BIOCHEMISTRY

Uncaging Akt

Sean J. Humphrey and David E. James*

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Many signal transduction pathways comprise protein kinase cascades in which the activity of each component is controlled by phosphorylation and dephosphorylation. A new layer of kinase regulation involving nucleotide binding has now been unraveled. Phosphorylation of Akt at two regulatory sites plays a major role in kinase activation. New findings show that adenosine triphosphate (ATP) binding to Akt induces an intramolecular interaction between these phosphorylation sites and other domains in the protein, creating a cage around the phosphate group and restricting the access of phosphatases to these sites. ATP hydrolysis and substrate phosphorylation open the cage, which permits dephosphorylation and inactivation of the kinase. This switchlike mechanism provides important new insights into the biology of protein kinases.

In defining the behavior of complex systems, a major goal is to identify "controllers" or molecules whose activity coordinates many outcomes. Not surprisingly, the activity of the controllers is subject to intense scrutiny by regulatory events often involving posttranslational modifications, and disturbances in this balance are at the heart of disease. Since its identification as a serine-threonine kinase (*1*, *2*), Akt has emerged as a critical regulator of diverse cellular processes, including cellular growth and proliferation, metabolism, gene expression, and differentiation. More than 100 substrates of Akt have been identified to date, which makes this kinase a key regulator of a vast number of essential biological functions. Consistent with the diverse roles of Akt, gain-of-function mutations in Akt are linked to cancer in humans (*3*, *4*), whereas loss-of-function mutations cause insulin resistance and type 2 diabetes (*5*). Indeed, in view of the identification of Akt as a proto-oncogene (*6*), it is of little surprise that the pharmaceutical industry has applied considerable effort to identify small-molecule inhibitors of this member of the AGC kinase family. The AGC kinase family contains 60 family members that, on the basis of sequence alignment of the catalytic kinase domain, are most closely related to cyclic adenosine monophosphate (cAMP)–dependent protein kinase A (PKA), cyclic guanosine monophosphate (cGMP)–dependent protein kinases (PKG), and protein kinase C (PKC) (*7*). A challenge in this regard is to develop Akt inhibitors that block tumorigenesis without causing diabetes and other disorders. To achieve this, every facet of Akt regulation must be divulged in order to find a tumor-specific vulnerability that can be therapeutically manipulated.

Two studies now show that the naturally occurring nucleotides ATP and adenosine diphosphate (ADP) themselves play a key role in regulating Akt activity by governing the accessibility of activating phosphorylated residues in Akt to their cognate phosphatases (*8*, *9*). These findings give rise to a model whereby ATP and ADP regulate a conformational switch in Akt that, by con-

