Signals and signal-transduction systems in the control of development in *Hydra* and *Hydractinia*

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ABSTRACT Pattern control in *Hydra* has traditionally been assigned to the determining influence of morphogens and neuropeptides. However, at present, arachidonic acid and its derivative 12-S-HETE are the only identified, potential signal molecules known to promote head and bud formation. More potent factors might exist but are not yet identified. Nonetheless, it is possible to evoke the development of an almost unlimited number of supernumerary head structures and to induce ectopic foot formation by interference with the PI-PKC signal transducing system. Such an interference can also rescue the regeneration-deficient mutant *reg-16*. Regarding signals in the development of *Hydractinia*, metamorphosis is induced by an external key stimulus, i.e. a lipid derived from environmental bacteria. The reception of this stimulus involves PKC-mediated responses. Upon its reception, a neuropeptide is released as an internal, synchronising signal. Members of the novel LWamide family of peptides appear to represent this internal signal. In post-metamorphic development, a glycoprotein SIF serves as an inducer of stolon formation.

KEY WORDS: *pattern formation, metamorphosis, neuropeptides, protein kinase C, hydrozoa*

Pattern control in *hydra:* historical aspects, traditional and new theoretical concepts

The beginning

It has now been more than 250 years since regeneration studies in *Hydra*, performed by the Swiss scholar Abraham Trembley (1744), rang in the era of experimental Developmental Biology. In the meanwhile, the interest in the amazing regenerative capacities of this low metazoan organism and its marine relatives has fluctuated but never came to a standstill. At the turn of the century, marine hydroids of the *Tubularia* group were predominant attracting the attentions of personalities such as Hans Driesch in Germany, Jacques Loeb and Thomas Hunt Morgan in the USA. Their dispute on the significance of the red pigment that accumulates in the regenerating hydrant, as a *formative stuff*, led T.H. Morgan (1904) to propose a mechanism of pattern control based on competition for "a formative or nutritive material". In his view, the polyp at the apical end of a stem holds in check the development of a polyp at the basal end by using up nutritive material. However, this idea was not developed further. Only recently has one of the present authors (W.A.M) developed a model which revives the idea of competition for factors (Müller, 1994a,b; briefly outlined below).

Morphogen concepts

For some 30 years, pattern formation and pattern control in *Hydra* have been attributed to the determining influence of morphogens. Although morphogens have not yet been identified in *Hydra*, they are the constitutive elements of theoretical models based on positional information (Wolpert *et al.*, 1974) or on Turing-type reaction-diffusion systems (Turing, 1952; Gierer and Meinhardt, 1972; MacWilliams, 1982; Meinhardt, 1982, 1993; Shimizu *et al.*, 1993).

In the positional information model of Wolpert, the long-range gradient of a hypothetical morphogen S serves as a nominal gradient which is used to adjust a second, more stable gradient of positional values P (Wolpert *et al.*, 1974). Likewise, the Gierer-Meinhardt model uses hypothetical long-range morphogens to adjust, in a second, separate step of pattern control, the more stable gradient in the density of morphogen sources (Meinhardt, 1993).

In particular, the restriction of head structures to the apical end and the inhibitory influence of the parental head on bud formation have been ascribed to the action of a putative short-range head activator which is flanked by a long-range head inhibitor.

Likewise, the restriction of foot formation to the lower end of the body has been attributed to the action of a mirror-image countersystem comprising a short-range foot activator and a long-range foot inhibitor (Meinhardt, 1993, and this issue; Schaller *et al.*, this issue, and references therein).

A new model

In contrast to these hypotheses, the most recent model of pattern control (Müller, 1995a,b) does not separate labile prepatterns ('positional information') from more stable secondary postpatterns

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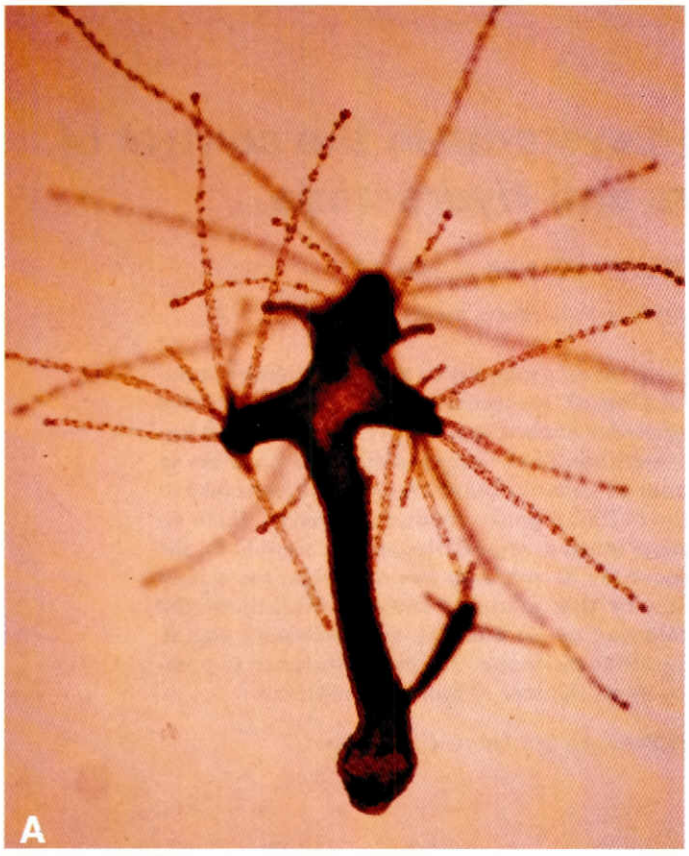
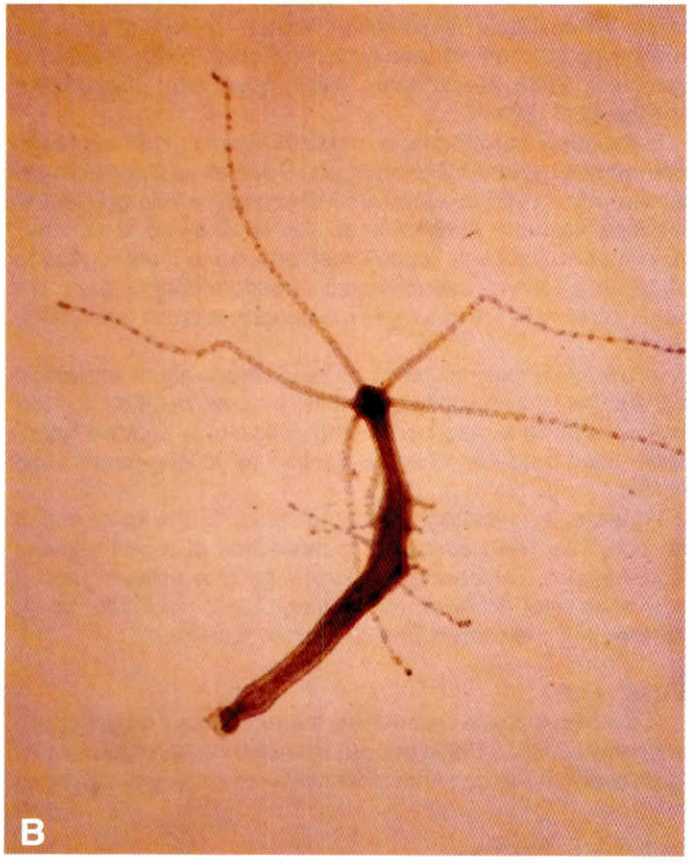




