosphorylation**

Akt is activated by a wide range of stimuli. For example, activation of a receptor tyrosine kinase by mitogenic growth factors leads to the activation of phosphatidylinositol 3-kinase (PI3K), which catalyzes the formation of a membrane-associated second messenger phosphatidylinositol (3,4,5)-triphosphate ($PIP_3$). $PIP_3$ binds to the PH domain of Akt and recruits Akt to the membrane, where it is activated by phosphorylation on two key residues. Phosphorylation of the activation loop at threonine 308 by PDK1 is essential for Akt kinase activity. Phosphorylation of serine 473 in the C-terminal hydrophobic motif by the mammalian target of rapamycin complex 2 (mTORC2) also contributes to Akt activation. Akt phosphorylated on these two sites is fully active and can subsequently phosphorylate its downstream target proteins, which include GSK3, BAD, and TSC2. The inhibition of GSK3 by Akt regulates cellular metabolism, cell survival, and proliferation (Cross et al., 1995). Phosphorylation of BAD by Akt contributes to cell survival by preventing BAD from binding to Bcl2 family proteins (Datta et al., 1997), whereas phosphorylation of Foxo1 by Akt promotes its retention in the cytoplasm (Brunet et al., 1999). Akt phosphorylates TSC2 on multiple sites and inhibits its tumor suppressor function (Inoki et al., 2002). Schenck et al. (2008) reveal a mechanism by which the substrate specificity of Akt is regulated. They show that binding of Akt to the adaptor protein Appl1 recruits Akt to the endosome where it phosphorylates GSK3 to promote cell survival.

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