ANALYSIS OF A *HYDRA* MUTANT WHICH PRODUCES EXTRA HEADS ALONG ITS BODY AXIS

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The fresh water polyp *Hydra* provides many advantages for studying control of pattern formation including its simple architecture and its high capacity to regenerate. The polyp is a tube with two different ends, the head and the foot. Current opinion of pattern control is that a *Hydra* has only one head because an existing head prevents the formation of a further one along the whole body axis. However, there exists a mutant (multi headed, mh-1) which produces with increasing age an additional head or even more than one close to the original one (fig.1). Our aim is to understand how pattern control is affected in these animals and this may help to understand pattern control in principle in *Hydra*.

In *Hydra* the phorbolester 12-o-tetradecanoylphorbol-13-acetate (TPA) was found to cause the formation of head structures along the body axis (Müller, 1990). Following repeated pulse treatments with high concentration over one week leads to the formation of tentacles and later on also of hypostomes and feet. A single treatment with TPA caused no supernummary heads to form neither in the wildtype nor in immature mh-1 animals. However, a single pulse treatment for 30 minutes with cantharidin an inhibitor of protein phosphatases, in particular of PP2A (Li and Casida, 1992), in concentration of 25µM caused the development of second heads in up to 50% of the treated mh-1 animals (fig.2). The wildtype still kept their normal morphology. Simultaneous treatment with both the PKC activator and the PP2A inhibitor showed no synergistic effects. It appears that there is a target which in its phosphorylated form is able to cause secondary heads, probably via a local stimulation of an increase of the positional value. Whether or not a PKC is involved in that control is yet unclear.

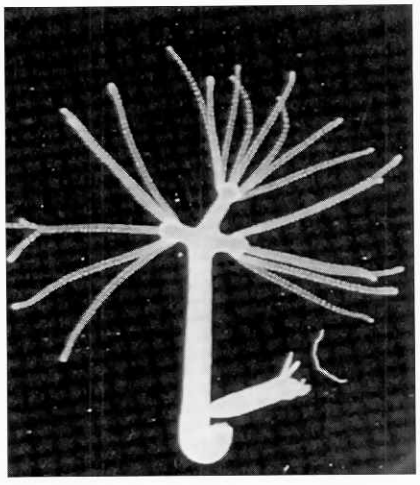

Fig. 1. Mh-1 polyp with two additional heads and a normal developing bud.

A further approach to understand the control of this event is to ask which cell types are responsible for that phenotype. The body wall of *Hydra* consists of two cell layers made out of some few cell types including interstitial cells (i-cells). The i-cells give rise to nerve cells, nematocytes, gland cells and germ cells.

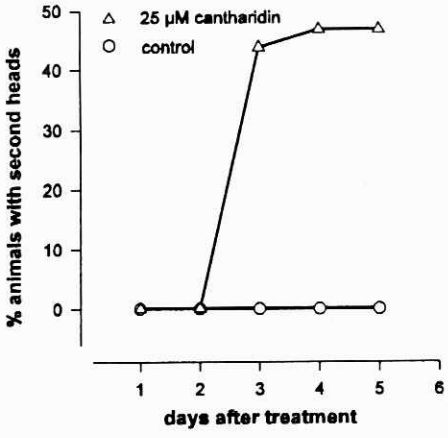

Fig. 2. Response of mh-1 animals to cantharidin. Three day old mh-1 were treated with 25µM cantharidin for 30 minutes. cantharidin (n=41, Δ), control (n=40, O)

We treated mh-1 and wildtype animals for 5 days with 1mM LiCl according to Hassel and Berking (1988). This treatment caused the animals to loose their i-cells and all their derivatives. The so called epithelial hydras were used to produce chimeric animals (Marcum and Campbell, 1978). Treated and untreated tissue was transplanted together. The transplant was kept for 72 hrs, then the untreated tissue was removed in such a way that no epithelial cells of the untreated region were included. During the period of transplantation some i-cells have immigrated into the i-cell free tissue. By separation of the tissue and after some time of regeneration we obtained chimeric animals consisting of epithelial cells of one type and i-cells and their derivatives of the other type. Following feeding these animals produced buds which were analysed by treatment with cantharidin (25µM for 30 minutes) whether or not they are able to form additional heads (fig 3). It turned out that the presence of mh-1 epithelial cells is necessary for expression of the mh-1 phenotype whereas the interstitial cells and their derivatives appear to have no influence on the mh-1 phenotype.

Fig. 3. Response of chimeric animals to 25µM cantharidin (30 minutes)

source of epithelial cells	source of interstitial cells and derivatives	number of treated animals	number of mh-1 phenotype
wt	wt	36	0
wt	mh-1	38	0
mh-1	wt	35	11
mh-1	mh-1	44	16

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