# Lipid activation of protein kinases

Alexandra C. Newton<sup>1</sup>

Department of Pharmacology, University of California at San Diego, La Jolla, CA 92093-0721

Abstract Lipids acutely control the amplitude, duration, and subcellular location of signaling by lipid second messengerresponsive kinases. Typically, this activation is controlled by membrane-targeting modules that allosterically control the function of kinase domains within the same polypeptide. Protein kinase C (PKC) has served as the archetypal lipidregulated kinase, providing a prototype for lipid-controlled kinase activation that is followed by kinases throughout the kinome, including its close cousin, Akt (protein kinase B). This review addresses the molecular mechanisms by which PKC and Akt transduce signals propagated by the two major lipid second messenger pathways in cells, those of diacylglycerol signaling and phosphatidylinositol-3,4,5-trisphosphate (PIP<sub>3</sub>) signaling, respectively.—Newton, A. C. Lipid activation of protein kinases. J. Lipid Res. 2009. 50: S266–S271.

 $\label{eq:supplementary key words protein kinase C \bullet Akt \bullet diacylglycerol \bullet phosphatidylinositol-3,4,5-tris phosphate$ 

Cellular membranes form a platform of intense signaling activity. Serving as the site where extracellular signals are first received by the cell, they not only recruit and activate effector molecules, but they also provide a spring board to launch activated effector molecules throughout the cell. Protein kinases comprise one of the most common classes of effector molecules that transduce signals emanating from the plasma membrane. These kinases can be embedded in the plasma membrane, exemplified by the tyrosine kinase growth factor receptors, or can be either soluble or amphipathic membrane proteins that translocate on and off cellular membranes in response to appropriate signals. Soluble proteins are recruited to membranes by protein scaffolds, but there exists a class of amphipathic membrane kinases whose members directly bind lipid second messengers via specific membrane-targeting modules. It is this latter class of lipid-controlled kinases that forms the focus of this review.

Despite the enormity of the kinome, few kinases (approximately 10%) directly bind and transduce lipid second messenger signals. Yet they transduce signals in two of the most pivotal signaling pathways in cells, notably the di-

acylglycerol and phosphatidylinositol 3 kinase pathways. To this end, lipid second messenger-regulated protein kinases contain modules that bind with high specificity and affinity to the relevant lipid second messengers (1, 2): the C1 domain is the cell's diacylglycerol sensor (3) and the pleckstrin homology (PH) domain, or related modules such as the phox (PX) domain, sense 3'-phosphoinositides (4). Other domains such as the C2 domain can assist in membrane recruitment of the kinase by interaction with specific phospholipids, in some cases by a Ca<sup>2+</sup>-triggered mechanism (5, 6). Thus, lipid second messenger-regulated kinases contain one or more membrane-targeting modules whose membrane engagement results in protein kinase activation, typically by relieving autoinhibitory constraints.

## CONTROL OF KINASES BY MEMBRANE-TARGETING MODULES

Figure 1 illustrates the modular architecture of some of the major lipid second messenger-sensing Ser/Thr protein kinases. With the exception of protein kinase D, which belongs to the Ca<sup>2+</sup>/calmodulin kinase branch of the kinome, these kinases are all members of the AGC (protein kinases A, G, and C) branch of the kinome, where they account for approximately one-third of the members of this branch. Members include the protein kinase C (PKC) family, of which there are 10 isozymes distributed among three subfamilies, conventional, novel, and atypical (7, 8). Conventional and novel PKC isozymes contain diacylglycerol-binding C1 domains (orange) and transduce signals that trigger diacylglycerol production. Protein kinase D also contains a diacylglycerol-binding C1 domain (9, 10). But some variants of this domain, found in atypical PKC isozymes and in Raf, do not bind diacylglycerol (3); however they still maintain the natural affinity of C1 domains for the anionic phospholipid, phosphatidylserine (11). Other kinases in this group contain domains that recognize various 3'-phosphoinositides. Notably, members of the Akt family of kinases contain a PH

**OURNAL OF LIPID RESEARCH** 

This work was supported in part by National Institutes of Health GM43154. Manuscript received 28 October 2008 and in revised form 14 November 2008. Published, JLR Papers in Press, November 24, 2008. DOI 10.1194/jlr.R800064-JLR200

Abbreviations: AGC kinases, protein kinases A, G and C; PDK-1, phosphoinositide-dependent kinase-1; PH, pleckstrin homology; PHLPP, PH domain Leucine-rich repeat protein phosphatase; PIP<sub>3</sub>, phosphatidyl-inositol-3,4,5-trisphosphate; PKC, protein kinase C; PX, phox.

