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Metamorphosis and pattern formation in *Hydractinia echinata*, a colonial hydroid

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ABSTRACT There are several reasons why *Hydractinia echinata* Hydrozoa, Cnidaria) is excellently suited to study developmental processes. In the laboratory fertilization takes place every morning in the seawater in thousands of eggs. Cleavage starting synchronously leads to a ciliated planula larva within 2 to 3 days. Onset of metamorphosis from the larval to the polyp stage must be triggered externally. There are several agents known to induce or to interfere with induction of metamorphosis thus allowing access to the biochemical basis of this process. The pattern of the resultant polyp can be influenced by certain treatments during the process of metamorphosis allowing access to a process of proportioning. The colony develops by elongation of hollow tubes at the base of the polyps, termed stolons on which in more or less regular intervals new polyps emerge. Two (main) types of polyps are formed allowing to study spacing by lateral inhibition and lateral dependence of each other. In the present paper current data and hypotheses concerning all these topics are discussed.

KEY WORDS: *S-adenosylmethionine, ammonium, polyamines, protein kinase C, serotonin*

Introduction

Hydra and other cnidarians are famous for their simple architecture and their high ability to regenerate. The body of the polyp is tube shaped with a mouth opening at one end surrounded by tentacles and – in the case of solitary cnidarians, like *Hydra*, a basal plate at the other end. The body is made up of two cell layers separated by a membrane, the mesogloea. Cnidarians consist of only some few cell types, including nerve cells. These simple features have attracted scientists for more than 200 years to study *Hydra* and other cnidarians. Most developmental biologists were particularly interested in control of pattern formation and cell differentiation.

Pattern formation in *Hydra* is usually studied by analyzing budding, a process of asexual reproduction and by analyzing regeneration. Marine colonial hydrozoa allow in addition an access to (1) sexual reproduction and embryogenesis, (2) metamorphosis from larva to polyp and (3) patterning of polyps within a colony. *Hydra*, and in particular its marine relatives, take up very efficiently substances from the culture medium. This feature greatly facilitates the biochemical approach to the control of these processes. One of the best studied marine cnidarians with respect to the mentioned topics is *Hydractinia echinata*.

Life cycle of *Hydractinia*

Hydractinia is a marine colonial hydroid frequently found in the North Sea. The colonies commonly cover shells inhabited by hermit crabs. Fertile colonies, male and female, produce eggs and sperm, respectively, and within less than three days the fertilized egg develops into a mature planula larva.

"Mature" larva means a larva that is able to metamorphose into a polyp, but under sterile laboratory conditions it will never do. It will rather die as it is unable to take up food. To continue its development, it needs an external trigger that appears to be provided in the natural habitat by certain sedentary bacteria of the genus *Alteromonas*. A lipophilic substance produced by these bacteria is thought to act as this trigger (Leitz and Wagner, 1993; Müller, 1973b). Microbial films are thought to play an important role in metamorphosis induction and settlement site selection for many marine invertebrate larvae (ZoBell and Allen, 1935; Crisp and Ryland, 1960; Burke, 1983).

Metamorphosis into a primary polyp, the founder of a new colony, is completed within one day. Stolons, hollow tubes connecting the polyps of the colony, grow out and new polyps emerge on them at more or less regular distances. Usually within some few months this new colony has reached sexual maturity, and the life cycle is closed (Fig. 1)

Abbreviations used in this paper: DAG, 1,2-diacyl-*sn*-glycerol; diC8, 1,2-dioctanoylglycerol; MGBG, methylglyoxal-*bis*-(guanylhydrazone); p-CPA, p-chlorophenylalanine; PAF, proportion altering factor; PKC, protein kinase C; SAM, S-adenosylmethionine; SIF, stolon inducing factor; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

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Pattern formation during embryogenesis

Morphological pattern of the planula

Oocytes and sperm from female and male animals, respectively, are released into the seawater triggered by light (Ballard, 1942). After fertilization cleavage starts and continues every 50 min almost synchronously until the 9th cleavage. Then the cleavage becomes asynchronous, different cell types appear and the almost spherical cell assembly starts to elongate. Finally a spindle shaped larva develops consisting of about 10000 cells (Plickert *et al.*, 1988).

In *Hydractinia* the anterior-posterior polarity of the larva could be traced back to the polar organization of the oocyte. The pronucleus lies excentrically. Close to it is the only entry point for spermatozoa (Freeman and Miller, 1982). At that position the first cleavage furrow starts to appear and finally the larval posterior forms from membrane and cytoplasm from that position (Teissier, 1931; Freeman, 1980, 1987; Schlawny and Pfannenstiel, 1991). In the related species *Phialidium gregarium*, in which axis formation is similar, gastrulation takes place by polar invagination at the former pronucleus position (Freeman, 1981a). In *Hydractinia* gastrulation takes place by ingression at multiple points and tangential division of cells (Teissier 1931; Van de Vyver 1964). In *Obelia* the axis of the larva is found not to be fixed in the first cleavage stages (Ostroumova and Beloussov, 1971).

Occasionally in *Hydractinia* and other hydrozoan larvae with more than two ends are found. Such animals were produced artificially by pulse-treatment of zygotes of *Phialidium* with cytochalasin B, which blocks cleavage. Following treatment cleavage often starts simultaneously at two positions. If these sites are not too close to each other larvae with two posterior ends develop (Freeman, 1981a). If cleavage occurs consecutively a normal larva develops. It appears that the initiation of the first cleavage establishes conditions, which do not allow the formation of a secondary axis.

Treatment of early cleavage stages with a low molecular weight factor isolated from *Eudendrium*, termed Proportion Altering Factor, PAF, (Plickert 1987) also causes the development of larvae with more ends. In most cases posterior ends were formed in addition (Kroiher and Plickert, 1992). Treatment with vanadate result in roughly the same type of animals (Leitz and Wirth, 1991). The reasons for these developmental effects are unknown.

