# This Week's Citation Classic®

**Cohen P.** The role of cyclic-AMP-dependent protein kinase in the regulation of glycogen metabolism in mammalian skeletal muscle. *Curr. Topics Cell. Regul.* 14:117-96, 1978.
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The regulation of phosphorylase kinase and glycogen synthase by cyclic AMP-dependent protein kinase is reviewed. The protein phosphatases that dephosphorylate these enzymes are also described, with emphasis on their substrate specificities and response to thermostable inhibitor proteins. [The *SCI*® indicates that this paper has been cited in over 290 publications.]

## Multisite Phosphorylation in Glycogen Metabolism

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After postdoctoral work with Ed Fischer at the University of Washington studying comparative and evolutionary aspects of the control of glycogen metabolism, I had returned to Britain in October 1971. My initial aim was to characterise phosphorylase kinase in greater detail, since Ed Krebs and Fischer had already shown that this enzyme was activated by $Ca^{2+}$ and cyclic AMP and therefore was the key to the neuronal and hormonal control of glycogenolysis. From 1972 to 1975, I and a graduate student, Steve Yeaman, demonstrated that the activation of phosphorylase kinase by cyclic AMP-dependent protein kinase (PK-A) was accompanied by phosphorylation of two serine residues *in vitro* and *in vivo*, one on the $\alpha$ and one on the $\beta$ subunit, changes in activity correlating with reversible phosphorylation of the latter component. Another student, John Antoniw, identified two enzymes, termed protein phosphatases 1 and 2 (PP1, PP2) that dephosphorylated the $\alpha$ and $\beta$ subunits, respectively.

Phosphorylation of an enzyme at more than one site was a novel concept in the early 1970s and prompted a search for further examples of this phenomenon. This led a postdoctoral fellow, Hugh Nimmo, and a further student, Chris Proud, to show that glycogen synthase was regulated by two protein kinases which phosphorylated residues distinct from those labelled by PK-A. Antoniw found that the major enzyme dephosphorylating glycogen synthase (and glycogen phosphorylase) was PP1, demonstrating that a single enzyme catalysed each of the dephosphorylation events that stimulated glycogen synthesis and inhibited glycogenolysis. Following the discovery of inhibitors 1 and 2 by Walter Glinsmann,[1] Dr. Gillian Nimmo and I purified these proteins and showed that they inhibited PP1 specifically, PP2 being unaffected.

The review summarizes the work of my laboratory from 1972 to 1977, and I was surprised when told that it has become the second most highly cited paper to have been published in *Current Topics in Cellular Regulation*. One reason may be that it disclosed new levels of complexity in the regulation of a metabolic pathway that many had believed was already solved. Although phosphorylase kinase and glycogen synthase were the first enzymes shown to be regulated by "multisite phosphorylation," this phenomenon is now known to be an important general mechanism for integrating the effects of different extracellular signals into key regulatory proteins. The review was among the first to emphasize that protein phosphatases were likely to be under hormonal control, and it introduced criteria for distinguishing these enzymes that have subsequently been used very widely.[2] The article also contained the first suggestion that insulin stimulates glycogen synthase by promoting the dephosphorylation of residues distinct from those phosphorylated by PK-A.

Soon after submission we discovered that phosphorylase kinase contained the $Ca^{2+}$-binding protein calmodulin as an integral subunit.[3] This finding, which helped to explain how glycogenolysis and muscle contraction are synchronized, appears as a "note added in proof." It took 4 more years before PP2 was found to be regulated by $Ca^{2+}$/calmodulin[4] and 10 years before the full complexity of the glycogen synthase system was revealed. This enzyme is now known to be phosphorylated on nine serines *in vivo* by at least six different protein kinases.[5] A "contaminant" in glycogen synthase, termed component C in the review, turned out to be complexed to the enzyme and was identified as the priming protein (glycogenin) required for the *de novo* synthesis of glycogen in 1987. The concept of protein phosphorylation as a regulatory device developed enormously over the next five years.[6] PP1 and PP2 were found to regulate many other cellular processes and the study of these enzymes is becoming a very active research area.[2]

For the work described in the review, I received two young investigator awards, an Anniversary Prize of the Federation of European Biochemical Societies in Copenhagen (1977) and the Colworth Medal of the British Biochemical Society (1978).

1. **Huang F L & Glinsmann W H.** Separation and characterization of two phosphorylase phosphatase inhibitors from rabbit skeletal muscle. *Eur. J. Biochem.* 70:419-26, 1976. (Cited 215 times.)
2. **Cohen P.** The structure and regulation of protein phosphatases. *Annu. Rev. Biochem.* 58:453-508, 1989.
3. **Cohen P, Burchell A, Foulkes J G, Cohen P T W, Nairn A C & Vanaman T C.** Identification of the calcium-dependent modulator protein as the fourth subunit of rabbit skeletal muscle phosphorylase kinase. *FEBS Lett.* 92:287-93, 1978. (Cited 515 times.)
4. **Stewart A A, Ingebritsen T S, Manalan A, Klee C B & Cohen P.** Discovery of a $Ca^{2+}$ and calmodulin-dependent protein phosphatase: probable identity with calcineurin (CaM-$BP_{80}$). *FEBS Lett.* 137:80-4, 1982. (Cited 215 times.)
5. **Poulter L, Ang S G, Gibson B W, Williams D H, Holmes C F B, Caudwell F B, Pitcher J & Cohen P.** Analysis of the in vivo phosphorylation state of rabbit skeletal muscle glycogen synthase by fast atom bombardment mass spectrometry. *Eur. J. Biochem.* 175:497-510, 1988. (Cited 10 times.)
6. **Cohen P.** The role of protein phosphorylation in the neural and hormonal control of cellular activity. *Nature* 296:613-20, 1982. (Cited 805 times.)




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