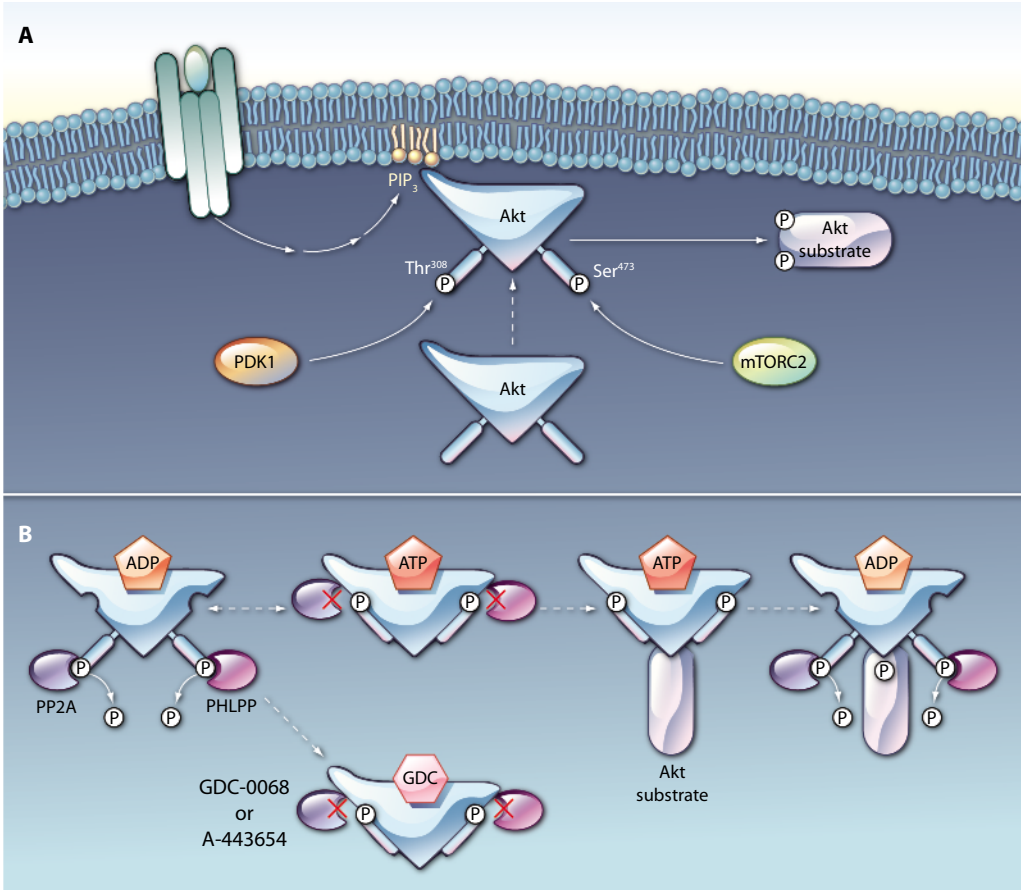

Fig. 1. (**A**) Activation of Akt involves translocation to the plasma membrane and binding to PIP_3 through its PH domain. Upstream kinases PDK1 and mTORC2 phosphorylate Akt at activating residues Thr^{308} and Ser^{473}, respectively. (**B**) When Akt binds ATP or ATP-analog compounds (GDC-0068 or A-443654), it adopts a conformation in which the phosphorylated residues Thr^{308} and Ser^{473} are protected from attack by phosphatases (PP2A and PHLPP). When Akt phosphorylates a substrate, ATP is hydrolyzed to ADP, and Akt returns to a conformation in which Thr^{308} and Ser^{473} are accessible to phosphatases. Solid lines indicate signaling events; dashed lines indicate transitions between different states. P, phospho.

Diabetes and Obesity Program, Garvan Institute of Medical Research, Darlinghurst, New South Wales 2010, Australia.

***Corresponding author. E-mail, d.james@garvan.org.au**

trolling access to inhibitory phosphatases to activating sites in Akt, determines kinase activity. This means that under physiological conditions, Akt toggles back and forth between "open" and "closed" conformations in step with substrate phosphorylation. According to this model, the availability of ATP, as well as that of the substrate, will have a major influence on maintaining kinase activity. In the ATP-bound form, interactions between phosphorylated residues in the catalytic domain of Akt create a cage around these activating residues, blocking their dephosphorylation and keeping the kinase "on." In this way, kinase activity, availability of cellular energy (ATP), and unphosphorylated substrate are coupled together, which ensures kinase fidelity.

Activation of Akt is a multistep process involving translocation to the plasma membrane after a growth factor stimulus and subsequent phosphorylation at key residues Thr^{308} and Ser^{473} by the kinases phosphoinositide-dependent protein kinase 1 (PDK1) and mammalian target of rapamycin complex 2 (mTORC2), respectively (Fig. 1). Thr^{308} lies in the activation or "T-loop" and Ser^{473} in the hydrophobic motif in the C-terminal tail. Phosphorylation of the T-loop is essential for kinase activity, as it stimulates enzymatic activity by >100-fold, whereas phosphorylation of the hydrophobic motif promotes activity a further 10-fold (*10*). Movement of Akt to the membrane is mediated by its lipid-binding pleckstrin homology (PH) domain (*11*), thus linking phosphoinositide-3 kinase (PI3K) activity to activation of Akt (*12*). Moreover, the association of Akt with phosphoinositidyl-3,4,5-phosphate [$PI(3,4,5)P_3$ or PIP_3] not only provides an anchoring mechanism but also promotes a conformational change in Akt that increases kinase activity (*13*, *14*). The diverse regulatory mechanisms governing Akt activity provide distinct targets for small-molecule inhibitors, including those that target the PH domain or the ATP binding pocket, as well as substrate-competitive and allosteric inhibitors.

