Akt is a serine-threonine kinase first identified in mice as the cellular homologue of the v-akt oncogene and identified independently as a kinase related to protein kinases A and C. As a consequence of the latter discoveries, Akt is also referred to as protein kinase B (PKB). There are three closely related mammalian Akts (Akt1/PKBα, Akt 2/PKBβ, and Akt 3/PKBδ) with similar substrate specificities but distinctive tissue distributions. They all share a common structure that consists of an N-terminal regulatory domain with pleckstrin homology (PH), a hinge region connecting the PH domain to the kinase domain, and a C-terminal region required for the induction and maintenance of the kinase activity. Akt sits strategically at the hub of insulin and insulin-like growth factor-1 (IGF-1) signaling, communicating directly with phosphatidylinositol 3-kinase (PI3-kinase) through the membrane lipids phosphatidylinositol 4,5 bisphosphate (PIP2) and phosphatidylinositol 3,4,5 triphosphate (PIP3). The pathway is also activated by growth factors including PDGFβ, cytokines (leukemia inhibitory factor [LIF-1], and cardiotropin), and by adrenergic stimulation. The src homology-2 (SH-2) domain-containing inositol phosphatase SHIP-1/2 and the PTEN phosphatase dephosphorylate Akt are critical mediators of the activity of the pathway.

Studies on the pro-oncogenic activities of Akt kinase first established its pivotal role in cell growth and survival. Akt was shown to promote transformation by suppressing apoptosis and differentiation and increasing cell cycle progression. These activities were subsequently extended to nontumor cells including hematopoietic cells, neuronal cells, and cardiac myocytes, and Akt regulation is now being explored as a possible therapeutic strategy for degenerative diseases to prevent cell loss. Stimulation of the PI3-kinase pathway by IGF-1 or overexpression of constitutively active Akt in the heart protects against ischemia and multiple other apoptotic stimuli. Unfortunately, chronic activation of this pathway can also lead to hypertrophic and dilated cardiomyopathy, heart failure, and sudden death. Therefore if the prosurvival therapeutic potential of Akt is to be realized, it will be necessary to tease apart the different functions and targets of the pathway.

Akt Has Multiple Cytoplasmic and Nuclear Substrates

Akt phosphorylates substrates including regulators of apoptosis and growth in both cytoplasmic and nuclear compartments. Overexpression of constitutively active Akt protects against ischemic damage in vitro and in vivo and because Akt is a central component of insulin signaling, it has been hypothesized that key substrates may be glucose transporters and key enzymes of glycolysis. Phosphorylation of the main cardiac glucose transporter GLUT4 by Akt promotes its membrane translocation and can increase glucose transport by 10- to 40-fold. This may be essential for survival during ischemia; indeed, it has been suggested that survival downstream of Akt relies directly on glucose metabolism. Other potential cytoplasmic Akt targets that are direct regulators of apoptosis include the Bcl-2 family proteins Mcl-1, BAD, and Bcl-XL, caspase-9, c-FLIP, which is a regulator of caspase-8, the apoptosis signal-regulating kinase-1 (ASK-1), and glycogen synthase kinase-3 (GSK-3) (reviewed in ). Potential nuclear targets include the Fas ligand (FasL), Forkhead transcription factors (FOXO), the transcriptional regulator Bcl-6, and the cell cycle regulator p27Kip1. If common Akt target pathways regulate both apoptosis and hypertrophy, it may be difficult to separate the prosurvival properties from the deleterious side effects, especially if both targets are in the same intracellular compartment. The article by Shiraishi et al in this issue of Circulation Research indicates that this may not be the case. In this elegant study, these authors show that Akt targeted exclusively to the nucleus using a nuclear targeting sequence, was at least as effective in protecting cardiac myocytes from ischemic damage as cytoplasmic Akt, but chronic overexpression of the nuclear-targeted Akt did not cause cardiac myocyte hypertrophy or cardiomyopathy. Therefore, it seems probable that one or more essential regulators of apoptosis associated with staurosporine, deoxyglucose, insulin-withdrawal hypoxia, and ischemia are present in the nucleus whereas the cell growth/hypertrophy response requires cytoplasmic as well as nuclear targets.