('positional value' or 'source density'). The new model is based on receptor-mediated competition for factors, and has been developed to account for new findings on long-range interactions between head, bud and foot formation. Thus, while head regeneration and the onset of budding are mutually inhibitory, fully developed heads and advanced buds cooperatively support foot formation. In head regeneration this foot-promoting activity displays the same kinetics as the apparent long-range 'head inhibition' determined in classical transplantation studies (e.g. MacWilliams, 1983). The model includes the following hypotheses:

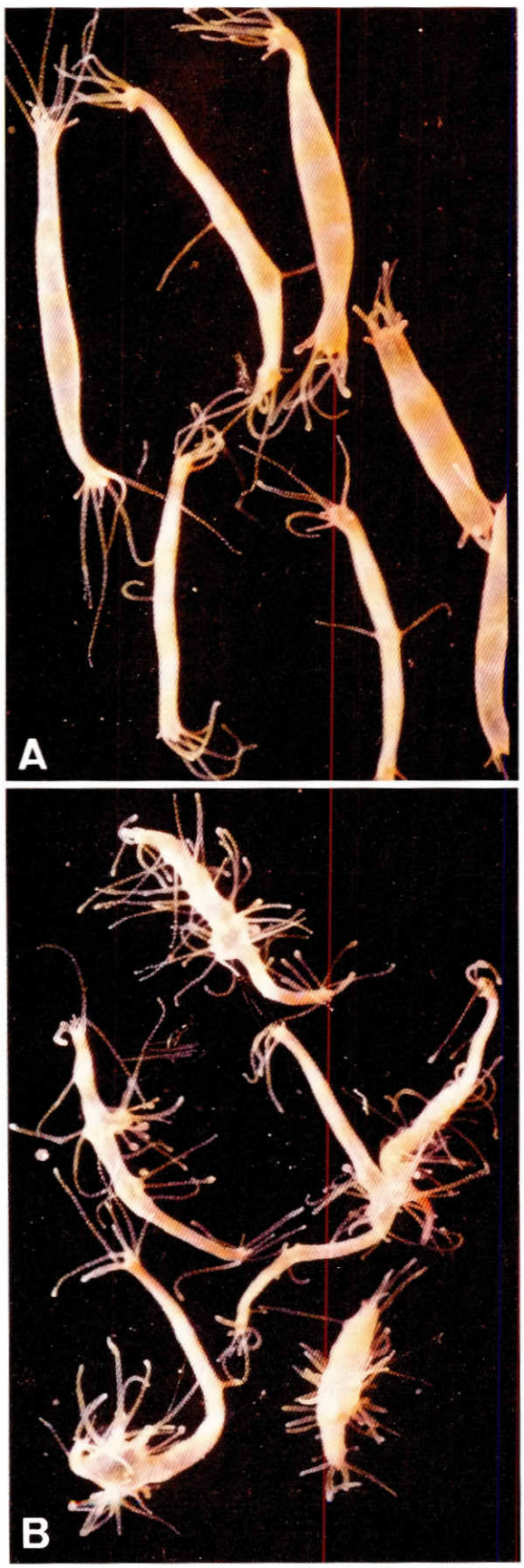
(1) Heads and buds compete for resources, such as precursor cells and soluble head-promoting factors that are distributed in the interstitial spaces. (2) The ability to make use of these resources is associated with positional value and decreases down the body column. The decreasing capability is attributed to a decreasing complement of receptors for the head-promoting factors. (3) Feet are made by body regions that lose out in the competition for these factors. (4) Superiority in the ability to compete for the locally available factors enables transplants to develop head structures, inferiority causes them to form a foot. (5) Depletion of the head-promoting factors in the whole body column is a significant component of the 'head inhibition potential' and mediates the assistance of heads and buds in foot formation. (6) A surplus of resources causes supernumerary head structures and delays or prevents foot regeneration. These interpretations have reference to a new receptor-based computer model of pattern control (Sherratt *et al.*, 1996) as well as to experimental results which are partly reviewed in the following.

