Protein kinase C

ARTICLE *in* INTERNATIONAL UNION OF BIOCHEMISTRY AND MOLECULAR BIOLOGY LIFE · NOVEMBER 2008

Impact Factor: 3.14 · DOI: 10.1002/iub.118 · Source: PubMed

1 AUTHOR:

Alexandra C Newton

University of California, San Diego

177 PUBLICATIONS **15,650** CITATIONS

SEE PROFILE

IUBMB *Life*, 60(11): 765–768, November 2008

My Favorite Enzyme

Protein Kinase C

Alexandra C. Newton
Department of Pharmacology, University of California San Diego, La Jolla, CA, USA

Alexandra C. Newton

I wanted to be a ballerina, but somehow, I ended up being a spectator and a student to the most intricate dance of all—that of an enzyme that performs center stage in the theater of cell signaling. Protein kinase C moves between cellular compartments, interacting with binding partners and second messengers, in an exquisitely choreographed dance of perfection and precision. The enzyme displays remarkable flexibility, undergoing global conformational changes as it pirouettes around the cell, from partner to partner. It is my prima ballerina assoluta and my favorite enzyme, all in one.

Dan Koshland, in an autobiographical article in *Annual Reviews in Biochemistry* (*1*) recounts that he felt like a character in the Mikado, "wafted by a favoring gale" from place to place. I too feel that the breezes were favorable as I was wafted from a B.Sc. in Biochemistry from Simon Fraser University in my hometown of Vancouver, to a Ph.D. in Chemistry at Stanford with Wray Huestis, and into the lab of Dan Koshland at the University of California, Berkeley for my postdoctoral studies. Koshland recruited me to solve memory. I succeeded Daria Mochly-Rosen, who was his first postdoctoral fellow to work on protein kinase C in his lab (1983–1985). The enzyme had been discovered a few years earlier by Yasutomi Nishizuka and his group in Kobe, Japan (*2*). We overlapped for 1 month during which she provided me with intense and rigorous training in protein kinase C—memorable for the 5 day-long, round the clock, purifications of protein kinase C (imagine, no tags!) from rat brain; I will not forget the day a decapitated rat head bit my glove to the music of the Talking Heads. As Daria recounts in an article on his life we wrote together for this journal (*3*), Koshland had decided that protein kinase C was the memory enzyme because repetitive painting on mouse skin of phorbol esters, which had just been shown to activate protein kinase C, caused cancer. Reasoning there was a mechanism to "remember" the previous application, he charged me with solving memory mediated by protein kinase C.

For the first two weeks in the Koshland lab, I diligently set forth to develop a system to repetitively stimulate protein kinase C in cells and study desensitization, the closest I could think of to memory. I was getting nowhere quickly. After I had been in the lab about a week, Koshland asked if had solved memory yet. I replied that yes, I had, but I had forgotten the answer. After two weeks, it stopped being funny. So, I secretly started looking at the lipid regulation of protein kinase C. My graduate training was in lipid biochemistry, something much more tangible than memory. By then it was well known from Nishizuka's discoveries that protein kinase C was activated by lipids and

Received 16 June 2008; accepted 16 June 2008
Address correspondence to: Alexandra C. Newton, Department of Pharmacology, University of California San Diego, La Jolla, CA 92130-0721, USA. Tel: 858-534-4527. Fax: 858-822-5888. E-mail: anewton@ucsd.edu

ISSN 1521-6543 print/ISSN 1521-6551 online
DOI: 10.1002/iub.118

Yusuf Hannun, then in Robert Bell's lab, was publishing some beautiful papers on the lipid regulation of the enzyme. I thought this was something I could do. But the problem was that Koshland was relentlessly unforgiving about my training in "grease," so I had to be furtive in these studies. One day I had accidentally left a graph on my desk showing the activity of protein kinase C as a function of mol fraction phosphatidylserine in Triton mixed micelles. Koshland saw it and was ecstatic: the data were described by a perfect sigmoidal curve with a Hill coefficient of 12. Cooperativity is one of the many concepts that Koshland had made seminal contributions to our understanding. I think that for a moment he forgot that the *x* axis was "grease." Regardless, lipids were redeemed, and I had found a project.