<sup>&</sup>lt;sup>1</sup>To whom correspondence should be addressed.

e-mail: anewton@ucsd.edu

Copyright © 2009 by the American Society for Biochemistry and Molecular Biology, Inc.



**Fig. 1.** Domain composition of major Ser/Thr kinase families with membrane-targeting modules. Membrane-targeting modules are the C1 domain (orange), C2 domain (yellow), pleckstrin homology (PH) domain (dark purple), phox (PX) domain (light purple); the pseudosubstrate of protein kinase C (PKC) family members is shown in green, the kinase core in cyan, the C-terminal tail (CT) in pink. Additional protein-interaction domains present on these kinases are shown in gray [antiparallel coiled coil (ACC); Ras binding domain (RBD); regulator of G protein signaling (RGS); coiled coil (CC); Bem1 (PB1)]. ROCK, Rho-activated kinase. GRK-2, G-protein coupled receptor kinase-2; PDK-1, phosphoinositide-dependent kinase-1; PKD, protein kinase D; PKN/PRK, protein kinase N/protein kinase C-related kinase; SGK-3, serum/glucocorticoid regulated kinase 3.

domain (purple) that selectively recognizes phosphatidylinositol-3,4,5-trisphosphate (PIP<sub>3</sub>), thus serving as one of the major mechanisms to transduce signals that activate phosphatidylinositol 3 kinase (12). A PH domain is also found in one of the G protein receptor coupled kinases, GRK2 (13). Phosphoinositide-dependent kinase-1 (PDK-1), the upstream kinase for most of the AGC kinases shown in Fig. 1, also has a PH domain. Although it binds phosphoinositides with high affinity, this kinase is constitutively active in cells, with substrate conformation controlling down-stream signaling (13, 14). Not all PH domains bind phosphoinositides: although a PH domain is found in the Rho-activated kinase, it functions as a protein-binding module (15). The PH domain is the module most commonly used to sense 3'-phosphoinositides by protein kinases, but note that some kinases such as serum glucocorticoid kinase-3 have a related PX domain (blue); the selective affinity of the PX module for phoshatidylinositol-3-monophosphate localizes SGK-3 to endosomes, where this lipid accumulates (16). Some lipid-activated kinases employ a third lipidbinding module, the C2 domain (yellow) (6, 17). In the case of conventional PKC isozymes, this module binds anionic phospholipids in a Ca<sup>2+</sup>-dependent manner. In the case of novel PKC isozymes and protein kinase N, the domain does not appear to function as a lipid-sensing module. It is noteworthy that for each of the major lipid-binding modules noted above, the C1, C2, and PH domains, there exist variants that do not bind lipids, as exemplified by the novel C2 domain found in novel PKCs and protein kinase N, the atypical C1 domains found in atypical PKC isozymes and Raf, and the protein-interacting PH domain in Rhoactivated kinase.

A common theme in the regulation of amphipathic membrane kinases that translocate on and off membranes is the coordinated use of two membrane-targeting domains (18). The affinity of one interaction is typically too low to allow membrane recruitment and activation, but the coordinated binding of both modules to the membrane is sufficient to activate the kinase there. This is best illustrated by the conventional PKC isozymes, which are activated by signals that elevate diacylglycerol and intracellular  $Ca^{2+}$  (19, 20); the affinity of each membrane-targeting module (the C1 and C2 domains) is too low to allow efficient membrane recruitment when only one second messenger is elevated (each domain has an apparent membrane binding constant on the order of  $10^3 \text{ M}^{-1}$ ). But when both second messengers engage their respective modules, the kinase is effectively recruited to membranes through a highaffinity membrane interaction, which provides the energy to release autoinhibition of the kinase (apparent binding constants on the order of  $10^6 \text{ M}^{-1}$ ) (21). The myristoylelectrostatic switch, which reversibly controls the membrane interaction of the tyrosine kinase Src, is another example (22); for this protein, an N-terminal myristic acid and an adjacent stretch of basic residues comprise the two membranetargeting modules. As with the membrane-binding domains of conventional PKC isozymes, each determinant binds membranes with too low affinity to allow efficient membrane recruitment (binding constants on the order of  $10^3-10^4$  M<sup>-1</sup> per module), but the coordinated engagement of both determinants allows high-affinity membrane binding (binding constants on the order of  $10^7$  M<sup>-1</sup>) (23). This coordinated use of two modules allows ultrasensitivity