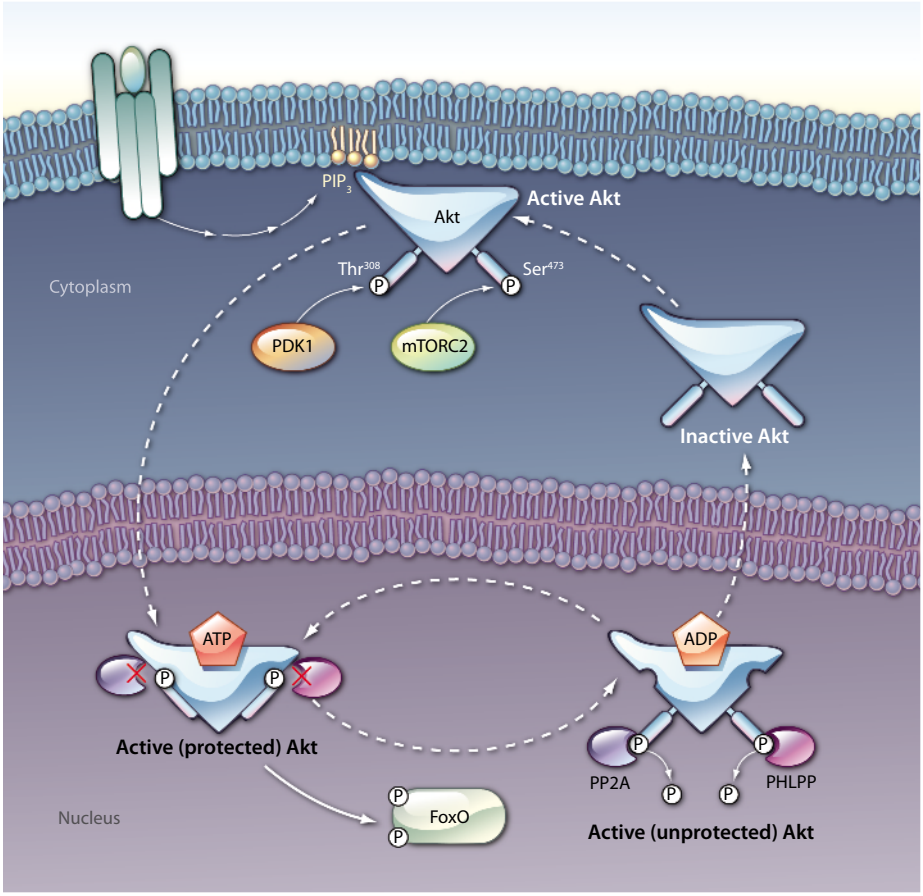

Fig. 2. Once activated at the plasma membrane by PDK1 and mTORC2, Akt can move to distal locations in the cell to phosphorylate its substrates (depicted here phosphorylating FoxO in the nucleus). ATP-dependent caging of the activating residues Thr^{308} and Ser^{473} may serve to protect Akt from inactivation by phosphatases until substrate phosphorylation and concomitant ATP hydrolysis have occurred. At this point, ADP-ATP exchange may occur rapidly, which ensures that Akt remains active, or phosphatases may inactivate Akt necessitating its return to the plasma membrane for reactivation. Solid lines indicate signaling events; dashed lines indicate transitions between different states or translocation between subcellular compartments.