TABLE 1

SUBSTANCES AND CONDITIONS INDUCING METAMORPHOSIS IN *HYDRACTINIA*

Metamorphosis inducible by	Reference
Alteromonas-lipid	Spinder and Müller, 1972
1,2-diacyl-*sn*-glycerol (DAG)	Leitz and Müller, 1987
not by: 1-oleyl-2-acetyl-*sn*-glycerol (OAG)	Müller, 1985
phorbol esters: TPA, PDB, RPA, EPA, PDD	Müller, 1985
not by: methyl-TPA	
vanadate	Leitz and Wirth, 1991
not by: $InsP_3$	Leitz and Müller, 1987
not by: Ca^{2+}	Freeman and Ridgway, 1987
Li^+, K^+	Spindler and Müller, 1972
Cs^+, Rb^+	Müller and Buchal, 1973
NH_4^+, methylamin	Berling, 1988b
tetraethylammonium (TEA^+)	
Ba^{2+}, Sr^{2+}, amiloride	
Mg^{2+}-deficiency	Müller, 1985
temperature shift	Kroiher *et al.*, 1992
methylglyoxal-*bis*-(guanylhydrazone) (MGBG)	present paper

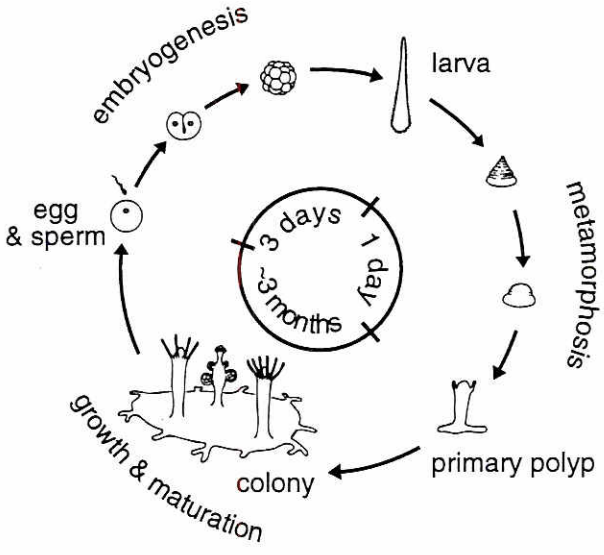

Fig. 1. The life cycle of *Hydractinia echinata*. *A larva is about 1 mm long. A mature colony consists of several hundred polyps of different types (only two gasterozooids and one gonozooid are shown).*

If embryos or larvae are excised transversely they regenerate the missing body parts maintaining the original polarity (Teissier 1933; Müller *et al.*, 1977; Schwoerer-Böhning *et al.*, 1990). Interestingly, aggregates from dissociated blastula cells are able to form normal shaped planulae. A sorting out of the cells was not detected during formation of the new anterior-posterior axis. A proper development was observed when an aggregate has about the size of an egg. Larger aggregates develop into larvae with several ends, anterior and posterior ones. Pieces of blastula tissue implanted into an aggregate were found to have a strong polarizing influence. The resultant polarity of the developing planula coincides with the polarity of the implant (Freeman, 1981b).

Morphogenesis at the cellular level

Initially cleavage is synchronous. From the 10th division onwards (9th h of development) cleavage slows down and becomes asynchronous. At that time two cell types can be distinguished, epithelial cells and cells that keep their embryonic appearance (Plickert *et al.*, 1988). As development proceeds the embryonic cells cleave more slowly and the relative frequency of the epithelial cells increases. From the 12th hour of development onwards some cells leave the cell cycle and start to differentiate (M. Kroiher, unpublished). Cycling cells become more and more restricted to the endoderm of the central region of the larva (Plickert *et al.*, 1988; Kroiher *et al.*, 1990). At first the posterior pole becomes free of cycling cells followed by the anterior pole (Kroiher *et al.*, 1990). Treatment of embryos with PAF at an early stage of development hinders the regular pattern of cell proliferation, a later treatment with the factor delays the local slowing down of proliferation (Kroiher and Plickert, 1992).

The mature planula is composed of an ecto- and an entodermal layer separated by a basal lamina, the mesogloea. Such a larva consists of about 10 000 cells, including epithelial cells, interstitial stem cells (I-cells), nerve cells, nematocytes and gland cells, some of which are distributed in a graded manner along the anterior-posterior axis (Van de Vyver, 1964; Weis *et al.*, 1985). A treatment with PAF in particular increases the number of I-cells and their derivatives (Kroiher and Plickert, 1992).

Metamorphosis

Initial steps and their control

Metamorphosis is the transition from the very simply designed larva to the primary polyp, a substantially higher organization possessing well defined apical and basal structures and the capabilities to feed and to reproduce (asexually at first, finally sexually). In *Hydractinia* this structure forming process is completed within less than one day. It can be started at any time you like by simply applying a suitable inducer to the larvae. Thus thousands of individuals can be synchronized in their development. To date a lot of artificial inducers are known (Table 1), but there are about ten to twenty times more substances that have been tested without finding any inducing capacity, often without finding any influence on metamorphosis at all.

Obviously, except for the bacteria-derived substance and perhaps for ammonium, in the normal habitat of the animals these substances are not found, at least in sufficient concentrations for metamorphosis induction. So they cannot act as inducers of metamorphosis in nature. However, utilizing such agents helps to learn something about the biochemical processes involved in metamorphosis initiation. And exactly this is the focus of our interest.