Role and Mechanism of Akt in Survival

Discrete pathways for the different functions of Akt in the myocardium may have eluded investigators in many instances because of the numerous, sometimes redundant, and seemingly moving intracellular targets of Akt kinase. Negoro et al reported that Akt activation by the interleukin-6–related cytokine LIF-1 protected neonatal cardiac myocytes from doxorubicin-induced apoptosis by phosphorylating BAD and increasing the effective levels of antiapoptotic Bcl-XL. Protection was blocked by dominant-negative Akt. Wu et al reported that IGF-1 pretreatment also protected cardiac myocytes against doxorubicin through PI3-kinase and Akt, but they did not find any change in the phosphorylation of...
BAD.12 Matsui et al observed no change of BAD phosphorylation in hearts overexpressing myr-Akt, even though there was protection against ischemia.6 In contrast, several other groups confirmed that BAD is a substrate for Akt in the heart and can mediate protection by IGF-1 or myr-Akt.13,14 The discovery by Shiraishi et al that cytoplasmic but not nuclear Akt increased BAD phosphorylation while both conditions afford protection suggests that there are multiple targets in both compartments that can confer protection through Akt.10 In a previous study, the Sussman laboratory also reported that Akt levels were higher in the nuclei of young women compared with men or postmenopausal women.15 They suggested that this may contribute to the reduced cardiovascular disease risk in the former group. Importantly, nuclear Akt also correlated with increased cytoplasmic levels of phosphorylated Forkhead transcription factors. These studies as well as an increasing body of data from other sources raise the possibility that FOXO factors are part of the apoptotic pathway activated by ischemia and may be the targets for nuclear Akt-mediated protection.

**Akt Targets Nuclear FOXO**
The FOXO subfamily of Forkhead transcription factors including AFX (acute-lymphocytic-leukemia-1 fused gene from chromosome X), FKHR (Forkhead in rhabdomyosarcoma), and FKHR-L1 (FKHR-like 1) are directly phosphorylated by Akt resulting in nuclear export and inhibition of FOXO-mediated transcription. The FOXO signaling pathway was first identified as a component of life-span regulation in the nematode *Caenorhabditis elegans*. The *C. elegans* Forkhead-related DAF-16 protein transduces insulin receptor signals in a PI3-kinase/Akt homologous signal cascade and regulates the expression of SCL-1, a protein implicated in longevity and stress resistance.16 In higher eukaryotes, the FOXO factors also play key roles in the regulation of cell cycle progression and survival (reviewed in9,16). FOXO factors can activate two different subsets of genes, those that contain FOXO consensus binding sites and others that are regulated independently of FOXO DNA binding, probably by protein-protein interaction. The latter include transcriptional modulators and adaptors such as the p300/CREB-binding protein and steroid receptors. The preferred core sequence for FOXO proteins is 5′-TTGTTTAC-3′, and two copies of this sequence are present in the promoter of the proapoptotic protein Bim, a BH3-only member of the Bcl-2 family. Bim is a target for nuclear FOXO3a (FKHR-1) and initiates apoptosis through the intrinsic (mitochondrial) pathway in T cells during development9 and in sympathetic neurons subjected to nerve growth factor withdrawal or hyperpolarization.17,18 IGF-1 treatment protects against neuronal death by blocking nuclear translocation of FOXO3a and preventing the induction of Bim. In T cells and lymphocytes, FOXO3a can activate both intrinsic and extrinsic death programs by increasing the expression of Bim and Fas ligand, respectively.8,9 The FasL promoter is also induced by FOXO through a consensus binding sequence. Other targets that are regulated by FOXO factors include the CDK inhibitor p27kip,

the retinoblastoma-like protein p130, and factors controlling cytokinesis and cell cycle M to G1 transition. Cardiac myocytes that are terminally differentiated and quiescent may respond to cell cycle induction by undergoing apoptosis.19