in reversible control of membrane translocation. In the case of conventional PKC, a drop in either diacylglycerol or  $Ca^{2+}$  lowers the affinity of PKC for membranes sufficiently to inactivate the kinase; in the case of Src, phosphorylation of the basic segment alters the electrostatic



Fig. 2. PKC and Akt are regulated by two mechanisms in common: lipid second messengers and phosphorylation. A: Cartoon showing details of the regulation of conventional PKC: newly-synthesized conventional PKC associates with the membrane in an open conformation in which the pseudosubstrate (green rectangle) is expelled from the substrate-binding cavity of the kinase domain (blue circle) and the upstream kinase, phosphoinositide-dependent kinase-1 (PDK-1) (pink/purple), is docked on the C-terminal tail. Phosphorylation at the activation loop (pink circle, Thr500 in PKC  $\beta$ II) is generally proposed to be first and to be followed by two ordered phosphorylations at the C-terminal tail, the turn motif (orange circle, Thr641 in PKC βII) and then the hydrophobic motif (green circle, Ser660 in PKC βII) (see step "1. Phosphorylation"). The phosphorylation of the turn motif depends on the mTORC2 complex (orange oval); this phosphorylation triggers autophosphorylation of the hydrophobic motif. The fully-phosphorylated "mature" PKC is released into the cytosol in a closed conformation in which the pseudosubstrate occupies the substrate-binding cavity, thus autoinhibiting the kinase (bottom left species of PKC). Signals that cause hydrolysis of phosphatidylinositol-4,5-bisphosphate result in translocation of PKC to the membrane (see step "2. Translocation"). Specifically, binding of  $Ca^{2+}$  to the C2 domain (yellow) recruits PKC to the membrane by a low-affinity interaction where it binds diacylglycerol via the C1 domain (orange). Engaging both the C1 and C2 domains on the membrane results in a high-affinity membrane interaction that results in release of the pseudosubstrate, allowing downstream signaling (top right species of PKC). Membrane translocation is reversible and driven by changes in second messenger levels. The membrane-bound conformation is highly phosphatase-sensitive, so that prolonged membrane binding results in dephosphorylation of PKC by PH domain Leucine-rich repeat protein phosphatase (PHLPP) (red) and PP2A, and subsequent degradation (see step "3. Dephosphorylation"). Binding of Hsp70 (yellow) to the dephosphorylated turn motif on the C terminus stabilizes PKC, allowing it to become rephosphorylated and reenter the pool of signaling-competent PKC. Note that the phosphorylation step is constitutive, and the translocation and dephosphorylation are agonist-evoked. PKC that is not rescued by Hsp70 is ubiquitinated by E3 ligases such as the recently discovered RINCK and degraded. B: Cartoon showing details of the regulation of Akt; newly synthesized Akt is phosphorylated on the turn motif (orange circle, Thr450 in Akt1) by a mechanism that depends on mTORC2 (orange oval). Signals that generate phosphatidylinositol-3,4,5-trisphosphate (PIP<sub>3</sub>) engage the PH domain and thus recruit Akt to the plasma membrane (see step "1. Translocation"). Membranebinding exposes the activation loop, resulting in phosphorylation by PDK-1 (pink circle, Thr308 in Akt1) and subsequent phosphorylation on the hydrophobic motif (green circle, Ser473 in Akt1) (see step "2. Phosphorylation"). The fully phosphorylated species of Akt is locked in an active conformation and diffuses throughout the cell to mediate down-stream signaling (bottom right species). Signaling is terminated by dephosphorylation of the lipid second messengers and direct dephosphorylation of Akt, catalyzed in part by the recently discovered PH domain Leucine-rich repeat protein phosphatase phosphatases (PHLPP) (red), which directly dephosphorylate the hydrophobic motif (see step "3. Dephosphorylation").