Currently known signal molecules

The new model proposed implies that there exists a growth-factor like molecule which is needed to gain and maintain positional value. Its presence enables or promotes head formation, its absence causes foot formation. The existence of such a factor is suggested by studies with aggregates of cells from the gastric region in *Hydractinia* (Müller *et al.*, 1986). Above a critical mass, aggregates form only tentacles, below this critical mass they form only stolons. The interpretation of this finding is that small aggregates lose too much of the putative soluble factor due to the relatively large surface area, while larger aggregates can accumulate it. However, the factor is not yet identified chemically.

Efforts to identify supposed morphogens or head-promoting factors in *Hydra* have succeeded in the presentation of two chemically defined, potential signal molecules that are capable of evoking supernumerary tentacles and ectopic head formation. Arachidonic acid may evoke ectopic head formation in *H. magnipapillata*, strain *wt105* (Müller *et al.*, 1993). If supported by herbimycin A, a known inhibitor of tyrosine-specific protein kinases, a derivative of arachidonic acid, 12-S-HETE (12-S-hydroxy-

Fig. 1. Supernumerary head structures evoked by diacylglycerol in non-regenerating *Hydra vulgaris*. *The intact animals were treated with 0.2 mM 1,2-dioctanoyl-sn-glycerol (DAG) for 1-2 h, and the treatment was daily repeated over about two weeks. In H. vulgaris supernumerary heads predominantly arise by splitting of the existing, enlarged head* **(A)**. *Body tentacles occur only occasionally in H. vulgaris* **(B)** *but frequently in H. magnipapillata, strain wt105 (Müller, 1998, 1990). The experiments with H. vulgaris were done, and the photos taken, by Marcus Frohme.*



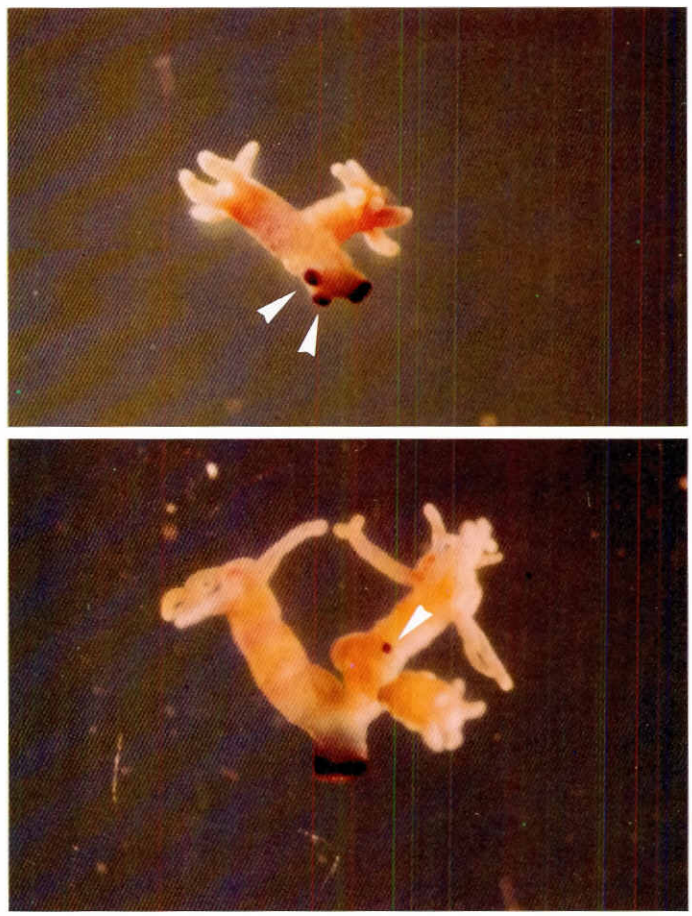


Fig. 3. Supernumerary feet evoked by multiple heads. *Multiheaded H. vulgaris were produced by surgically splitting the existing head. In addition, the heads of buds assisted in the evocation of supernumerary feet. These occurred about 1-2 weeks after the splitting of the parental head. The supernumerary feet were made visible by a peroxidase stain (Hoffmeister and Schaller, 1985).*

eicosatetra-enoic acid), may also cause the development of ectopic tentacles. However, the low effectiveness of these eicosanoids suggests that more effective factors might exist.

While having only slight inducing capacity of their own, arachidonic acid and 12-S-HETE strongly enhance DAG-induced (see below) ectopic head formation. The common denominator in the action of these polyunsaturated fatty acids might be their capability of activating protein kinase C in an additive way (for review see Asaoka *et al.*, 1992).

Other eicosanoids such as the 5-(S,R)-HETEs and the 11-(S,R)-HETEs reversibly inhibit head and bud formation (Leitz *et al.*, 1994c, and as yet unpublished results).

There is no known endogenous substance capable of inducing foot formation in *Hydra*. In *Hydractinia*, the factor SIF is able

Fig. 2. Gastric segments excised from DAG-treated wt105 polyps. *The polyps had been pre-treated with 0.1 mM DAG on 5* **(A)** *or 7* **(B)** *successive days. When the segments were excised the animals did not yet display ectopic tentacles but still looked quite normal. The supernumerary tentacles emerged during abnormal 'regeneration' of the excised segments.*