A larva remains a larva until external cues enable the transition into a polyp. So there might be some endogenous compounds that stabilize the larval stage and that must be antagonized to allow metamorphosis to commence. Larvae kept in seawater containing Cs^+ (3-19 mM for 24 h) undergo metamorphosis, but simultaneous application of homogenates of larvae or adult polyps reduces the rate of metamorphosing animals in a concentration dependent manner. By means of this assay system and applying chromatographic methods on the tissue homogenates, four activities were isolated and identified: *N*-methylpicolinic acid (homarine), *N*-methylnicotinic acid (trigonelline), *N*-trimethylglycine (betaine) and β-aminoethane sulfonic acid (taurine) (Berking, 1986a,b, 1987, 1988a). These substances all have in common that their concentrations in larval tissue are extremely high (up to 90 mM for taurine, 3-25 mM for the others) and that they are able to inhibit metamorphosis if they are present in micromolar concentrations in the medium. Besides this, homarine, trigonelline and betaine have another feature in common: they have a labile methyl group that can be transferred to suitable acceptors. Related substances lacking the methyl group (e.g. picolinic acid, nicotinic acid) were unable to antagonize metamorphosis induction. Betaine is known to be the most important source of methyl groups for the synthesis of methionine from homocysteine in animal cells (Saunderson and Mackinlay, 1990), and the methyl group from homarine was shown to be transferred via *S*-adenosylmethionine to several target molecules in shrimp (Netherton and Gurin, 1982). *S*-adenosylmethionine (SAM) is the universal donor molecule of methyl groups in biological transmethylation reactions. It is formed from methionine and ATP. Methionine is taken from food protein or synthesized from homocysteine and the methyl group of a suitable donor like betaine (Fig. 2). Methionine has an inhibitory effect on metamorphosis induction, too, while homocysteine has not. Cycloleucine (1-aminocyclopentane-1-carboxylic acid), an inhibitor of SAM synthesis, abolishes the inhibitory effects of methionine or the methyl donors. Sinefungin, the well known inhibitor of SAM-dependent transmethylations, does the same. These findings have led to the hypothesis that the methylation of some unknown target molecule(s) via SAM stabilizes the larval state. But transmethylation inhibitors like cycloleucine, sinefungin or adenosine dialdehyde (Walther, unpublished) could at best enhance the rate of metamorphosing animals in combination with some other inducer. They were never found to be inducing themselves. This might either indicate that these agents are not efficient enough in blocking methylation, or that blocking methylation is not sufficient to trigger metamorphosis, or even that SAM does not act via transmethylation. SAM is not only the source of methyl groups (Fig. 2), its aminopropyl group is used for another very important synthesis pathway: it can be transferred on putrescine resulting spermidine. Spermidine can accept another aminopropyl group from another molecule of SAM forming spermine. Polyamines like these are found in all cells from bacteria to mammals and are known to be indispensable for cell proliferation, growth and development (for review see: Tabor and Tabor, 1984). Different inhibitors of this polyamine synthesis pathway (*bis*-(cyclohexylammonium) sulfate, methylthioadenosine, methylglyoxal-*bis*-(guanylhydrazone)) increase the rate of metamorphosis when applied in combination with inducers like Cs^+ or DAG, and at least one of them, methylglyoxal-*bis*-(guanylhydrazone) (mitoguazone, MGBG) does induce metamorphosis alone, though with a relatively low yield. Consistently, the polyamines spermidine and spermine act inhibitory (Walther, in preparation).

The alkali and earth alkali ions inducing metamorphosis have been argued to act by causing a membrane depolarization

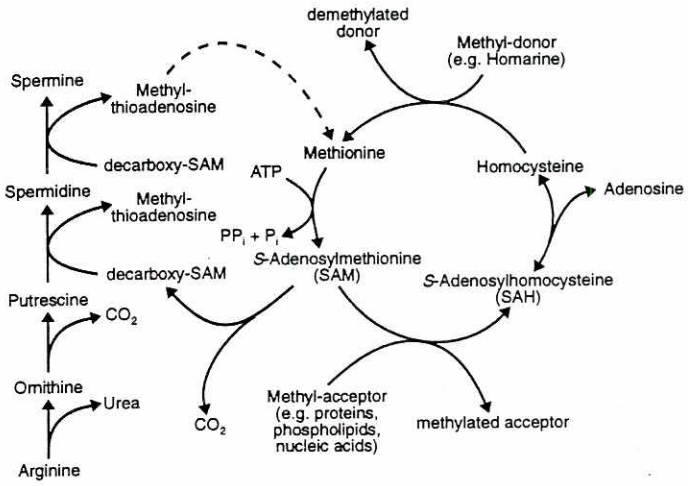

Fig. 2. Methyl group cycle and polyamine synthesis pathway. *The dotted line indicates that conversation of methylthioadenosine to methionine takes more than one reaction step.*

(Müller, 1973a; Leitz and Klingmann, 1990). A membrane depolarization has been hypothesized to be the primary event in larvae from different species of marine invertebrates (Yool *et al.*, 1986; Pechenik and Heyman, 1987; Freeman, 1993). Diacylglycerols (DAGs) like dioctanoylglycerol (diC_8) and tumor promoting phorbol esters like 12-*O*-tetradecanoylphorbol-13-acetate (TPA), well known activators of protein kinase C-like (PKC-like) enzymes, are potent inducers of metamorphosis in *Hydractinia*. Consistently, inhibitors of PKCs like sphingosine and K-252a act inhibitory on metamorphosis induction (Müller, 1985; Leitz and Klingmann, 1990). Based on these data it was proposed, that the bacterial inducer binds to a receptor which is coupled to the PI system. This would cause the release of the second messenger DAG and hence activation of a PKC. Just recently, a generation of DAG following contact with bacteria was found, as well as the presence of PKC-like activities (Schneider and Leitz, 1994). As this kinase is known to close K^+ channels in mammalian cells by phosphorylating them (Kaczmarek, 1987), it was supposed to do the same in *Hydractinia*, thus causing a membrane depolarization (Leitz, 1993). However, some experimental results were difficult to explain in terms of this model. Inhibitors of PKCs do not only block metamorphosis induction by activators of this kinase but also induction by Cs^+. This indicates that the PKC acts downstream from Cs^+, not that both do the same as proposed by the model (to cause depolarization via