SBMB

Downloaded from www.jlr.org at Univ of California - San Diego Serials/Biomed Lib 0699 on May 12, 2009



Fig. 2.—*Continued*.

potential sufficiently to lose membrane tethering, releasing Src from the membrane. Thus, the coordinated use of two membrane-targeting modules is an effective mechanism to decrease basal signaling and to provide high sensitivity in the reversible membrane interaction in response to appropriate signals.

### PKC AND AKT

Engaging their membrane-targeting modules with the appropriate lipid ligand allosterically controls the activity of PKC and Akt, but by different mechanisms for each kinase. In the case of PKC, engaging the membrane-targeting modules on the membrane, with sufficiently high affinity, produces a conformational change that releases an autoinhibitory pseudosubstrate segment from the substrate-binding cavity, thus allowing substrate binding and downstream signaling. This active conformation depends acutely on lipid binding. In the case of Akt, engaging the PH domain on membranes serves the purpose of allowing priming phosphorylations of Akt. Once phosphorylated at two key positions, Akt is locked in an active conformation, and, unlike PKC (which is constitutively phosphorylated at priming sites), activity is independent of lipid binding. Rather, activity is acutely controlled by the phosphorylation state of Akt. Thus, lipid allosterically controls the activity of a constitutively phosphorylated PKC, whereas lipid allosterically controls the agonist-evoked phosphorylation of Akt, an event that dissociates Akt activity from lipid binding.

The different ways that lipids control the activity of PKC and Akt are presented in **Fig. 2** where the maturation and activation cycle of each kinase is illustrated. Conventional PKC is constitutively phosphorylated at three key sites, the activation loop, the turn motif, and the hydrophobic motif, by mechanisms that depend on the upstream kinases PDK-1 and mTORC2, a complex containing the kinase TOR (24-27) (Fig. 2A). The fully phosphorylated enzyme localizes to the cytosol in an autoinhibited conformation in which an autoinhibitory pseudosubstrate occupies the substrate-binding cavity (Fig. 2A, bottom left molecule). Signals that cause hydrolysis of phosphatidylinositol-4,5-bisphosphate, in particular, elevate intracellular  $Ca^{2+}$  and diacylglycerol. The immediate consequence for PKC is the binding of  $Ca^{2+}$  to the C2 domain of PKC, an event that alters the electrostatic potential of the domain so that upon the next diffusion-driven membrane encounter it is retained at the membrane. The enzyme then diffuses back and forth in two-dimensional space until it engages its membrane-bound ligand, diacylglycerol, on the C1 domain. Binding of the C1 and C2 domains to membranes provides the energy to release the pseudosubstrate from the substrate-binding cavity, thus allowing substrate phosphorylation (Fig. 2A, top right molecule). Because release of the pseudosubstrate simply depends on the binding energy of PKC to the membrane, ligands such as phorbol esters, which bind the C1 domain with two orders of magnitude higher affinity than diacylglycerol, are able to recruit PKC to membranes with sufficiently high affinity to release the pseudosubstrate, in the absence of C2 domain binding to membranes. Similarly, sufficiently high levels of Ca<sup>2+</sup> can recruit PKC to membranes and cause pseudosubstrate release in the absence of C1 domain engagement. PKC reversibly translocates on and off membranes in response to second messenger levels. However, the membranebound conformation is sensitive to dephosphorylation and, upon prolonged activation, becomes dephosphorylated, ubiquitinated, and degraded. The dephosphorylated species binds the molecular chaperone Hsp70, which allows the enzyme to become rephosphorylated and re-enter the

**JOURNAL OF LIPID RESEARCH** 

pool of signaling-competent enzyme. Thus, for PKC, lipid binding allosterically controls the activity of the constitutively phosphorylated enzyme by modulating the pseudosubstrate.