The lipid regulation of protein kinase C was a major focus of our research during my first faculty position in the Chemistry Department at Indiana University. Using enantiomeric lipids, we showed protein kinase C specifically bound *sn*-1,2-phosphatidyl-L-serine and that this interaction produced a conformational change in the enzyme that released an autoinhibitory pseudosubstrate sequence, thus allowing activation. It also became apparent during this time that the activity of protein kinase C itself depended on phosphorylation, and a mass spectrometric analysis revealed that it is processed by three priming phosphorylations. One at the activation loop, which our lab and Peter Parker's lab had shown was a key site modified by a heterologous kinase, later identified as the phosphoinositide-dependent kinase, PDK-1. But there were also two sites at the C-terminus which we named the turn motif (by analogy with the protein kinase A structure, it was predicted to be positioned at a turn at the top of the upper lobe of the kinase core) and hydrophobic motif (flanked by hydrophobic residues); these were also conserved amongst most AGC family members (a branch of the kinome containing protein kinases A, G, and C), suggesting an important role in the function of these kinases. In 1995, as we were delving deeper and deeper into this new regulation of protein kinase C by phosphorylation, our lab moved to the Pharmacology Department at the University of California, San Diego. Here the breezes were truly favorable and we were in kinase heaven with Susan Taylor as a neighbor. Conversations with her have been influential as we have tried to understand how protein kinase C's catalytic activity is regulated by phosphorylation. We also began a wonderful and direction-changing collaboration with Roger Tsien that empowered us to visualize the activities of protein kinases B, C, and D in real time in live cells at specific intracellular locations.

Our work on protein kinase C led us to its close cousin, Akt, also regulated by phosphorylation and lipid second messengers. Specifically, we were intrigued at how acute termination of the Akt signal depended on removal of the hydrophobic motif phosphorylation site, one of the two C-terminal phosphorylation sites we had originally mapped on protein kinase C. Some controversy existed as to the identity of the kinase modifying this site on Akt. Because we had shown previously that this site is regulated by an intramolecular phosphorylation on protein kinase C βII, we wondered whether the same mechanism might hold for Akt, so that the key regulator of this site could be a phosphatase rather than a kinase. In an example of chance favoring the prepared mind, we reasoned that such a phosphatase may have a lipid-second messenger sensing module just as Akt and its upstream kinase PDK-1 have; they both contain a PH (pleckstrin homology) domain. A query of the sequence databases revealed the presence of precisely one gene family predicted to encode a phosphatase with a PH domain and, in addition, Leucine-rich repeats. We named the protein PHLPP (PH domain Leucine-rich repeat Protein Phosphatase; pronounced "flip") after its domain composition (*4*). We went on to show that this phosphatase controls the acute agonist-evoked activity of Akt, and chronically controls the stability of protein kinase C (*5*). Although PHLPP research monopolizes much of my students' research at the moment, protein kinase C remains my favorite enzyme, as would be apparent to anyone driving in San Diego who might see a car with the license plate "KINASE C."

My hero in the protein kinase C field was Yasutomi Nishizuka who discovered protein kinase C with beautifully rigorous, classic biochemistry. Specifically, he and his team had chromatographically purified a protein kinase that appeared to be constitutively active, requiring only Mg^{2+} for activity. So they called it protein kinase M (PKM) after its only cofactor, Mg^{2+} (*6, 7*). Curiously, much higher levels of PKM were purified from stocks of frozen brain compared to brains from freshly-sacrificed rats; and yields improved when the researchers skimped on protease inhibitors. Drawing from his experience with protein kinase G, which Nishizuka's group had previously shown becomes constitutively active following proteolysis, the Nishizuka team hypothesized that PKM was a proteolytic fragment of a larger kinase that had lost an autoinhibitory region. In 1979, Nishizuka's group identified the proenzyme and named it protein kinase C, because it was activated by cleavage with the Ca^{2+}-dependent protease calpain (*2*). Thus was named my favorite enzyme.