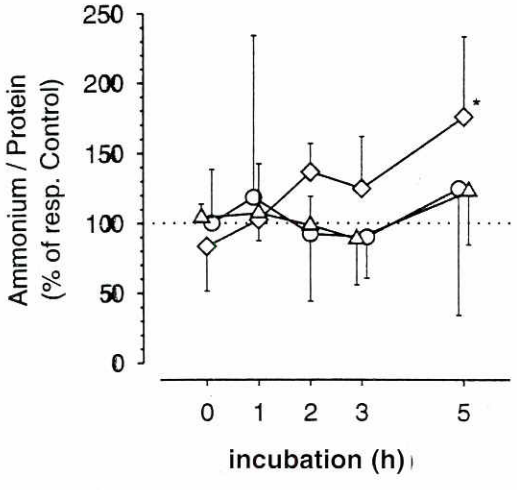

Fig. 3. Internal concentration of total ammonium (NH_3+NH_4^+). *Values are nanomoles of ammonium per mg of protein as a percentage of the respective control (untreated larvae of the same batch). ◊ 100 mM CsCl, ○ 0,1 mM diC_8, Δ artificial seawater containing 2,5 mM Mg^{2+} (about 5% of the content of natural seawater). Ammonium was measured using the determination kit "Spectroquant" (Merck, Darmstadt, FRG). About 100 animals/sample were incubated in the respective medium (seawater, seawater with reduced Mg^{2+}-concentration or containing CsCl or diC_8) for the times noted, the supernatant was removed subsequently. The animals were washed once with seawater, transferred to 1,1 ml of deionized water and disintegrated by sonication. 100 µl of the resulting homogenate was used for protein determination using the Bradford method (Bradford, 1976), the remaining 1 ml for ammonium determination. Each symbol is the mean of 5 samples, error bars are standard deviations. The significance of a difference from the control was judged by a homoscedastic two-sample Student's t-test. The * marks a p<0,05.*

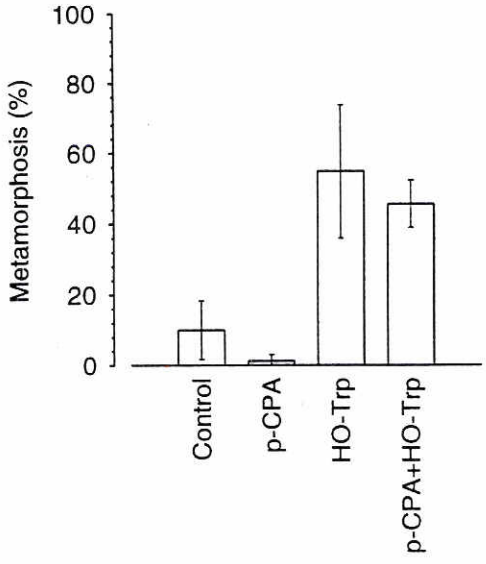

Fig. 4. Simultaneous treatment (3 h) with 0.1 mM p-chlorophenylalanine (p-CPA) reduces the number of animals undergoing metamorphosis when induced with seawater having 33% of the Mg^{2+}-concentration of normal seawater (control). *This inhibitory effect is antagonized by simultaneous application of 0,3 mM hydroxytrytophan (p-CPA + HO-Trp). HO-Trp has a stimulating effect on metamorphosis induction by Mg^{2+}-deficiency. The figure represents experiments done in triplicate from larvae of the same batch. Error bars represent standard deviations.*

closing K^+ channels). On the other hand, no translocation of PKCs to the membrane, which is thought to be a prerequisite for PKC activation, was found when larvae were treated with Cs^+ (Schneider and Leitz, 1994).

The finding that ouabain, a blocker of the Na^+/K^+-antiport, increases the rate of metamorphosis when applied simultaneously with diC_8 (Berking and Walther, 1994), but reduces it when applied in combination with Cs^+, NH_4^+, Rb^+, Li^+, vanadate, amiloride, and seawater with reduced Mg^{2+} concentration (Müller, 1973b; Berking, 1988b; Leitz and Wirth, 1991) further supports the hypothesis that PKC activators and the inorganic ions act at different sites. As metamorphosis induction by bacteria or the bacterial factor is antagonized by ouabain (Müller, 1973b), its mode of action appears not to be a direct stimulation of PKCs.

In contrast to the other metamorphosis inducers known, NH_4^+ is an endogenous substance. It is constantly produced in the course of protein turnover. As a K^+-like ion, NH_4^+ is usually exported from cells via K^+ channels. We propose that several of the metamorphosis inducers act due to their ability to block these channels thus increasing the internal concentration of NH_4^+ (Berking, 1988b). Cs^+, Rb^+, Sr^{2+}, Ba^{2+} and TEA^+ are known to act in this way (Stanfield, 1983; Latorre *et al.*, 1984). As discussed above, activation of a PKC might do the same.

When *Hydractinia* larvae were treated with Cs^+ to induce metamorphosis a significant increase of the internal concentration of NH_4^+ was measured (Fig. 3), i.e. the concentration doubled within 5 h. Inducing treatments using diC_8 or seawater with reduced Mg^{2+} concentration did not cause significant changes.

Kroiher *et al.* (1992) found that metamorphosis induction by Cs^+ or NH_4^+ is accompanied by the synthesis of heat shock pro-

teins (HSPs) while induction with diC_8 or seawater with low Mg^{2+} concentration is not. Both these findings indicate that stimulation of PKCs does not cause a rise in the internal concentration of NH_4^+. Again activators of PKCs and Cs^+ appear to induce metamorphosis via different modes of action.