The role of lipids in Akt activation is to allow key priming phosphorylations. Akt is constitutively phosphorylated at only one site, the turn motif, by a mechanism that (like that of PKC) depends on mTORC2 (28). However, full phosphorylation requires membrane binding (Fig. 2B). Specifically, the PH domain of Akt binds agonist-produced PIP<sub>3</sub>, an event that exposes the activation loop for phosphorylation by PDK-1, the same upstream kinase that modifies PKC; this phosphorylation is rapidly followed by phosphorylation of the hydrophobic motif. Once fully phosphorylated, Akt is locked in an active conformation and diffuses throughout the cell phosphorylating downstream substrates. Dephosphorylation catalyzed in part by the phosphatase PH domain Leucine-rich repeat protein phosphatase terminates signaling by the kinase. Thus, for Akt, lipid binding allows phosphorylation, the key event in locking Akt in a catalytically-competent conformation.

SBMB

**OURNAL OF LIPID RESEARCH** 

The advent of genetically encoded reporters revolutionized the study of the spatiotemporal dynamics of PKC signaling (19, 29-32). The ability to simultaneously visualize PKC translocation, PKC activity, and the second messengers diacylglycerol and Ca<sup>2+</sup> has revealed that PKC isozymes have a unique signature of activation depending on the cellular location (33). In response to agonists such as uridine-5'-triphosphate that activate G protein-coupled receptors and cause Ca<sup>2+</sup> and diacylglycerol levels to rise, conventional PKCs are rapidly recruited to, and activated at, the plasma membrane, with the kinetics of activation (**Fig. 3A**, blue line) mirroring the rise in  $Ca^{2+}$  (Fig. 3, black line) (33). This rise in  $Ca^{2+}$  is followed by a rise in plasma membrane diacylglycerol, and it is the diacylglycerol levels (Fig. 3A, orange line) that then sustain the activity of membrane-bound PKC. Some agonists cause oscillations in Ca<sup>2+</sup> levels, which in turn cause oscillations in PKC activity; if diacylglycerol levels remain elevated, PKC can remain membrane bound but the activity oscillates depending on whether Ca<sup>2+</sup> levels are high and the C2 domain is membrane-engaged (and thus the pseudosubstrate is expelled from the substrate-binding activity), or low such that the C2 domain is not membrane-engaged (and thus the pseudosubstrate occupies the substratebinding cavity) (31). Diacylglycerol levels at the Golgi are significantly elevated compared with the plasma membrane under basal conditions, and, in addition, agonist-evoked increases of this lipid second messenger are much more sustained at the Golgi compared with the plasma membrane. The unique profile of diacylglycerol at Golgi produces, in turn, a PKC signature unique to Golgi; not only is there preferential recruitment of novel PKCs, which have an intrinsically higher affinity for diacylglycerol because of a C1 domain tuned for tighter binding to diacylglycerol (34, 35), but the agonist-evoked activity at Golgi is much more prolonged than at the plasma membrane (33).

Reporters for Akt activity have revealed a wave of phosphorylation that emanates from the plasma membrane to the nucleus (36). Thus, PDGF-treatment of NIH3T3 cells



**Fig. 3.** Initial activation of PKC and Akt is driven by second messenger levels but only PKC requires sustained increase in second messenger levels for sustained activity. A: Graph showing the agonist-evoked rise in intracellular Ca<sup>2+</sup> (black line), diacylglycerol levels at the plasma membrane (orange line), and PKC activity at the plasma membrane (blue line) simultaneously reported by probes for Ca<sup>2+</sup>, diacylglycerol, and PKC activity. Initial activation is driven by Ca<sup>2+</sup>, which recruits conventional PKC to the plasma membrane, but sustained activity depends on diacylglycerol. Adapted from Gallegos et al. (33). B: Graph showing agonist-evoked rise in PIP<sub>3</sub> levels at the plasma membrane (orange line), Akt activity at the plasma membrane (dark blue line), in the cytosol (medium blue line), and in the nucleus (light blue line). Adapted from Kunkel et al. (36).

triggers rapid Akt activation at the plasma membrane (Fig. 3B, dark blue line), with kinetics that parallel PIP<sub>3</sub> formation (half-time of approximately 0.5–1 min) (Fig. 3B, orange line); activity then migrates to the cytosol with a half-time of 3–5 min (medium blue line), and, finally, activity is observed in the nucleus with a half-time of approximately 10 min (light blue line). Activity at the plasma membrane and nucleus is long-lived, whereas that in the cytosol is short-lived.

