BIOCHEMISTRY

# A perspective on AKT 25-plus years after its discovery

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**Since its discovery more than 25 years ago, the kinase AKT has become a central figure in cell signaling. We highlight some of the landmark findings in those 25 years that contributed to our understanding of the regulation and function of AKT in directing cellular processes and behavior. Future progress toward fully understanding the roles of AKT in cell, tissue, and organismal biology will depend on technological innovations and the combination of in-depth reductionist analyses with systems-based strategies.**

In the course of studies focusing on the factors responsible for the induction of thymomas in AKR mice, Staal *et al.* isolated in 1977 the acute transforming retrovirus AKT8 (*1*). Thirteen years later, it was shown that the oncogene transduced by this virus encodes a serine/threonine protein kinase (AKT) (also known as PKB), which harbors an N-terminal regulatory domain (now known as the PH domain) and exhibits a high degree of homology with the kinases PKC and PKA (*2*–*5*). Soon after its discovery, it became evident that the AKT kinase, which has three closely related but functionally distinct isoforms encoded by paralogous genes (*6*), defines a critical node in the network of cell signaling and that its deregulation gives rise to multiple diseases, including cancer and metabolic, neuropsychiatric, degenerative, and immune dysfunction–associated diseases.

The observation that placed AKT in center stage came a few years after its initial discovery, when it was shown that its activation by external signals depends on phosphatidylinositol 3-kinase (PI3K)–generated phosphoinositides and that the process of activation is PH domain–dependent (*7*). Linking AKT activation to PI3K-generated phosphoinositides via the PH domain not only identified AKT as the first bona fide PI3K target but also identified a novel protein domain that relays PI3K-transduced signals. Defining the function of the PH domain, in turn, led to the identification and characterization of a multitude of PI3K-dependent signaling molecules.

Today, we know that the abundance of PI3K-generated D3-phosphorylated phosphoinositides depends, in addition to PI3K, on several phosphoinositide-modifying enzymes, including the phosphatases PTEN, SHIP, and INPP4B and the kinases PIP4K, PIP5K, PIK3C2A, and IP6K1, and that these enzymes also contribute to AKT regulation. In addition, we know that the binding of the PH domain of AKT to membrane-associated $PI(3,4)P_2$ and $PI(3,4,5)P_3$, which are induced via PI3K activation, is required for the translocation of AKT to the membrane, where it is activated via T loop phosphorylation ($Thr^{308}$) by phosphoinositide-dependent kinase 1 (PDK1) (*8*) and hydrophobic motif phosphorylation ($Ser^{473}$) by mammalian target of rapamycin complex 2 (mTORC2) (*9*). In addition to illuminating the mechanism of AKT activation by external signals, this work generated a blueprint for deciphering the mechanism of activation of other PH domain–containing kinases.

The discovery of these basic aspects of AKT regulation, followed by the discovery of a multitude of AKT targets, linked AKT to major signaling networks that control membrane, cytoplasmic, organellar, nuclear, and cytoskeletal activity. This work is the subject of more than 60,000 publications over the last 25-plus years, linking the PI3K-AKT pathway to cell, tissue, and organismal biology.

Despite significant progress to date, important questions on the regulation and function of AKT remain. Some of these questions relate to the topology of AKT activation, which have not been addressed adequately because of technological limitations. Such questions include, but are not limited to, the mechanism(s) by which external signals promote the rapid transport of AKT to the membrane, where it is anchored by binding to D3-phosphorylated phosphoinositides; the mechanism(s) coordinating AKT phosphorylation at $Thr^{308}$ and $Ser^{473}$ by kinases that may be localized in different cellular membranes (PDK1 in the plasma membrane and mTORC2 in the endoplasmic reticulum) (*8*, *10*); the mechanism(s) by which AKT activated in cellular membranes reaches its targets in different cellular compartments; and the mechanism(s) by which AKT may be activated directly in endomembranes in different subcellular locations.

Other outstanding questions relate to the complexity of AKT signaling and biological systems where AKT is operating. Such questions focus on the mechanism(s) responsible for the differences between AKT isoforms and the biological importance of these differences in complex biological systems, such as cancer, the complexity of AKT posttranslational modifications and their biological output (Fig. 1), and the complexity emerging from the integration of AKT signals with the cellular signaling landscape. This complexity is likely to be responsible for the fact that the predicted impact of targeting AKT in cancer and other human diseases is, as yet, an unfulfilled promise. Future success of AKT-targeted therapies will depend on the imaginative application of new research tools that combine in-depth reductionist analyses and systems-based strategies.