**Regulation of FOXO by Akt**
FOXO transcription factors are phosphorylated on multiple threonine (T1 and T2) and serine residues (S1 through S5). Three of these, T1, S1, and S2, are targets for Akt.9,16 Akt-mediated phosphorylation of FOXOs occurs in the nucleus where it both prevents DNA binding and creates docking sites for 14-3-3 proteins. Interaction with 14-3-3 proteins drives nuclear export and anchors the inactive FOXO–14-3-3 complex in the cytoplasm. When FOXO3a is neutralized by sequestration in the cytoplasm, Bim- and FasL-dependent apoptosis are inhibited. In principle, this could be the mechanism for the suppression of apoptosis by nuclear Akt described in the Shiraishi study, although neither FOXO nor Bim has been directly implicated in the models of cardiac myocyte apoptosis described. Indeed, cardiac Bim has not yet been reported. Apoptosis involved in ischemia and infarction has been linked with caspases -9, -8, and -3, and contributions from both intrinsic and extrinsic pathways have been proposed. Modulators of proand antiapoptotic Bcl-2 proteins that regulate the mitochondrial permeability transition pore and changes in the activity of FasR/FasL, respectively, are central to the regulation of these pathways. Therefore, FOXO-regulated Bim and/or FasL are clear candidates for protection by nuclear Akt. Although Shiraishi et al demonstrate that an Akt-regulable nuclear activity is required in each of their models of myocardial apoptosis,16 they have not yet revealed the subcellular location of FOXO factors nor whether Bim or FasL activities are changed in these models.

The inhibition of Bim and FasL by Akt-mediated phosphorylation of FOXO factors are both loss-of-function responses. Because nuclear Akt protected against multiple stimuli with distinct death pathways, it is possible that Akt promotes a gain-of-function response. Protection of neonatal cardiac myocytes against insulin-withdrawal hypoxia is particularly intriguing because this pathway of death is prevented if insulin, IGF-1, or serum is present during hypoxia20 and K.A. Webster, unpublished data). Protection includes Akt-mediated increases of glucose transport and glycolysis with the targets (GLUT4, hexokinase, PFK2) primarily in the cytoplasm. The demonstration that nuclear Akt also protects against this stress may be another example of the multiple survival pathways that are activated by Akt. Clearly, although the Akt/FOXO pathway is an attractive candidate for protection by nuclear-targeted Akt, there may be other targets for this promiscuous, opportunistic kinase that are not yet identified.

**Nuclear Targeting of Akt Avoids Cytoplasmic Targets for Hypertrophy**
Inactive Akt is located principally in the cytoplasm. During receptor-mediated activation, Akt is recruited to the plasma membrane where it undergoes a double phos-
phosphorylation involving autophosphorylation (or phosphorylation by an unidentified kinase) on Ser473 and by PDK1 on Thr308, probably in that order. Phosphorylated Akt leaves the membrane and targets substrates in the cytoplasm and nucleus. Myristillation of the N-terminus of Akt facilitates activation by promoting the association of myristyl-Akt with the plasma membrane but may reduce nuclear translocation and retain more activity in membrane-cytoplasmic compartments. Because nuclear Akt is apparently free of the cardiomyopathic side effects associated with untargeted Akt, the latter may result from aktivation in the cytoplasm. Cytoplasmic targets of Akt that may be responsible for pathological hypertrophy include calcineurin/NFAT, glycogen synthase kinase-3β/GATA4, and the mammalian target of rapamycin (mTOR) (reviewed in ). Glycogen synthase 3-kinase (GSK-3β) is an essential negative regulator of cardiac hypertrophy suppressing the activity of transcription factors (GATA4, NFAT, and MEF2) and translation factors (eEF2Be, 4E-BP, and eEF2). This suppression is relieved when GSK-3β is phosphorylated by Akt so that a major constraint on hypertrophy pathways is removed. Overexpression of Akt in cytoplasmic and nuclear compartments will promote the phosphorylation of GSK-3β, but GSK-3β should remain active and suppression of hypertrophy would persist if Akt overexpression were confined to the nucleus. The spatial and functional dichotomy that may be responsible for the results of Shiraishi et al. is illustrated in the Figure. On a side note, it is interesting that nuclear targeting of Akt is compatible with aktivation because this process is generally believed to take place at the plasma membrane, requiring membrane-bound PI3-kinase and PIP3.

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