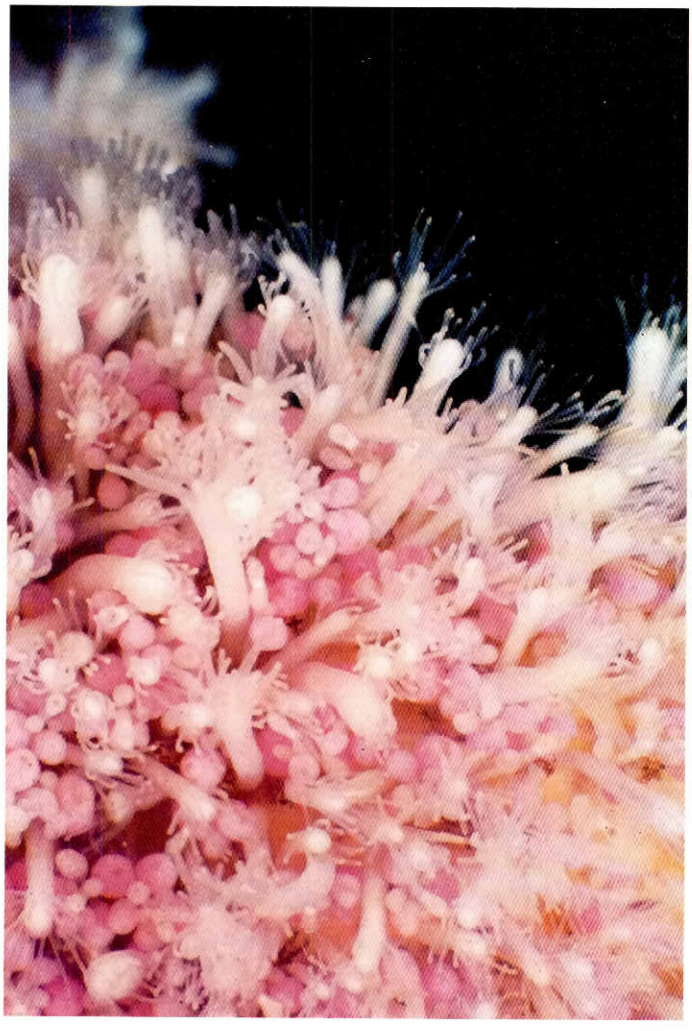


Fig. 4. Mature colony of *Hydractinia*. *In between the Hydra-like gasterozooids sexual polyps are interdispersed. These produce reddish oocytes in transparent globular containers (= sessile, reduced medusae).*

to transform hydranths into stolons which topographically correspond to the foot in *Hydra*. However, most probably SIF is a natural inducer but not a morphogen (see below).

The "head activator" and "foot activator" referred to in the paper of Schaller et al. (this volume) do not induce head and foot formation in the strains of *Hydra* used in our laboratory *(H. vulgaris, H. magnipapillata)*. Furthermore, in regenerating *H. oligactis* the head activator does not qualitatively alter head morphology (Javois and Tombe, 1991).

Induction of ectopic head and foot formation by interference with the PI-PKC signal transduction system

Evocation of supernumerary head structures

In the hydroid *Hydractinia echinata* extracts from hydranths may cause excised segments of hydranths to regenerate heads not only at the oral but also at the aboral end (Müller, 1969). Attempts to bypass or replace the putative head-promoting component by interference with signal transduction systems yielded evidence of a role for the PI-PKC system (phosphatidylinositol-protein kinase C system) in the control of the body pattern. The tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA), a known agonist of the PI-PKC system, can be used as a very potent substitute for the endogenous, head-inducing factor (Müller, 1985).

The PI-PKC system includes protein kinase C (PKC) as a key enzyme. From a *Hydra* cDNA library two isoforms of PKC have been cloned (Hassel *et al.*, unpublished), one being paralogous to calcium-dependent and one to calcium-independent mammalian isoforms. At least one of the PKCs appears to be involved in the regulation of positional value and, hence, of head formation:

Activators of PKC such as TPA, diacylglycerol (DAG) and arachidonic acid, if periodically applied for several days, induce supernumerary head formation. In *H. magnipapillata*, strain *wt105*, the supernumerary head structures arise ectopically in the gastric region, in *H. vulgaris* they may occur by multiplication of the existing head, which occasionally splits into two or more (Fig. 1). Before supernumerary tentacles emerge on the intact animals, segments excised from the gastric region of pre-treated *wt105* polyps may regenerate head structures at their basal end instead of a foot, or sprout tentacles all around (Fig. 2; Müller, 1989, 1990, 1991, 1995a). Even a single pulse treatment with TPA induces an enhanced expression of the head-specific epithelial gene, *Ks1* (Weinziger *et al.*, 1994).

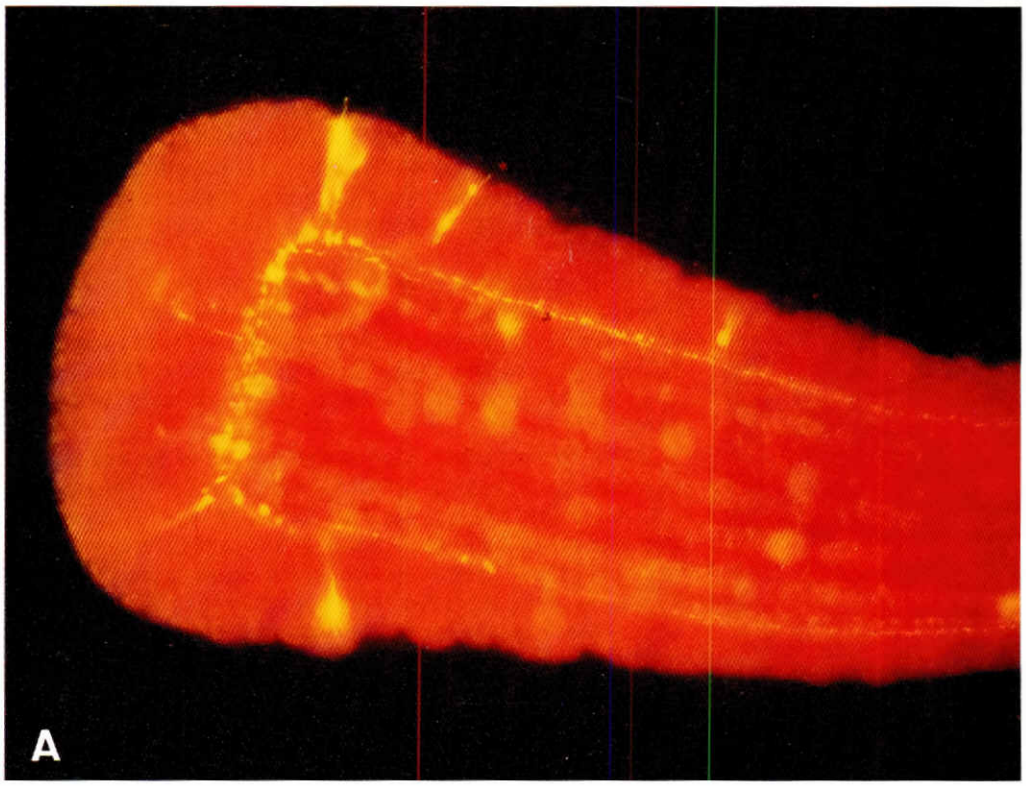
In addition, activators of PKC delay or prevent a decrease in positional value at the lower end of a segment excised from the body column, and at the base of a bud. Therefore, buds may fail to detach and branched animals result. This effect is enhanced by inhibitors of non-C-type protein kinases such as staurosporine and genistein (Perez and Berking, 1994). An inhibitor of protein-tyrosine kinases, herbimycin A, supports activators of PKC in their capability of inducing the formation of supernumerary tentacles (Müller, not yet published).

