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This Week's Citation Classic

Walsh D A, Perkins J P & Krebs E G. An adenosine 3',5'-monophosphate-dependent protein kinase from rabbit skeletal muscle. *J. Biol. Chem.* 243:3763-5, 1968.
[Department of Biochemistry, University of Washington, Seattle, WA]

A protein kinase that catalyzes an adenosine 3', 5'-monophosphate (cyclic AMP)-dependent phosphorylation of casein and protamine was purified from rabbit skeletal muscle. The K_m values of cAMP for these reactions are 1×10^{-7} and 6×10^{-8}, respectively. The protein kinase markedly increases the rate of the cAMP-dependent activation and phosphorylation of phosphorylase kinase by ATP. [The *SCI*® indicates that this paper has been cited over 675 times since 1968.]

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"I joined the laboratories of Ed Krebs and E.H. Fischer as a postdoctoral fellow in the autumn of 1966. Krebs was the past master of having fellows 'choose' the project that he thought best for them. So I 'selected' to work on the activation of phosphorylase kinase. Possibly, I was spurred on by Krebs's comment that he did not think a 'phosphorylase kinase kinase' existed. At that time, phosphorylase kinase was already highly purified; the stimulation by cAMP of the ATP-dependent activation was well recognized. As is the axiom of biochemistry, the conditions of an experiment should be optimized; so since the activation and phosphorylation of phosphorylase kinase were faster at higher concentrations of ATP-Mg^{2+}, it was examined under those conditions. After the fact, we understand that high ATP, in fact, masked the specificity of cAMP-dependent activation.

"Four observations led to the discovery of the cAMP-dependent protein kinase. The first was the conviction that at high ATP there were two catalytic processes to activate phosphorylase kinase. The second was the unpublished observation of Bob Kemp that there was far less than a stoichiometric binding of cAMP to phosphorylase kinase. The third, albeit unrecognized by the investigators,[1] was that anomalously phosphorylase kinase was reactivated at a much faster rate after dephosphorylation by a crude protein phosphatase preparation than it was in the initial activation.

"The fourth observation was the most crucial. One night, while 'watching' a column, I picked up Carmen Gonzalez's MS thesis. This was work done in 1962, unpublished because it was difficult to interpret, and somewhat forgotten. Carmen had described how a heat stable factor, first described because it interfered with the assay of cAMP,[2] modified phosphorylase kinase activation. What became apparent was that if phosphorylase kinase activation involved two catalysts, Carmen's inhibitor only blocked one of them. Could it be that this inhibitor would block the activator that was present, but unrecognized, in the crude extracts in the phosphatase experiment? Could it be that the activating factor in crude extracts was a mediator of cAMP? Could it be that phosphorylase kinase preparations were contaminated with this mediator of cAMP? Well, the history now is well known and the answer to all three questions was yes. The first definitive experiment was designed and from that single experiment came the identification of both the cAMP-dependent protein kinase and the heat-stable inhibitor protein of that enzyme. Ironically, this experiment was not published until later,[3] and the first paper was built upon experiments that were possible only after this cAMP-dependent 'phosphorylase kinase kinase' was purified.

"The identification of the cAMP-dependent protein kinase was an experiment whose time had come and I was fortunate to have been at the right place at the right time. Its discovery marked the end of an era of investigation with all the links in the chain of the glycogenolytic cascade now known.[4] Krebs had chosen to investigate this system recognizing that it served as a model for cAMP-mediated hormonal action. Because, with the final link in, Krebs thought it not erudite to point out the obvious, he has not been given the unique recognition that he deserves."

1. **Riley W D, DeLange R J, Bratvold G E & Krebs E G.** Reversal of phosphorylase kinase activation. *J. Biol. Chem.* 243:2209-15, 1968.
2. **Posner J B, Hammermeister K H, Bratvold G E & Krebs E G.** The assay of adenosine-3',5'-phosphate in skeletal muscle. *Biochemistry* 3:1040-4, 1964.
3. **Walsh D A, Perkins J P, Brostrom C O, Ho E S & Krebs E G.** Catalysis of the phosphorylase kinase activation reaction. *J. Biol. Chem.* 246:1968-76, 1971.
4. **Walsh D A & Cooper R H.** The physiological regulation and function of cAMP-dependent protein kinases. (Litwack G, ed.) *Biochemical actions of hormones.* New York: Academic Press, 1979. Vol. 6. p. 1-75.