The metamorphosis inducing effect of ammonia appears to be widespread among marine invertebrate larvae of different tribes. It has been found to induce metamorphosis in echinoid larvae (Gilmour, 1991) and in *Ciona* (tunicata) (Berking and Herrmann, 1990), and to induce partial metamorphosis in buds of the scyphozoan *Cassiopea* (Berking and Schüle, 1987). In larvae of oysters (mollusca) it causes at least settlement behavior, for final settlement and metamorphosis additional cues are necessary (Coon *et al.*, 1990). In the natural habitats of these animals, in areas of high biological activity and low water current (as in boundary layers near surfaces and in small cavities) the total concentration of (NH_3+NH_4^+) may reach levels sufficient for induction or at least facilitating it. Concentrations of 10 mM were measured in interstitial waters from marine sediments (Bruland, 1983), 300-400 µM have been reported for samples taken from surfaces of oyster shells in a Georgia salt marsh (Fitt and Coon, 1992). High ammonia concentrations resulting from reduced mixing may be the cause for the obvious preference of many marine invertebrate larvae to settle in grooves and pits in the substrate (Burke, 1983).

The specificity of *Hydractinia* for certain bacteria shows that the natural inducer is not simply ammonia, but high concentrations of it could facilitate metamorphosis in an environment with high biological activity.

The biochemical mode of action of NH_4^+ is unknown. Several hypotheses are discussed. It was proposed to act as acceptor of methyl groups, thus reducing the methylation potential (Berking, 1988b). It appears not to simply increase the cytoplasmic pH by entering as NH_3 and taking up protons to re-establish the NH_3/NH_4^+-ratio inside the cell. The concentration of NH_4Cl necessary to induce metamorphosis is shifted to lower values if the incubation is made in seawater with a pH that is lower than usual, though the ratio NH_3 / NH_4^+ is more on the side of NH_4^+. Metamorphosis can be induced by amiloride, a blocker of the Na^+/H^+-antiport which can only cause cytoplasmic acidification. The fact that ouabain antagonizes induction by NH_4^+ indicates that it must enter the cells via active transport in ionic form to exert its action.

Signal uptake and metamorphosis

Under natural conditions, a planula larva propels itself slowly over the substrate by beating cilia and the thick leading end appears to investigate the substrate. From time to time the larva adheres to the substrate but may restart moving. When a suited place is found metamorphosis commences. Based on these observations several authors have proposed that the anterior end is specialized to take up the inducing signal.

Neurosensory cells in the anterior part of the planula have been hypothesized to have the function of sensing the environmental cues triggering attachment and metamorphosis (Thomas *et al.*, 1987; Edwards *et al.*, 1987; Plickert 1990). Edwards *et al.* (1987) found that exogenously applied catecholamines cause metamorphosis in *Halocordyle*. They proposed that the bacterial inducer is sensed by neurosensory cells of the RFamide type.

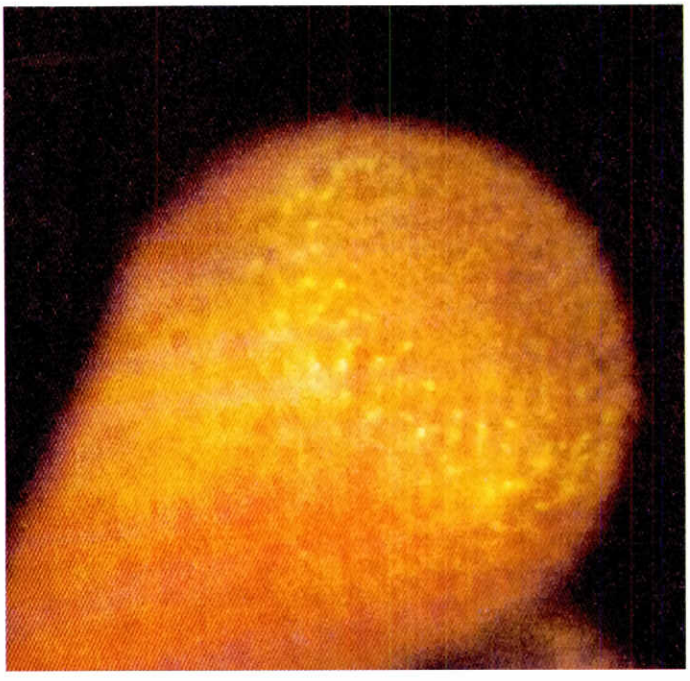

Fig. 5. Larval anterior end with cells showing immunoreactivity for serotonin. *Fixation was carried out with 0,4 % paraformaldehyde in seawater. Immunostaining according to Grimmelikhuijzen (1983). The anti-serotonin antibody was a polyclonal rabbit serum (Dianova, Hamburg, FRG).*

Cells displaying immunoreactivity for the neuropeptide FMRFamide are found in the anterior region of planulae of *Hydractinia* (Plickert, 1989). RFamide released is argued to act on ganglion cells, causing the release of catecholamines which subsequently act on epithelio-muscle cells and mucous cells, resulting in attachment, contraction and finally transformation into a polyp. Exogenously applied RFamide has not been demonstrated to induce metamorphosis, neither in *Hydractinia* nor in other species.

However, the mechanism in *Hydractinia* appears to be different from that as catecholamines do not induce metamorphosis. Only a slight inhibition of Cs^+-induced metamorphosis was found at millimolar concentrations of epinephrine or dopamine which is very unlikely to be a specific action. But also for *Hydractinia* some indications regarding an involvement of neurotransmitters do exist. Serotonin and its precursor hydroxytryptophan increase the rate of metamorphosis in combination with other inducers. P-chlorophenylalanine (p-CPA), a specific inhibitor of serotonin synthesis (Koyuncuoglu *et al.*, 1975) inhibits metamorphosis induced by Cs^+ or Mg^{2+}-deficiency. This inhibition is compensated by simultaneously applying hydroxytryptophan, the precursor of serotonin whose synthesis is blocked by p-CPA. (Fig. 4). Cells showing serotonin-like immunoreactivity were found to be arranged in a circle around the anterior pole of the larva, the region hypothesized to be responsible for the perception of the metamorphic stimuli (Fig. 5).