Inhibitors of PKC such as chelerythrine cause the gradual reduction of existing head structures but do not induce ectopic foot formation (Müller *et al.*, 1993). Foot formation appears to involve (also) other subroutes of the PI-PKC system.

Evocation of ectopic foot formation

The head of the parental body and the heads of advanced buds promote foot formation in both the parent and the bud, whereas feet do not promote head formation (Müller, 1995b). Supernumerary head structures may eventually evoke the development of supernumerary feet, regardless of how the supernumerary heads had been generated. Even multiplication of the heads by surgical splitting may be followed by the formation of supernumerary feet (Fig. 3).

Another method applies lithium ions. While pulse treatment of *H. vulgaris* with LiCl elevates positional value, long-term exposure of the animals to lithium leads to its decrease and eventually to the formation of ectopic feet (Hassel and Berking, 1989, 1990; Hassel *et al.*, 1993). Lithium is known to block signal transduction through the PI-PKC system, for instance by interference with G-protein receptor coupling and by inhibition of inositol phosphatases (references in Hassel *et al.*, 1993). Quantitative determinations of PI-metabolites in normal animals and in systemically lithium-treated polyps revealed the appearance of a



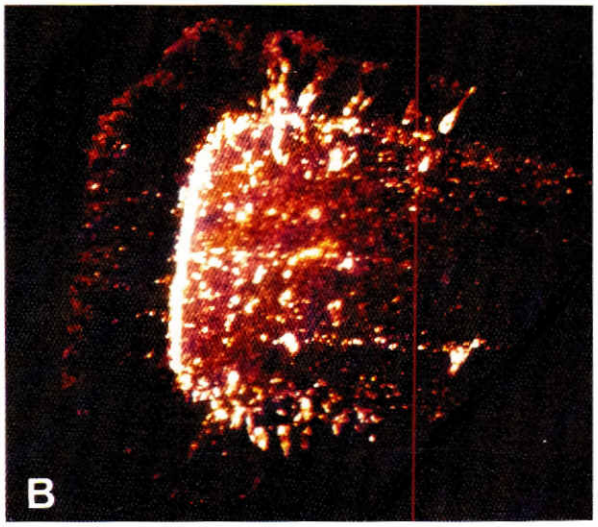


Fig. 5. Localisation of neuropeptides in the planula larva of *Hydractinia* by means of immunostaining. (A) *Conventional fluorescence microscopy of the anterior half of a planula showing neurosensory cells which contain a peptide with the C-terminal sequence RF-amide. Such neuropeptides are common in coelenterates (Grimmelikhuijzen et al., 1992).* **(B)** *Confocal laser scanning image of cells showing LWamide immunoreactivity. For methods see Leitz and Lay (1995).*

particular inositolpolyphosphate during head regeneration and a redistribution of PI-metabolites along the body column as a consequence of prolonged lithium treatment (Hassel, in preparation).

Conclusion

The results collected so far suggest that activation of the PI-PKC system leads to an increase in positional value and eventually to head formation, while inhibition of the PI-PKC system causes a decrease in positional value and eventually foot formation.

Rescue of a regeneration deficient mutant

The mutant strain *reg-16* of *Hydra magnipapillata* has less capacity to regenerate a head than the wild-type strain *wt105* when examined in a standard test. Moreover, its head-forming potential is severely impaired by the presence of a just-emerging or latent bud. Periodic treatment with dioctanoylglycerol (DAG) plus arachidonic acid (AA) enabled *reg-16* to insert additional tentacles into their original whorl and to store potentials for head and bud formation: gastric segments excised from pretreated animals formed more tentacles than untreated *wt105*, and even formed supernumerary head structures; in addition, segments excised from all body regions quickly resumed budding in spite of starvation and while they regenerated a head (Müller, 1995a).

The phenomenon is explained in terms of the new model outlined above: a regenerating head and a beginning bud compete for hormone-like factors that enable the cells to increase positional value, and for precursor cells. Periodic treatment with activators of PKC plus AA leads to an augmentation of these resources, and head structures and buds can be produced simultaneously. Traditional terms are reinterpreted correspondingly: the high level of 'head inhibition' in reg-16 is interpreted as a low level of resources, in particular of head-promoting factors, the low 'head activation' level as a low ability to make use of resources.

Control of development in *Hydractinia*

In *Hydra* embryonic development is almost inaccessible to experimental and biochemical studies. Embryogenesis and metamorphosis are studied in the marine colonial hydroid *Hydractinia echinata* (Fig. 4). In the present review the significance of signals and signal transducing systems in metamorphic and postmetamorphic development are addressed.