#### CONCLUSION

Lipid second messengers relay an abundance of signals that control cell growth and survival, among other key cellular functions. Direct binding of protein kinases to these lipid second messengers serves as a first step to transduce information into the depths of the cell. The activity of these kinases is exquisitely controlled, and deregulation of this activity results in pathological states, most notably cancer (37, 38). This review has highlighted how two of the major lipid-regulated kinases, PKC and Akt, use membrane-targeting modules to directly bind lipid second messengers, triggering conformational changes that permit activation.

I thank members of my lab and Tony Hunter for helpful discussions.

#### REFERENCES

 Hurley, J. H. 2006. Membrane binding domains. *Biochim. Biophys.* Acta. 1761: 805–811.

BMB

**JOURNAL OF LIPID RESEARCH** 

- Lemmon, M. A. 2008. Membrane recognition by phospholipidbinding domains. Nat. Rev. Mol. Cell Biol. 9: 99–111.
- Hurley, J. H., A. C. Newton, P. J. Parker, P. M. Blumberg, and Y. Nishizuka. 1997. Taxonomy and function of C1 protein kinase C homology domains. *Protein Sci.* 6: 477–480.
- Misra, S., G. J. Miller, and J. H. Hurley. 2001. Recognizing phosphatidylinositol 3-phosphate. *Cell.* 107: 559–562.
- Hurley, J. H., and T. Meyer. 2001. Subcellular targeting by membrane lipids. *Curr. Opin. Cell Biol.* 13: 146–152.
- Newton, A. C. 1995. Protein kinase C. Seeing two domains. *Curr. Biol.* 5: 973–976.
- 7. Nishizuka, Y. 1995. Protein kinase C and lipid signaling for sustained cellular responses. *EASEB J.* **9:** 484–496.
- Gould, C. M., and A. C. Newton. 2008. The life and death of protein kinase C. *Curr. Drug Targets.* 9: 614–625.
- Yang, C., and M. G. Kazanietz. 2003. Divergence and complexities in DAG signaling: looking beyond PKC. *Trends Pharmacol. Sci.* 24: 602–608.
- Wang, Q. J. 2006. PKD at the crossroads of DAG and PKC signaling. Trends Pharmacol. Sci. 27: 317–323.
- 11. Johnson, J. E., J. Giorgione, and A. C. Newton. 2000. The C1 and C2 domains of protein kinase C are independent membrane targeting modules, with specificity for phosphatidylserine conferred by the C1 domain. *Biochemistry*. **39**: 11360–11369.
- Manning, B. D., and L. C. Cantley. 2007. AKT/PKB signaling: navigating downstream. *Cell.* 129: 1261–1274.
- Ribas, C., P. Penela, C. Murga, A. Salcedo, C. Garcia-Hoz, M. Jurado-Pueyo, I. Aymerich, and F. Mayor, Jr. 2007. The G protein-coupled receptor kinase (GRK) interactome: role of GRKs in GPCR regulation and signaling. *Biochim. Biophys. Acta.* 1768: 913–922.
- Mora, A., D. Komander, D. M. van Aalten, and D. R. Alessi. 2004. PDK1, the master regulator of AGC kinase signal transduction. *Semin. Cell Dev. Biol.* 15: 161–170.
- Schmandke, A., A. Schmandke, and S. M. Strittmatter. 2007. ROCK and Rho: biochemistry and neuronal functions of Rho-associated protein kinases. *Neuroscientist.* 13: 454–469.
- Tessier, M., and J. R. Woodgett. 2006. Serum and glucocorticoidregulated protein kinases: variations on a theme. *J. Cell. Biochem.* 98: 1391–1407.
- Ponting and Parker. 1996. Extending the C2 domain Family: C2's in PKCs δ, ε, η, θ, phospholipases, GAPs and perforin. *Protein Sci.* 5: 162–166.
- 18. Newton, A. C., and J. E. Johnson. 1998. Protein kinase C: a para-

digm for regulation of protein function by two membrane-targeting modules. *Biochim. Biophys. Acta.* **1376**: 155–172.