Inactivation of Akt kinase activity in cells is facilitated by dephosphorylation of Thr^{308} and Ser^{473} by protein phosphatase 2A (PP2A) and PHLPP (PH domain and leucine-rich repeat protein phosphatase) (*15*, *16*), respectively. Unexpectedly, several ATP competitive inhibitors caused increased phosphorylation of these two residues while concomitantly reducing Akt kinase activity (*17*). This effect is unlikely to be due to off-target interference with other regulatory mechanisms because a kinase-dead mutant of Akt that can bind an ATP-analog compound maintained the same sustained phosphorylation phenomena in the absence of pathway feedback (*18*), which suggests that this effect occurs through an intrinsic mechanism. Experimentally, Lin *et al*. (*8*) showed, using crystallography and immunoprecipitation assays, that occupancy of the ATP-binding pocket of Akt by the ATP-competitive inhibitor GDC-0068 promotes a conformational change in which the activating residues Thr^{308} and Ser^{473} are protected from phosphatase attack. This explains the observed sustained phosphorylation of these activating sites, despite inactivation of the kinase. The authors also demonstrated that, like the GDC compound, ATP itself promotes the caged conformation that restricts phosphatase access, whereas ADP has the opposite effect. In equally elegant experiments, Chan and colleagues used another ATP-competitive Akt inhibitor (A-443654) and site-directed mutagenesis to demonstrate this intrinsic mechanism of phosphatase caging (*9*). Both studies demonstrated that a key residue, Arg^{273} in Akt1 or Arg^{274} in Akt2, interacts with phosphorylated Thr^{308} and that mutation of this residue obviates the ATP-dependent caging mechanism. This is interesting because this same residue is mutated in patients with an autosomal dominant form of diabetes (*5*), which demonstrates the relevance of this mechanism in maintaining functional Akt activity.

Furthermore, the caging mechanism may not be specific to Akt but may apply to other AGC kinases or protein kinases in general, as similar phenomena of sustained phosphorylation have been observed for PKC (*19*) and mitogen-activated protein kinase kinase (*20*).

One further layer of regulation that remains to be evaluated concerns the membrane binding of Akt and how its PH domain influences the ATP-dependent caging mechanism. Most of the studies by Chan *et al.* (*9*) utilized Myr-Akt, which is constitutively membrane bound. Chan and colleagues also noted that A-443654 blocked dephosphorylation of Akt when attached to beads but not in solution, which raises the possibility that membrane targeting enhances the ATP-dependent caging mechanism. Previous studies that used fluorescent Akt substrate reporters indicate that Akt activity is confined to membranes but not the cytosol (*21*), which suggests that Akt is more susceptible to phosphatase attack in the cytosol, despite the fact that the concentration of ATP should be quite high in the cytosol. This raises a conundrum for kinase regulation because, although some Akt substrates are thought to be phosphorylated at the plasma membrane, others such as the forkhead (FoxO) proteins are thought to be phosphorylated elsewhere in the cell, necessitating a tortuous crossing by Akt of the phosphatase-rich cytosol to these destinations while maintaining its activity. The mechanism described by Lin *et al*. and Chan *et al*. may provide Akt with a means of traversing this route while remaining active until it reaches its substrates at distal locations (Fig. 2).

An intriguing interpretation of these data is that Akt can only phosphorylate one or several molecules of substrate before it is inactivated and must go through a complete on-off cycle before it can continue phosphorylating further substrates, in effect putting the brakes on enzymatic amplification. More likely, however, there is rapid exchange of ADP and ATP after substrate phosphorylation that enables Akt to retain full activity for many cycles. Notably, Chan and colleagues showed that in the case of ATP, a concentration of ~1 mM was required to sustain the phosphatase blocking step (*9*), which approximates the concentration of ATP found in most cells. This introduces one final layer of regulation of kinase activity—the intracellular ATP concentration itself. Many studies have examined the possibility that ATP concentrations may vary in different cellular locations (*22*–*24*). Here again, in the context of tumor cells, an intriguing observation is that in glucose-rich environments, cells maintain their intracellular ATP principally through glycolysis (*24*). One wonders then if this could represent another rationale for the Warburg effect in which tumor cells amplify glycolytic flux to ensure that growth-promoting kinases such as Akt retain maximal enzymatic activity in the face of extreme energy demands.

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