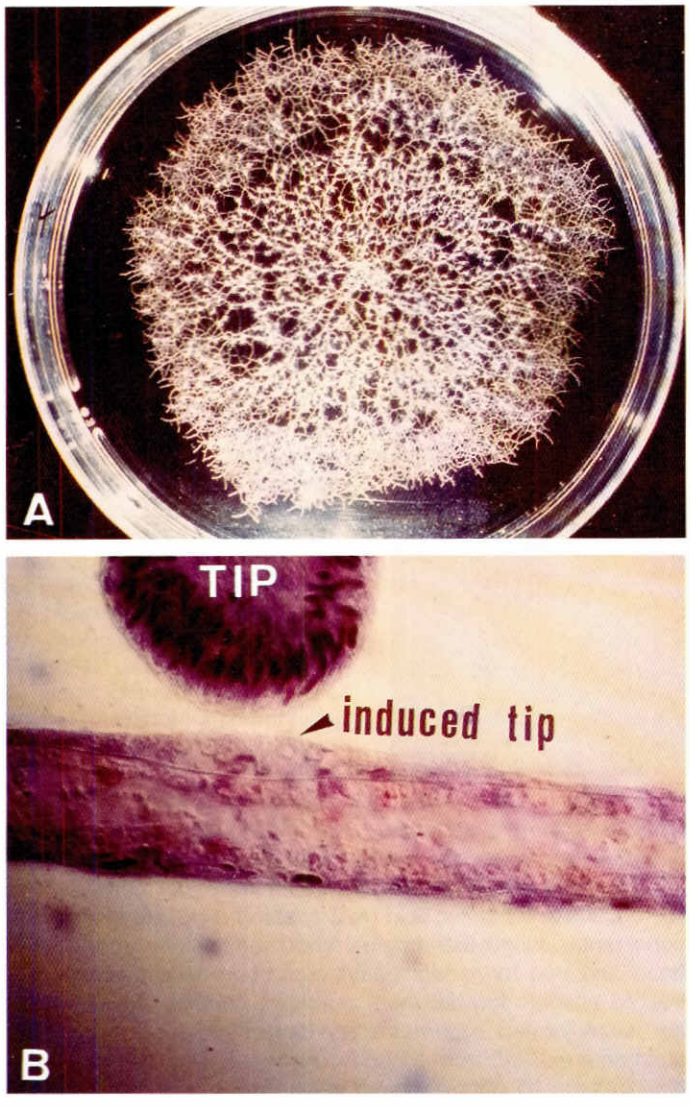


Fig. 6. The stolonal system and its development in *Hydractinia echinata*. **(A)** *A colony grown from a single metamorphosed planula larva in a Petri dish. The stolonal network connects secondary, nutritive polyps (gasterozooids). Through elongation and branching of the stolons, the network enlarges into the periphery, while in the centre of the colony the network becomes denser and denser.* **(B)** *A migrating stolon tip that approaches an established stolon emits a Stolon-Inducing Factor SIF, which induces the formation of another stolon tip in the established stolon. In encounters of intraclonal or histocompatible stolons both tips, the inducing and the induced, will fuse to form an anastomosis. In encounters of histo-incompatible colonies the induced tip will give rise to a stolon branch.*

Additional aspects are reviewed in the paper of Berking *et al.* (this issue).

Control of metamorphosis

As in most coelenterates, embryogenesis in *Hydractinia* terminates in a planula, a ciliated, cone-shaped, mouthless larva. The mature planula ceases cell proliferation, differentiation, and morphogenesis (Plickert *et al.*, 1988). It displays locomotory activity, and uses its sensory cells at the anterior end to prospect for a habitat suited for its future sessile phase of life.

Metamorphosis, the transformation of the larva into the sessile primary polyp, is dependent on the presence of an environmental key stimulus. Such a stimulus derives from bacteria of the genus Alteromonas which cover the substrate. The inducing stimulus is a lipid, the chemical identity of which is unknown at present (Müller, 1973; Müller *et al.*, 1976; Leitz and Wagner, 1993).

The mechanism of signal reception by the sensory equipment of the larva involves the PI-PKC system, and activators of PKC, such as TPA and DAG, can be used to replace the bacterial lipid for initiating metamorphosis (Leitz and Müller, 1987; Leitz and Klingmann, 1990; Leitz, 1993; Schneider and Leitz, 1994).

The reception of the external signal leads to the release of a molecule that transmits an internal signal to begin metamorphosis, from the anterior to the posterior end of the larva (Schwoerer-Böhning *et al.*, 1990). A first peptide with the capability of inducing metamorphosis in the posterior larval body as well as in entire larva has been extracted from a heterologous source *(Anthopleura elegantissima)* and its sequence was determined to be pEQPGLWamide (Leitz *et al.*, 1994b). The peptide, named metamorphosin A, was the first member of a new class of neuropeptides, now called LWamides. Using molecular probes derived from this peptide, a precursor of similar, autologous peptides was cloned. The cDNA structure of the precursor protein contains repeats of PPGLW-NH_2 and AKPPGLW-NH_2 (Gajewski *et al.*, 1996). Both homologous peptides trigger metamorphosis. Immunostaining with antibodies that recognize the aminoterminus (essential for function) assigns the LWamides to neurosensory cells in the anterior part of the larva, where the external, bacteria-born signal is received. These neurosensory cells extend their fibres into the posterior part of the larva (Fig. 5; Leitz and Lay, 1995), where they presumably release one or both of the LWamides to trigger and coordinate the events of metamorphosis.

Like the inducing factors in amphibians, the peptide(s) stimulate(s) – directly or by inducing cascades of subsequent signaling systems – pattern formation, cell proliferation, cell differentiation, and morphogenesis.

Some of these events may be initiated by arachidonic acid or its lipoxygenase products, of which several are found in *Hydractinia* (Leitz *et al.*, 1994a) as well as in *Hydra* (Müller *et al.*, 1993; Di Marzo *et al.*, 1994, and references therein).