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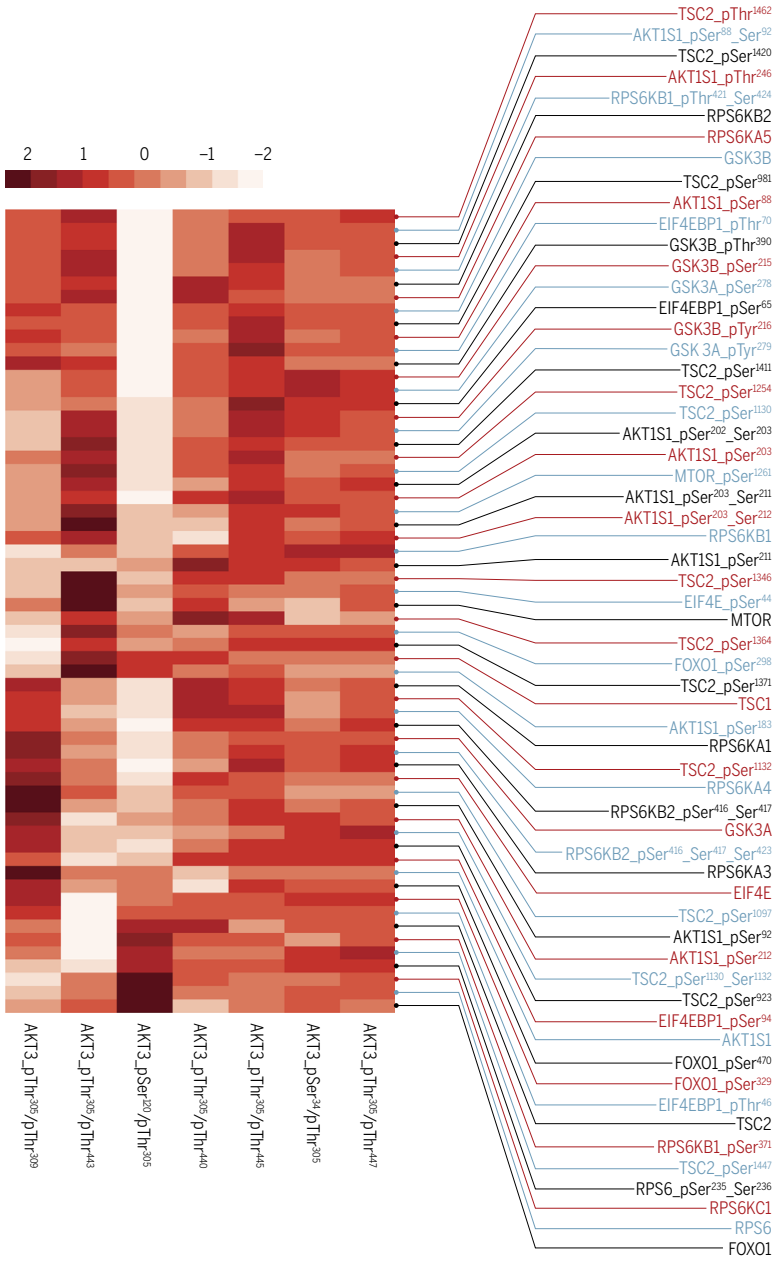
**Fig. 1. AKT targets are distinctly modulated by differential phosphorylation of active (Thr$^{305}$-phosphorylated) AKT3.** The phosphorylation of a set of AKT substrates and the abundance of a set of proteins encoded from potential AKT target genes are modulated differently when AKT3 is phosphorylated at sites in tandem with Thr$^{305}$ (the "T-loop" phosphorylation site required for kinase activation). Therefore, such phosphorylation events may alter the signaling output and the cellular behavior elicited by Thr$^{305}$-phosphorylated AKT. The present heatmap is proof of principle for a strategy that may offer clues to the biological significance of post-translational modifications of AKT that are frequently observed in various cancers. Further analyses are needed to thoroughly address statistical strength and confidence. The heatmap was generated from data on 105 cases of breast cancer available in the CPTAC Data Portal (https://cptac-data-portal.georgetown.edu/cptacPublic/) (*11*) that we accessed through the cBioPortal pipeline (https://github.com/cBioPortal/CPTAC-proteomics-pipeline) (*12*), which combined the proteomics and phosphoproteomics data in a single file and eliminated three tumors that had been assayed as technical replicates. [Identical results can be obtained by downloading the data from the cBioPortal repository (www.cbioportal.org/study?id=brca_tcga) in a format directly importable to downstream analyses.] The phosphorylation and expression events in the heatmap were selected because they were the most robust across the set of 102 tumors we analyzed. Each cell in the heatmap presents the mean relative abundance of a protein or a phosphorylated protein in tumors that exhibited dual phosphorylation of AKT3 at Thr$^{305}$ and another site. Relative values in individual cells were centered and standardized using row *z* scores; warmer colors correspond to higher relative abundance.





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