To explain the occurrence of completely metamorphosed animals one has to assume that either the respective inducing agents is taken up everywhere along the body axis or that there

is an internal signal which is generated where the external one is taken up and which spreads into all body regions. Such an internal signal has been proposed to be generated in anterior tissue where the larva is thought to take up the various inducing signals (Schwoerer-Böhning *et al.*, 1990). The model is mainly based on sectioning experiments. They showed, that anterior parts of sectioned larvae can be induced by various agents to metamorphose, while posterior ones remain in the larval state. Only seawater in which the Mg^{2+} concentration has been reduced causes posterior parts to metamorphose, as well (Schwoerer-Böhning *et al.*, 1990). Anterior parts give rise to stolons, a gastric region and, if the piece was large enough, to a small head. Posterior parts give rise to a polyp head and a gastric region. However, the results are not easy to interpret because the posterior parts become rapidly insensitive to Mg^{2+}-deficiency, with respect to metamorphosis induction. Further, the other agents which have been shown not to induce metamorphosis of posterior sections cause the irreversible induction of whole larvae after longer treatments than Mg^{2+} reduced seawater (Berking and Walther, 1994). Thus it is difficult to decide whether or not there is in principle a difference in metamorphosis induction of posterior parts by Mg^{2+} reduced seawater as compared to all other agents.

Fortunately it is possible to see whether there is a propagation of an internal signal from anterior to posterior in metamorphosis induction without cutting the animals. Due to some regimes of treatment (superoptimal concentrations of Li^+, NH_4^+, TEA^+, vanadate, inhibitors of protein synthesis, Spindler and Müller, 1972; Berking 1988b; Leitz and Wirth, 1991, Kroiher *et al.*, 1991) some larvae only undergo a partial metamorphosis. Some animals possessed a larval anterior and a polyp posterior, others were formed the other way round. It has also been found that only the middle part had transformed into polyp tissue (forming a ring of tentacles) and at the very anterior and posterior end, respectively, the tissue had remained in the larval state. SEM pictures show a distinct border between the areas of ciliated larval ectodermal cells and nonciliated adult ectodermal cells (Berking and Walther, 1994). We used partially metamorphosed animals, in which the anterior half had transformed into polyp tissue (basal plate and stolons) while the posterior half (the polyp's presumptive head) still consisted of larval tissue. When these animals were treated with NH_4^+ ions or DAG they completed metamorphosis (Berking and Walther, 1994). Obviously the inducing agents can be sensed by posterior tissue in the absence of larval anterior tissue. Thus the data do not give a hint for the existence of an internal signal.

The existence of partially metamorphosed animals demonstrates that metamorphosis can occur locally restricted. A group of larval cells may transform into polyp cells, but they are obviously unable to induce their neighbors to do the same. On the other hand, this group of cells is not hindered by those in close vicinity to transform into polyp cells. Larval and polyp cells can coexist close to each other, metamorphosis once performed, is not 'infectious', it does not spread like a bush fire.

Recently, a peptide has been isolated from anthozoan tissue which causes metamorphosis in *Hydractinia*. Some animals underwent complete, others a partial metamorphosis in a way that the anterior part remained in the larval state. This peptide is able to induce isolated posterior parts to metamorphose into

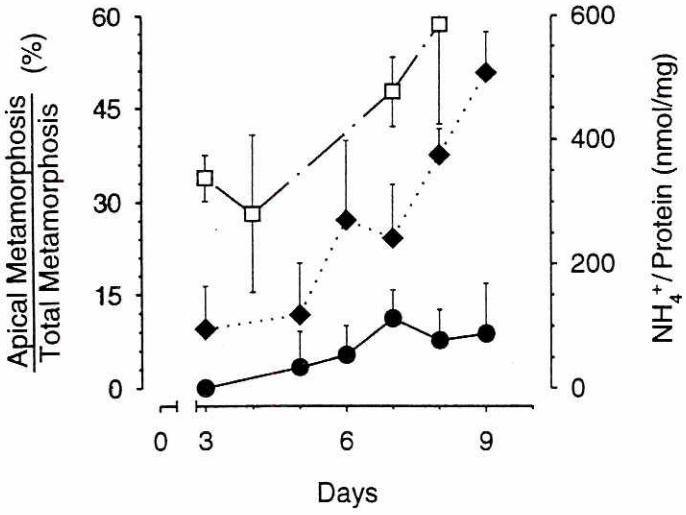

Fig. 6. Development of apical metamorphosis and concentration of NH_4^+ in *Hydractinia echinata* in relation to the developmental age. *Larvae of distinct age were induced to undergo metamorphosis by a treatment with artificial seawater containing 2.5 mM Mg^{2+} (5% of the content of normal seawater) at 18 °C (●) or 28 °C (◆) for 3 h or the NH_4^+ content (□) was measured according to the method described in Figure 3. The figure represents experiments done in triplicate from larvae of the same batch. Standard deviations are represented by bars whenever they were not to small.*

polyp heads like seawater in which the Mg^{2+} concentration has been reduced (Leitz *et al.*, 1994). The authors propose that the peptide is released internally from *Hydractinia* larval cells either when triggered by an internal signal or directly by exogenously applied inducing agents that were taken up at any site of the body column.