An inducing signal in postmetamorphosis

The primary polyp that emerges from the metamorphosing larva is the founder of a new colony. At its base tube-like stolons grow out. By increasing in length, branching, and forming anastomoses, the stolons form a network that interconnects the future members of the colony (Fig. 6A). These members, secondary polyps (hydranths) of various types (Fig. 4), emerge as buds on the stolons at more or less regular distances.

The processes of elongation, branching and reunification in the stolonal network display many analogies to angiogenesis in vertebrates. The elongation of stolons is accomplished by the locomotory activity of a particular pathfinding organ, the stolon tip (Fig. 6B). The spontaneous development of such a locomo-

tory organ at the flank of the stolons initiates the formation of a branch. On the other hand, stolon branching can also be externally induced by the approaching tip of another stolon that belongs to the same or to a neighbouring colony (Fig. 6B).

The stolon tip is the source of an inducing signal that causes branching by stimulating tip formation in adjoining stolons. Both tips, the inducing and the induced one, attract each other. In encounters of isogeneic, or allogeneic but histocompatible, stolons, the two tips fuse and an anastomosis results (Müller and Plickert, 1982; Müller *et al.*, 1987; Lange *et al.*, 1989).

We have succeeded in isolating a glycoprotein with tip-inducing capacity, and termed it SIF (stolon inducing factor). In various bioassays it turned out that the inducing potential of this factor is higher than expected. SIF can induce an ectopic sprouting of stolons in polyps (hydranth) and can even cause a complete transformation of the hydranth into a giant stolon (Lange and Müller, 1991).

References

ASAOKA, Y., NAKAMURA, S., YOSHIDA, K. and NISHIZUKA, Y. (1992). Protein kinase C, calcium and phospholipid degradation. *Trends Biochem. Sci. 17:* 414-417.

DI MARZO, V., GIANFRANI, C., DE PETROCELLIS, L.D., MILONE, A. and CIMINO, G. (1994). Polyunsaturated-fatty-acid oxidation in *Hydra*. Regioselectivity, substrate-dependent enantioselectivity and possible biological roles. *Biochem. J. 300:* 501-507.

GAJEWSKI, M., LEITZ, T., SCHLOβHERR, J. and PLICKERT, G. (1996). LWamides from Cnidaria constitute a novel family of neuropeptides with morphogenetic activity. *Roux Arch. Dev. Biol. 205:* 232-242.

GIERER, A. and MEINHARDT, H. (1972). A theory of biological pattern formation. *Kybernetik 12:* 30-39.

GRIMMELIKHUIJZEN, C.J., CARSTENSEN, K., DARMER, D., MOOSLER, A., NOTHACKER, H.P., REINSCHEID, R.K., SCHMUTZLER, C., VOLLERT, H., McFARLANE, I. and RINEHART, K.L. (1992). Coelenterate neuropeptides: Structure, action and biosynthesis. *Am. Zool. 32:* 1-12.

HASSEL, M. and BERKING, S. (1989). Nerve cell and nematocyte production in *Hydra* is deregulated by lithium ions. *Roux Arch. Dev. Biol. 197:* 471-475.

HASSEL, M. and BERKING, S. (1990). Lithium ions interfere with pattern control in *Hydra*. *Roux Arch. Dev. Biol. 198:* 382-388.

HASSEL, M., ALBERT, K. and HOFHEINZ, S. (1993). Pattern formation in *Hydra vulgaris* is controlled by lithium-sensitive processes. *Dev. Biol. 156:* 362-371.

HOFFMEISTER, S. and SCHALLER, H.C. (1985). A new biochemical marker for foot-specific cell differentiation in *Hydra*. *Roux Arch. Dev. Biol. 194:* 453-461.

JAVOIS, L.C., and TOMBE, V.K. (1991). Head activator does not qualitatively alter head morphology in regenerates of *Hydra oligactis*. *Roux Arch. Dev. Biol. 199:* 402-408.

LANGE, R.G. and MÜLLER, W.A. (1991). SIF, a novel morphogenetic inducer in Hydrozoa. *Dev. Biol. 147:* 121-132.

LANGE, R.G., PLICKERT, G. and MÜLLER, W.A. (1989). Histoincompatibility in a low invertebrate, *Hydractinia echinata:* analysis of the mechanism of rejection. *J. Exp. Zool. 249:* 284-292.

LEITZ, T. (1993). Biochemical and cytological bases of metamorphosis in *Hydractinia echinata*. *Mar. Biol. 116:* 559-564.

LEITZ, T. and KLINGMANN, G. (1990). Metamorphosis in *Hydractinia*: Studies with activators and inhibitors aiming at protein kinase C and potassium channels. *Roux Arch. Dev. Biol. 199:* 107-113.

LEITZ, T. and LAY, M. (1995). Metamorphosin A is a neuropeptide. (Submitted).

LEITZ, T. and MÜLLER, W.A. (1987). Evidence for the involvement of PI-signaling and diacylglycerol second messengers in the initiation of metamorphosis in the hydroid *Hydractinia echinata* Fleming. *Dev. Biol. 121:* 82-89.

LEITZ, T. and WAGNER, T. (1993). The marine bacterium *Alteromonas espeijana* induces metamorphosis in the hydroid *Hydractinia echinata*. *Mar. Biol. 115:* 173-178.

LEITZ, T., BECK, H., STEPHAN, M., LEHMANN, W.D., DE PETROCELLIS, L. and DI MARZO, V. (1994a). Possible involvement of arachidonic acid and eicosanoids in metamorphic events in *Hydractinia echinata* (Coelenterata; Hydrozoa). *J. Exp. Zool. 269:* 422-431.

LEITZ, T., MORAND, K. and MANN, M. (1994b). Metamorphosin A: a novel peptide controlling development of the lower metazoan *Hydractinia echinata* (Coelenterata, Hydrozoa). *Dev. Biol. 163:* 440-446.

