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Castagna M, Takai Y, Kaibuchi K, Sano K, Kikkawa U & Nishizuka Y. Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J. Biol. Chem.* 257:7847-51, 1982.
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Tumor-promoting phorbol esters such as 12-O-tetradecanoylphorbol-13-acetate (TPA) directly activate *in vitro* Ca^{2+}-activated, phospholipid-dependent protein kinase (protein kinase C), which normally requires unsaturated diacylglycerol. Using human platelets as a model system, TPA is shown to enhance the protein kinase C-specific phosphorylation associated with the release reaction in the total absence of phosphatidylinositol breakdown. [The *SCI*® indicates that this paper has been cited in over 1,450 publications.]

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It all started when I sat down with Yasutomi Nishizuka at the garden party given for the participants of the fourth International Conference on Cyclic Nucleotides and Protein Phosphorylation held in Brussels on July 25, 1980. Actually, I should say that the whole story started after completion of my PhD thesis, when I chose to study the epigenetic changes linked to cancer instead of DNA-associated irreversible alterations. Later, it was my postdoctoral training in the Department of Biochemistry of the Medical School at the University of California at Davis (headed by E.G. Krebs), where cAMP-dependent protein kinase was characterized and located in the adenylate cyclase pathway,[1] that "sensitized" me to search for a link between signal transduction and cancer.

When Nishizuka described to me the properties of an enzyme (which he referred to as protein kinase C) that had recently been discovered in his group at Kobe University, it struck me that this enzyme was the molecule I was looking for. One year previously, Peter Blumberg presented evidence for a high-affinity binding site in cell membranes that is specific for phorbol esters. From the biological effects of these tumor promoters, the features of this unknown receptor were predictable and amazingly similar to those Nishizuka was enumerating for protein kinase C. We agreed right away that the hypothesis deserved to be verified. However, it took a year before I was able to arrange a one-month leave at Kobe during my vacation time. Frankly, I should confess that when I boarded the plane to Japan with wrapped samples of phorbol esters in my purse I was more thrilled about the certainty of visiting Zen stone gardens in Kyoto than about the potential discovery of the receptor for tumor promoters.

I arrived in Kobe on August 1. We rapidly confirmed that phorbol esters such as thrombin were able to aggregate platelets and trigger serotonin release. Then we showed that, in contrast to thrombin, phorbol esters did not affect phosphoinositide metabolism. However, we provided evidence that phorbol esters enhanced phosphorylation of the 40 Kd protein, a major substrate of protein kinase C in these cells. Finally, we set up the experiment with purified protein kinase C on August 24. The prediction turned out to be right: phorbol esters substituted for diacylglycerol and in doing so mimicked physiological signals. I just had time before packing to write a draft manuscript and draw conclusions in a group seminar. When the plane took off from Osaka on September 1, I had not had much time for touring, but I had the feeling that I had completed my assignment.

The success of this project was made possible because I found in the Department of Biochemistry at Kobe Medical School a group of well-trained staff members headed by a restless, deeply dedicated scientist, who was able, due to his royal sense of hospitality, to create beneficial interactions among the participants of this study.

Back in France I applied for grants and working space. One year later I was ready to resume this research. The paper came out in June 1982. Interestingly, three reports in 1983 from American laboratories[2-4] extended our contribution, attesting to an amazing adjustment to a new concept.

The paper has been cited frequently because protein kinase C is a key enzyme in a signaling pathway common to neurotransmitters, hormones, mitogens, and many biological effectors. Phorbol esters are used as tools for investigating the transduction of these extracellular signals.

1. **Walsh D A, Perkins J P & Krebs E G.** An adenosine 3',5'-monophosphate-dependent protein kinase from rabbit skeletal muscle. *J. Biol. Chem.* 243:3763-74, 1968. (Cited 830 times.) [See also: **Walsh D A.** Citation Classic. (Barrett J T, ed.) *Contemporary classics in the life sciences. Vol. 2: the molecules of life.* Philadelphia: ISI Press, 1986. p. 153.]
2. **Niedel J E, Kuhn L J & Vandenbark G R.** Phorbol diester receptor copurifies with protein kinase C. *Proc. Nat. Acad. Sci. USA* 80:36-40, 1983. (Cited 680 times.)
3. **Kraft A S & Anderson W B.** Phorbol esters increase the amount of Ca^{2+}, phospholipid-dependent protein kinase associated with plasma membrane. *Nature* 305:621-3, 1983. (Cited 330 times.)
4. **Ashendel C L, Staller J M & Boutwell R K.** Identification of a calcium- and phospholipid-dependent phorbol ester binding activity in the soluble fraction of mouse tissues. *Biochem. Biophys. Res. Commun.* 111:340-5, 1983. (Cited 155 times.)

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