Attachment and metamorphosis

Metamorphosis can be induced by several agents which are well dissolved, like Cs^+ ions, but we never obtained freely floating polyps by such a treatment. It appears that contact to a substrate plays a crucial role in the process of metamorphosis. Either, a firm attachment strongly stimulates the onset of metamorphosis or attachment is an early step in the process of metamorphosis, even if induction takes place irreversibly during swimming. To decide on this alternative we tested various substrates and found that they have an influence on the efficiency of metamorphosis (Berking, 1988a; Berking and Walther, 1994). Polystyrene coated with heparin or bovine serum albumin (BSA) caused a high efficiency (but the influence of microbial growth on these substrates could not be excluded), while Teflon, known to prevent a strong contact to almost every substance, caused an extremely low efficiency. In addition, almost no metamorphosis was obtained when the larvae were hindered to reach the bottom during the inducing treatment, due to an increased viscosity of the seawater. The few resulting polyps were of normal shape. It appears that a tight contact supports the decision to metamorphose.

We propose that the substrate acts as a physical barrier reducing the release of constantly produced NH_4^+ at the site of tight contact (Berking and Walther, 1994). Therewith the internal concentration of NH_4^+ should rise. Because NH_4^+ is able to dif-

fuse rapidly within tissue its concentration may rise in the whole larva supporting metamorphosis everywhere. Partially metamorphosed animals may result when in a certain body region the conditions for metamorphosis are not met. Exogenously applied NH_4^+ induces metamorphosis within a narrow concentration range, superoptimal concentrations of ammonia do not allow metamorphosis, the larvae remain healthy larvae. In unresponsive tissue the concentration of NH_4^+ may thus be either to high or to low to initiate metamorphosis. We argue that ammonia is one component of the natural internal signal generated upon induction of metamorphosis.

Seawater in which the Mg^{2+} concentration has been reduced reliably causes complete metamorphosis. However, with increasing age of the treated larvae the frequency of one type of partial metamorphosed animals increased (Fig. 6). The animals have a larval anterior and a polyp's posterior (hypostome surrounded by tentacles). When the larvae were kept at 28°C during treatment the frequency of these animals was increased. It was strongly increased in old larvae but not in young ones. At the same time the frequency of animals with polyp structures (complete and partial metamorphosed animals) did not increase (not shown). It appears that in old larvae, enhanced by heat treatment, the anterior region is prevented to respond to the inducing condition. For we know that heat treatment increases the internal concentration of NH_4^+ (Kroiher *et al.*, 1992) we wondered whether this type of partial metamorphosis is due to an increased concentration of ammonia. Figure 6 shows that indeed the internal concentration of ammonia increases with the age of the larvae. Externally applied ammonia which by itself was not able to cause metamorphosis strongly increased the frequency of partially metamorphosed animals of the noted type when applied in seawater in which the Mg^{2+} concentration is reduced (Fig. 7). Thus we argue that the altered response of old larvae is due to their increased internal concentration of ammonia. It appears that the naturally occurring internal ammonia influences metamorphosis induction and pattern formation. Based on the observation that in old larvae it is the anterior end which does not respond we argue that in this region ammonia is more enriched than in the posterior one. This proposition also allows to understand that old larvae undergo metamorphosis spontaneously with low frequency: We and others found in addition to completely metamorphosed animals one type of partially metamorphosed animals, namely those in which the anterior end is in the polyp stage (stolons and gastric region) while the posterior persisted in the larval state.

Proportioning of polyps

Teissier (1931) showed that labeled cells in the posterior of a larva are found following metamorphosis in the head tissue of the polyp. Freeman (1981a) found that larvae with two posterior ends give rise to double-headed polyps. It appears that the pattern of the polyp is somehow preformed in the larva. Cell proliferation resumes after triggering of metamorphosis (Plickert *et al.*, 1988) but from both terminal regions of a larva, noncycling cells give rise to polyp structures without re-entering the cell cycle, indicating that these cells have been committed in the larval state for their proper future function in the polyp state (Kroiher *et al.*, 1990).

When the presumptive gastric tissue is excised from a larva and transferred to Mg^{2+} reduced seawater it gives rise to a complete polyp but with stolons and tentacles reduced both in number and mass (Schwoerer-Böhning *et al.*, 1990). The results indicate that during metamorphosis pattern forming processes can take place, at least in regenerating tissue.

Pattern formation in the course of metamorphosis and in the absence of regeneration can be induced by treating larvae following metamorphosis induction. The Proportion Altering Factor (PAF) caused the hypostom and the tentacle forming region to become large at the expense of the other regions (Plickert, 1987). Cs^+ and vanadate caused a similar effect but not as pronounced as PAF (Müller *et al.*, 1977; Berking, 1987; Leitz and Wirth, 1991). A 20 kDa glycoprotein isolated from supernatants of *Hydractinia* colonies, termed Stolon Inducing Factor (SIF) caused the opposite effect (Lange and Müller, 1991) The basal parts of primary polyps were larger at the expense of the other regions. At high concentrations the larva transforms completely into stolon tissue. The noted endogenous methyl donors have a similar effect, i.e. the size of the head is reduced while the region forming the stolons has become larger, but the effect is much less pronounced. (Berking, 1987). Simultaneous treatment of larvae with Cs^+ and crude extract of larvae (Berking, 1974) or the methyl donor homarine for 24 hours results in animals consisting of both a smaller head and a smaller stolon forming region. Higher concentrations of both agents (24 mM Cs^+, 20 µm homarine) cause the transformation of most larvae into gastric tissue without head and stolons.