LEITZ, T., MÜLLER, W.A., DE PETROCELLIS, L. and DI MARZO, V. (1994c). Enantiospecific synthesis of bioactive hydroxyeicosatetraenoic acids (HETE's) in *Hydra magnipapillata*. *Biochim. Biophys. Acta 1213:* 215-223.

MacWILLIAMS, H.K. (1982). Numerical simulations of *Hydra* head regeneration using a proportion-regulating version of the Gierer-Meinhardt model. *J. Theor. Biol. 99:* 681-703.

MacWILLIAMS, H.K. (1983a). *Hydra* transplantation phenomena and the mechanism of *Hydra* head regeneration. I. Properties of the head inhibition. *Dev. Biol. 96:* 217-238.

MacWILLIAMS, H.K. (1983b). Numerical simulation of *Hydra* head regeneration using a proportion-regulating version of the Gierer-Meinhard model. *J. Theor. Biol. 99:* 681-703.

MEINHARDT, H. (1982). *Models of Biological Pattern Formation*. Academic Press, London, New York.

MEINHARDT, H. (1993). A model for pattern formation of hypostome, tentacles, and foot in *Hydra*: How to form structures close to each other, how to form them at a distance. *Dev. Biol. 157:* 321-333.

MORGAN, T.H. (1904). An attempt to analyze the phenomena of polarity. *J. Exp. Zool. 1:* 587-591.

MÜLLER, W.A. (1969). Auslösung der Metamorphose durch Bakterien bei den Larven von *Hydractinia echinata*. *Zool. Jb. Anat. 86:* 84-95

MÜLLER, W.A. (1973). Induction of metamorphosis by bacteria and ions in the planulae of *Hydractinia echinata*; an approach to the mode of action. Publ. Seto Mar. Biol. Lab. 20, (Proc. 2nd Int. Symp. Cnidaria), pp. 195-208.

MÜLLER, W.A. (1985). Tumor-promoting phorbol esters induce metamorphosis and multiple head formation in the hydroid *Hydractinia*. *Differentiation 29:* 216-222.

MÜLLER, W.A. (1989). Diacylglycerol induced multihead formation in *Hydra*. *Development 105:* 306-316

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.

MÜLLER, W.A. (1991). Stimulation of head-specific nerve cell formation in *Hydra* by pulses of diacylglycerol. *Dev. Biol. 147:* 460-463.

MÜLLER, W.A. (1995a). Competition for factors and cellular resources as a principle of pattern formation in *Hydra*. I. Increase of the potentials for head and bud formation and rescue of the regeneration-deficient mutant *reg-16* by treatment with diacylglycerol and arachidonic acid. *Dev. Biol. 167:* 159-174.

MÜLLER, W.A. (1994b). Competition for factors and cellular resources as a principle of pattern formation in *Hydra*. II. Assistance of foot formation by heads and buds and a new model of pattern control. *Dev. Biol. 167:* 175-189.

MÜLLER, W.A. and PLICKERT, G. (1982). Quantitative analysis of an inhibitory gradient field in the hydrozoan stolon. *Roux Arch. Dev. Biol. 191:* 56-63.

MÜLLER, W.A., HAUCH, A. and PLICKERT, G. (1987). Morphogenetic factors in hydroids. I. Stolon tip activation and inhibition. *J. Exp. Zool. 243:* 111-124.

MÜLLER, W.A., LEITZ, T., STEPHAN, M. and LEHMANN, W.S. (1993). Arachidonic acid and the control of body pattern in *Hydra*. *Roux Arch. Dev. Biol. 202:* 70-76.

MÜLLER, W.A., PLICKERT, G. and BERKING, S. (1986). Regeneration in Hydrozoa: distal versus proximal transformation in *Hydractinia*. *Roux Arch. Dev. Biol. 195:* 113-518.

MÜLLER, W.A., WIEKER, F. and EIBEN, R. (1976). Larval adhesion, releasing stimuli and metamorphosis. In *Coelenterate Ecology and Behavior* (Ed. G.O. Mackie). Plenum Press, New York, pp. 339-346.

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

PLICKERT, G., KROIHER, M. and MUNCK, A. (1988). Cell proliferation and early differentiation in *Hydractinia echinata*. *Development 103:* 795-803.

SCHNEIDER, T., and LEITZ, T. (1994). Protein kinase C in hydrozoans: involvement in metamorphosis of *Hydractinia* and pattern formation of *Hydra*. *Roux Arch. Dev. Biol. 203:* 422-428.

SCHWOERER-BÖHNING, B., KROIHER, M. and MÜLLER, W.A. (1990). Signal transmission and covert prepattern in the metamorphosis of *Hydractinia echinata* (Hydrozoa). *Roux Arch. Dev. Biol. 198:* 245-251.

SHERRATT, J.A., MAINI, P.K., JÄGER, W. and MÜLLER, W.A. (1996). A receptor-based model for pattern formation in *Hydra*. *Forma.* (In press).

SHIMIZU, H., SAWADA, Y. and SUGIYAMA, T. (1993). Minimum tissue size required for *Hydra* regeneration. *Dev. Biol. 155:* 287-296.

TREMBLEY, A. (1744). *Memoires pour Servir a l'Histoire d'un Genre de Polypes d'Eau Douce, a Bras en Forme de Cornes.* J.U. Verbeek, Leyden.

TURING, A.M. (1952). The chemical basis of morphogenesis. *Philos. Trans. R. Soc. B237:* 37-72.

WEINZIGER, R., SALGADO, L.M., DAVID, C.N. and BOSCH, T.C.G. (1994). Ks1, an epithelial cell-specific gene, responds to early signals of head formation in *Hydra*. *Development 120:* 2511-2517.

WOLPERT, L., CLARKE, M.R.B. and HORNBRUCH, A. (1974). Positional signaling along *Hydra*. *Nature New Biol. 239:* 101-105.