I first met Nishizuka at a Lipid Second Messenger Keystone Meeting in Taos in 1994. I was an Assistant Professor in a Chemistry Department, so, to explain our research to my colleagues, I had drawn a cartoon of PKC activation using sea life for proteins (Fig. 1). For the meeting, I had taken a photo of the face of Nishizuka and placed it over the face of the octopus that represents protein kinase C. It was meant to indicate that Nishizuka was the king of protein kinase C, but, in retrospect, the success of the slide depended on Nishizuka having a sense of humor. Fortunately for my career, he totally loved the octopus. Later that evening, there was a band playing and meeting attendees were dancing. Nishizuka came up to me and said "Will you boogie-woogie with the bald-headed octopus?". I still had a lot of growing up to do. I laughed and laughed, and said no, I did not dance without a barre. Later, I was quite distraught about turning him down. So, at the next Keystone Meeting, again in Taos, I charged two of my students (their PhDs

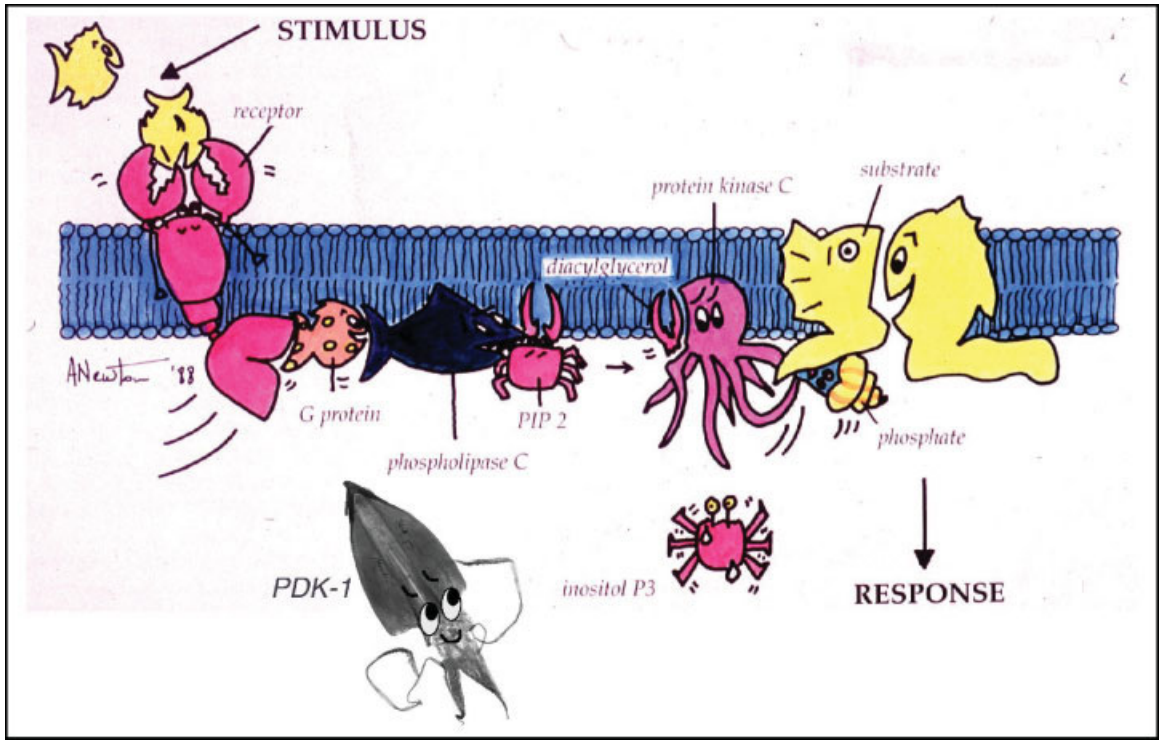

Figure 1. Aquatic representation showing the activation of protein kinase C (octopus) by diacylglycerol (crab claws) released by phospholipase C (shark)-catalyzed hydrolysis of phosphatidylinositol bis phosphate (crab) following agonist (yellow fish) binding to G-protein-coupled receptors (lobster). Also shown is the new cast member since the original drawing in 1988, the upstream kinase phosphoinositide-dependent kinase PDK-1 (represented by the dried autographed squid provided to the author by Yasutomi Nishizuka, see text).

depended on this) to ask Nishizuka to dance. They asked him, he accepted, and he had a blast "boogie woogieng." The photos are priceless.

I was invited to Kobe in 2001 on the occasion of Nishizuka retiring as President of Kobe University. It was so meaningful to be in the birthplace of protein kinase C—I was in awe of everything. And the funniest of all—to get to Kobe, you take a boat called the K-Cat. I asked Nishizuka if he had named it after Kinase-Catalytic domain, since that was the first bit of protein kinase C he had purified. I think Nishizuka thought it was all rather funny that I was so excited to be in the birthplace of protein kinase C. To humor me, he opened a glass cabinet in this office and pulled out the thesis of Takai, who had worked out the original purification of protein kinase C from rat brain (*2, 6*). I leafed through it, marveling at all the column profiles and activity assays while Nishizuka sat quietly smiling at me. I am sure he thought I was crazy. But—wow—it was historic!