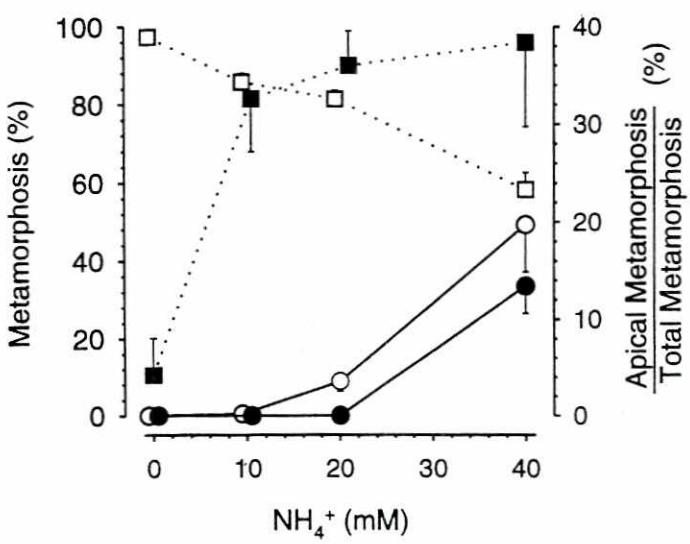

Fig. 7. Triggering of metamorphosis (open symbols) and appearance of apical metamorphosis (closed symbols) in *Hydractinia echinata*. *Planulae 7 day of age were treated simultaneously with 50 mM Mg^{2+}- seawater (○,●, i.e. the normal concentration of Mg^{2+} in seawater) or 2.5 mM Mg^{2+}-seawater (□,■) and distinct concentrations of NH_4^+ for 3 h. Note that the values of the apically metamorphosed animals (closed symbols) are applied to the total number of metamorphosis. The figure represents experiments done in triplicate from larvae of the same batch. Standard deviations are represented by bars whenever they were not to small.*

We argue that SAM plays a key role in the process controlling the range of positional information in tissue of metamorphosing animals.

Pattern forming systems of larva and polyp

Partially metamorphosed animals allow to look for interactions between the pattern forming systems of larvae and polyps. We have grown up posterior and anterior partial metamorphosed animals. Posterior metamorphosed animals have a mouth opening, they can feed and grow. However, only the polyp tissue grows. The gastric region elongates, polyp heads grow out laterally causing a branching but usually stolons do not form. The anterior part remains in the larval state and does not change its size. Posterior metamorphosed animals give rise to a colony with secondary polyps on the outgrowing stolons while the larval posterior persists in the center of the colony. In *Hydractinia* a stable situation is found only when all regions of the body are in contact with their normal neighbors. If the epithelium of one body region has contact with that of a normally not adjacent one intercalation of the missing parts occurs (Müller, 1982). Furthermore, almost all regions of larvae and polyps have the capacity to regenerate those parts which have been excised. Partially metamorphosed animals do not intercalate 'missing' polyp or larval tissue. They do not give rise to a complete larva and a complete polyp which finally separate from each other. These animals behave as if every region had contact with its normal neighbor region. It appears that the positional values, i.e. the memory of the positional information (Wolpert, 1969), are the same in polyps and larvae. Based on this view it is argued that pattern formation in larvae and adults is governed by the same positional information. It is the interpretation of the positional information, the specific response of cells to position signaling molecules which changes with the onset of metamorphosis (Berking, 1991). Interpretation of positional information is a cellular property. Thus the change of the interpretation rules can be locally restricted as it is observed in the partially metamorphosed animals.

Partially metamorphosed animals were also produced in the scyphozoan *Cassiopea andromeda* by means of applied ammonia. Polyps produce buds which look very similar to planula larvae. Following treatment animals which had a polyp's head and a bud's anterior were obtained. In these animals bud and polyp tissue, respectively, remained stable differentiated as in *Hydractinia,* but as in the natural situation the bud tissue separates from the polyp tissue resulting in a freely floating polyp head and a small freely swimming bud which can undergo metamorphosis into a complete polyp (Berking and Schüle, 1987).

Spacing of polyps in colonies and the formation of gonozooids

A colony grows by rhythmical elongation of its stolons (Beloussov *et al.*, 1989) and lateral stolon branching. On top of the new stolons some distance away from an existing polyp a new polyp forms. The positioning of the new polyp is argued to include lateral inhibition of polyps on the formation of new polyps. Factors affecting spacing have been postulated and partly purified by several authors (for review see Plickert, 1990), the chemical nature of the factors is largely unknown. In the thecate hydroid *Obelia* an almost fixed number of pulsations of the growing stolon has been observed to occur between one and the next polyp bud (Kossevitch, personal communication). This number is argued to determine the position of the new polyp bud. We found that in the process of spacing methyl donors play a decisive role. The experiments were performed with *Eirene*, a relative of *Hydractinia* which is particularly suited, because the spacing of polyps is very regular. Homarine applied externally at a concentration of 0.1 µM caused the formation of new polyps at increased distances from existing ones, while application of 0.1 µM of sinefungin, a competitor of SAM in transmethylation, had the opposite effect (Berking, 1986b). Therewith, patterning of larvae, patterning of polyps and spacing of polyps is argued to involve methyl donors. This proposition is supported by the finding that in colonies founded by partially metamorphosed animals the larval posterior end (which upon metamorphosis gives rise to a polyp's head) inhibits like a polyp secondary polyp formation in its close vicinity (Berking and Walther, 1994). Both larval and polyp tissue contain homarine in similar concentrations (Berking, 1987).

In the center of a large colony the mesh of stolons becomes denser, finally a mat forms on top of which interspersed between the residual polyps, termed gastrozooids, a new type emerges, the gonozooid. These polyps produce the germ cells. One may argue that gastrozooids somehow support the formation of gonozooids, but details of this control are completely unknown. In the hydroid *Thecocodium quadratum,* the interdependence of the two types of polyps this animal produces is even stronger. Gastrogonozooids can feed and can produce medusae but cannot catch the prey because they have no tentacles. The ten times more frequent dactylozooids have tentacles but no mouth. They can catch the prey but cannot feed themselves. The prey caught is taken over by the gastrogonozooids. The polyps depend on each other not only in the uptake of food but also in their development. A polyp regresses when it is separated from polyps of the other type and causes at the same time the formation of that type. (Pfeifer and Berking, 1995). We argue that such a lateral help (Meinhardt, 1982) in combination with lateral homo- and heterotypic inhibitions is a common feature of colonies consisting of different types of polyps.

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