- Oancea, E., and T. Meyer. 1998. Protein kinase C as a molecular machine for decoding calcium and diacylglycerol signals. *Cell.* 95: 307–318.
- Reither, G., M. Schaefer, and P. Lipp. 2006. PKCalpha: a versatile key for decoding the cellular calcium toolkit. J. Cell Biol. 174: 521–533.
- Newton, A. C. 2001. Protein kinase C: structural and spatial regulation by phosphorylation, cofactors, and macromolecular interactions. *Chem. Rev.* 101: 2353–2364.
- McLaughlin, S., and A. Aderem. 1995. The myristoyl-electrostatic switch: a modulator of reversible protein-membrane interactions. *Trends Biochem. Sci.* 20: 272–276.
- Resh, M. 1994. Myristylation and palmitylation of Src family members: the fats of the matter. *Cell.* 76: 411–413.
- Parker, P. J., and S. J. Parkinson. 2001. AGC protein kinase phosphorylation and protein kinase C. *Biochem. Soc. Trans.* 29: 860–863.
- Newton, A. C. 2003. Regulation of the ABC kinases by phosphorylation: protein kinase C as a paradigm. *Biochem. J.* 370: 361–371.
- 26. Sarbassov, D. D., S. M. Ali, D. H. Kim, D. A. Guertin, R. R. Latek, H. Erdjument-Bromage, P. Tempst, and D. M. Sabatini. 2004. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr. Biol.* 14: 1296–1302.
- Ikenoue, T., K. Inoki, Q. Yang, X. Zhou, and K. L. Guan. 2008. Essential function of TORC2 in PKC and Akt turn motif phosphorylation, maturation and signalling. *EMBO J.* 27: 1919–1931.
- Facchinetti, V., W. Ouyang, H. Wei, N. Soto, A. Lazorchak, C. Gould, C. Lowry, A. C. Newton, Y. Mao, R. Q. Miao, et al. 2008. The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C. *EMBO J.* 27: 1932–1943.
- Sakai, N., K. Sasaki, N. Ikegaki, Y. Shirai, Y. Ono, and N. Saito. 1997. Direct visualization of the translocation of the gamma-subspecies of protein kinase C in living cells using fusion proteins with green fluorescent protein. J. Cell Biol. 139: 1465–1476.
- Oancea, E., M. N. Teruel, A. F. Quest, and T. Meyer. 1998. Green fluorescent protein (GFP)-tagged cysteine-rich domains from protein kinase C as fluorescent indicators for diacylglycerol signaling in living cells. *J. Cell Biol.* 140: 485–498.
- Violin, J. D., J. Zhang, R. Y. Tsien, and A. C. Newton. 2003. A genetically encoded fluorescent reporter reveals oscillatory phosphorylation by protein kinase C. J. Cell Biol. 161: 899–909.
- Violin, J. D., and A. C. Newton. 2003. Pathway illuminated: visualizing protein kinase C signaling. *IUBMB Life*. 55: 653–660.
- Gallegos, L. L., M. T. Kunkel, and A. C. Newton. 2006. Targeting protein kinase C activity reporter to discrete intracellular regions reveals spatiotemporal differences in agonist-dependent signaling. *J. Biol. Chem.* 281: 30947–30956.
- 34. Giorgione, J. R., J. H. Lin, J. A. McCammon, and A. C. Newton. 2005. Increased membrane affinity of the C1 domain of protein kinase C delta compensates for the lack of involvement of its C2 domain in membrane recruitment. *J. Biol. Chem.* **76**: 411–413.
- Dries, D. R., L. L. Gallegos, and A. C. Newton. 2007. A single residue in the C1 domain sensitizes novel protein kinase C isoforms to cellular diacylglycerol production. *J. Biol. Chem.* 282: 826–830.
- Kunkel, M. T., Q. Ni, R. Y. Tsien, J. Zhang, and A. C. Newton. 2005. Spatio-temporal dynamics of protein kinase B/Akt signaling revealed by a genetically encoded fluorescent reporter. *J. Biol. Chem.* 280: 5581–5587.
- Yuan, T. L., and L. C. Cantley. 2008. PI3K pathway alterations in cancer: variations on a theme. *Oncogene*. 27: 5497–5510.
- Griner, E. M., and M. G. Kazanietz. 2007. Protein kinase C and other diacylglycerol effectors in cancer. *Nat. Rev. Cancer.* 7: 281–294.