EFFECTS OF CANTHARIDIN AND A PHORBOL ESTER ON BUD FORMATION IN *HYDRA VULGARIS*

Fernando PEREZ
Zoologisches Institut der Universität zu Köln, Weyertal 119, 50923 Köln, Germany

In the fresh water polyp *Hydra* new animals arise asexually as buds. Budding visibly starts by evagination of the double-layered tissue in the parent animal's gastric region. The protrusion elongates and forms a head at the tip and a foot at the base. Distal to the foot a constriction forms which allows the separation of the bud from the parent animal. The bud is made out of gastric tissue, thus the positional value at the tip has to increase in order to form the head and to decrease at the base in order to form the foot. We aim to understand how the positional value is controlled in *Hydra* tissue.

Müller (1990) found - by daily pulse treatments with 12-O-tetradecanoylphorbol-13-acetate (TPA) over one week - the positional value to increase in gastric tissue. We used foot formation at the buds base as testsystem and found that a single treatment with TPA of animals bearing a very young bud prevented the normal decrease of the positional value: The buds fail to form a constriction (which normaly occurs at about the 90th h) and they do not separate from the parent animal. Some of these buds form a foot patch instead of a belt-like foot at the position closest to the parent animal's foot (Pérez and Berking 1994).

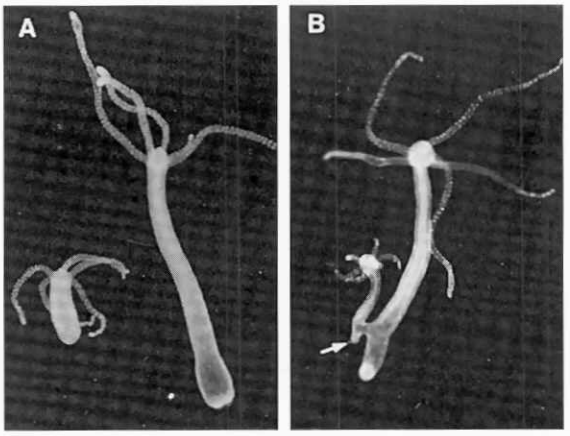

Figure 1. Effect of cantharidin on budding *Hydra*. (A) Untreated *Hydra* with a detached 7-day-old bud (*left hand*). (B) Branched animal 7 days after a treatment for 1 h with $3x10^{-5}$ M cantharidin. The *arrow* indicates a foot patch.

TPA is well known to stimulate serine/threonine protein phosphorylation by protein kinase C isoenzymes (PKCs; Gschwendt et al. 1991, and references cited therein). Thus, some unknown target protein was postulated to prevent in its phosphorylated form the normaly occuring decrease of the positional value. Processes that are reversibly controlled by protein phosphorylation require not only a protein kinase but also a phosphatase (for a review see Hunter 1995). Here it is shown that cantharidin, a serine/threonine protein phosphatase type 1 and 2A (PP-1 and PP-2A; Li and Casida 1992) inhibitor simulated the effects of TPA: The buds did not separate, they rather remain as a branch permanently connected to the parent animal (Figure 1). The sensitive periods of both treatments were similar (Figure 2). The substances act synergistically: Concentrations which displayed almost no effect had a strong effect when applied simultaneously (Figure 3). It is thus suspected that both substances act on the same pathway. TPA stimulates the phosphorylation of an (unknown) target protein, cantharidin prevents it from dephosphorylation. In normal development phosphorylation of that target protein is proposed to take place at a lower extent, as indicated by the cantharidin experiments. The TPA experiments indicate that in normal development the phosphatases prevent an increase of the phosphorylated form of the target protein. In conclusion, our data show that control of phosphorylation and dephosphorylation of a target protein is involved in control of the positional value in *Hydra*.

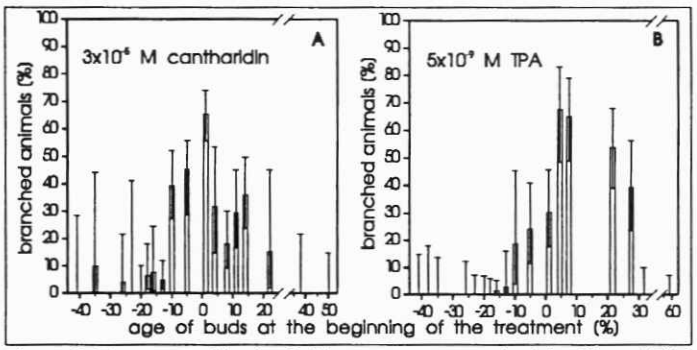

Figure 2. Response of buds of different ages to cantharidin (A) and TPA (B). Buds were incubated for 1 h and scored 4 days after treatment. Shown are the means and confidence-intervals. 0 h = time of visible bud appearence.

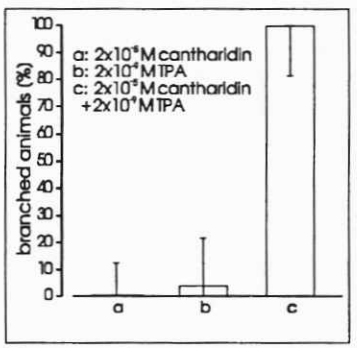

Figure 3. Frequency of branched animals following incubation of 3-6 h old buds with TPA and cantharidin.

References

Hunter, T. (1995). Protein kinases and phosphatases: The yin and yang of protein phosphorylation and signaling. Cell 80: 225-236.

Li, Y. and Casida, J.E. (1992). Cantharidin-binding protein: Identification as protein phosphatase 2A. Proc. Natl. Acad. Sci. 89: 11867-11870.

Müller, W.A. (1990). Ectopic head and foot formation in *Hydra*: Diacylglycerol-induced increase in positional value and assistance of the head in foot formation. Differentiation 42: 131-143.

Gschwendt, M., Kittstein, W. and Marks, F. (1991). Protein kinase C activation by phorbol esters: Do cysteine-rich regions and pseudosubstrate motifs play a role? Trends Biochem. Sci. 16:167-170.

Pérez, F. and Berking, S. (1994). Protein kinase modulators interfere with bud formation in *Hydra vulgaris*. Roux's Arch. Dev. Biol. 203: 284-289.