In addition to a science symposium, attended by Tony Hunter and Lew Cantley, as well as my protein kinase C partners in crime, Peter Blumberg and Alan Fields, there was a very formal banquet attended by university and government officials. The speeches were all in Japanese and the only word I understood, repeated over and over again was "protein kinase C." It was clear Nishizuka had added a new word to the Japanese language. During one of these speeches, Nishizuka left the table of honor and walked towards me very determinedly. When he got close, he produced a flat dried (and very smelly) squid out of a plastic shopping bag, then pulled out a pen from his jacket, and proceeded to write on the dehydrated cephalopod: "To Alexandra, thanks! Yasu." Amidst the decorum, there was one table resonating with laughter. This odorous squid, transported back from Japan and then scanned into an image, went on to assume the role of the upstream kinase, PDK-1, in the aquatic version of protein kinase C signaling (Fig. 1).

Unraveling the molecular and cellular mechanisms of protein kinase C has been a team effort contributed by a large number of labs. Over 45,000 articles have been published on this enzyme [for reviews see (8–12)]. It is a privilege that the breezes wafted me towards it, providing two delightful decades working with wonderful students to understand the choreography, role and technique of my favorite enzyme in the dance of cell signaling.

ACKNOWLEDGEMENTS

The author thanks the NIH for 20 years of uninterrupted funding (NIH GM 43154), my colleagues in the field for many stimulating discussions on my favorite enzyme, and

most importantly, the people past and present in my lab for their countless ideas, lively conversations, beautiful data, and friendship.

REFERENCES

1. Koshland, D. E. (1996) How to get paid for having fun. *Annu. Rev. Biochem.* **65**, 1–13.
2. Takai, Y., Kishimoto, A., Iwasa, Y., Kawahara, Y., Mori, T., and Nishizuka, Y. (1979) Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. *J. Biol. Chem.* **254**, 3692–3695.
3. Newton, A. C. and Mochly-Rosen, D. (2007) Induced fit and wit: celebrating the life of Daniel E. Koshland, Jr. (1920–2007). *IUBMB Life* **59**, 741–743.
4. Gao, T., Furnari, F., and Newton, A. C. (2005) PHLPP: a phosphatase that directly dephosphorylates Akt, promotes apoptosis, and suppresses tumor growth. *Mol Cell* **18**, 13–24.
5. Brognard, J. and Newton, A. C. PHLPPing the Switch on Akt and Protein Kinase C Signaling, *Trends Endocrinol. Metabol.* **19**, 223–230.
6. Takai, Y., Kishimoto, A., Inoue, M., and Nishizuka, Y. (1977) Studies on a cyclic nucleotide-independent protein kinase and its proenzyme in mammalian tissues. *J. Biol. Chem.* **252**, 7603–7609.
7. Inoue, M., Kishimoto, A., Takai, Y., and Nishizuka, Y. (1977) Studies on a cyclic nucleotide-independent protein kinase and its proenzyme in mammalian tissues. *J. Biol. Chem.* **252**, 7610–7616.
8. Griner, E. M. and Kazanietz, M. G. (2007) Protein kinase C and other diacylglycerol effectors in cancer. *Nat. Rev. Cancer*. **7**, 281–294.
9. Battaini, F. and Mochly-Rosen, D. (2007) Happy birthday protein kinase C: past, present and future of a superfamily. *Pharmacol. Res.* **55**, 461–466.
10. Violin, J. D. and Newton, A. C. (2003) Pathway illuminated: visualizing protein kinase C signaling. *IUBMB Life* **55**, 653–660.
11. Newton, A. C. (2003) Regulation of the ABC kinases by phosphorylation: protein kinase C as a paradigm. *Biochem. J.* **370**, 361–371.
12. Cameron, A. J., De Rycker, M., Calleja, V., Alcor, D., Kjaer, S., Kostelecky, B., Saurin, A., Faisal, A., Laguerre, M., Hemmings, B. A., McDonald, N., Larijani, B., and Parker, P. J. (2007) Protein kinases, from B to C. *Biochem. Soc. Trans.* **35**, 1013–1017.