Low-molecular-weight factors from colonial hydroids affect pattern formation

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Summary. Two morphogenetic factors have been isolated from tissue of colonial hydroids. Both exert strong effects on pattern formation during metamorphosis, regeneration and colony development. Polyp-inhibiting factor (PIF) is a bivalent inhibitor which strongly affects head and bud formation but acts weakly on stolon branching. Proportion-altering factor (PAF) is a distalizing factor. It counteracts the formation of stolon and promotes the formation of head structures during metamorphosis and regeneration. PIF and PAF antagonistically influence the spatial arrangement of polyps within a colony. They are capable of displo-cating structures and thus appear to interfere with or are even part of the pattern-controlling mechanism. Both fac-tors are of low molecular size (about 500 daltons), hydrophilic and probably not peptides.

Key words: Hydractinia echinata – Eirene viridula – Metamorphosis – Colonial hydroids – Pattern formation

Introduction
The cells of a multicellular organism have to decide which part of the genome they will activate in order to accomplish differentiation. It has been proposed that this decisive development step depends on signalling systems which at the appropriate times provide the cells with positional information (Wolpert et al. 1974; Gierer and Meinhardt 1972). During the embryonic development of vertebrates, cell commitment is sharply restricted to short time periods of sometimes only a few hours. Since the responsible control system apparently works only until cells are irreversibly committed, the time available to assay and to isolate signal substances is limited. Instead, in hydroids pattern formation in the genome they will activate in order to accomplish differentiation. It has been proposed that this decisive development step depends on signalling systems which at the appropriate times provide the cells with positional information (Wolpert et al. 1974; Gierer and Meinhardt 1972). During the embryonic development of vertebrates, cell commitment is sharply restricted to short time periods of sometimes only a few hours. Since the responsible control system apparently works only until cells are irreversibly committed, the time available to assay and to isolate signal substances is limited. Instead, in hydroids pattern formation occurs continuously, even in post-embryonic development.

1. Planulae undergo metamorphosis in response to a triggering stimulus. In the transforming larva, the axial pattern of the polyp (Fig. 1) forms for the first time.
2. Adult hydroid polyps regenerate distorted patterns.
3. Colonial hydroids form new patterns autonomously by stolon polyp budding and branching. Both processes depend on reorganization of the stolon tissue, cell commitment and cell differentiation.

Since new patterns can form at any time, the responsible control mechanisms must be permanently active. This is also indicated by the finding that two inhibitory signals are continuously present in the stolon. One extends from existing tips and controls branching of the stolon (Müller and Plickert 1982). Inhibition spreading out from polyp heads accounts for the control of polyp budding, i.e. the decision whether or not a new polyp will be formed as well as the spatial position of the bud. Experimental results strongly indicate that diffusion forms the physical basis of the transmission of this inhibitory signal (Plickert et al. 1987).

From coelenterates, several morphogenetically active biomolecules have already been isolated and identified. Schaller (1973) isolated a peptide from Hydra, termed head activator, which accelerates budding and head regeneration processes. Also from Hydra an inhibitor has been partially purified. It has inhibitory effects on regeneration and bud formation in Hydra and modifies patterning during metamorphosis of Hydractinia echinata (Berkling 1984). Recently, the compounds N-methylpicolinic acid, N-methylnicotinic acid and N-trimethylglycine were detected in coelenterates and were shown to influence pattern formation (Berkling 1987).

In this paper we show that colonial hydroids contain at least two further morphogenetic activities. They have remarkable effects on polyp budding, the spacing of polyps, and head/stolon tip development during metamorphosis as well as in regenerative pattern formation.

Materials and methods
Extracts were prepared from the colonial hydroids, Eirene viridula, Hydractinia echinata and Eudendrium species. For comparison, extracts were also prepared from Anthopleura elegantissima and Hydra attenuata. The material was sonicated and extracted several times in absolute methanol.

Hydractinia echinata and El. viridula were reared in artificial sea-water (made from spring-water and a salt mixture from Tropicarium Buchschlag) and fed with brine shrimp larvae. Bulk material of several Eudendrium species collected from different locations was kindly supplied by Dr. H. Zibrowius (Station Marine d’Endoume, Marseille, France). Frozen A. elegantissima were purchased from the California Supply Company.

Chromatography
Crude extracts were chromatographed on Sephadex LH20 in absolute methanol. Three different column sizes were used according to the amount of material: 2.6×145 cm, V = 750 ml; 7×100 cm, V = 3000 ml (for the large scale
preparation of about 10 kg Eudendrium material, wet weight); 1 x 30 cm, V = 20 ml (for analytical preparations).

Active fractions were pooled, dried, redissolved in 0.1 M acetic acid and chromatographed on Biogel P2, 200-400 mesh or -400 mesh, using 0.1 M acetic acid as the elutant. The column sizes used were: 5 x 100 cm, V = 1700 ml; 2.6 x 145 cm, V = 750 ml; 1 x 30 cm, V = 20 ml (all P2, 200-400 mesh); and 1 x 100 cm, V = 75 ml (P2, -400 mesh).

Biological assays

During the purification procedure fractions were assayed for morphogenetic activity on stolon tip or polyp head formation. Activities were traced by the Hydractinia echinata metamorphosis assay (Berking 1984), then assayed for potential effects on head/stolon tip regeneration (foot regeneration) in Ei. viridula and Hydra attenuata. Activities were further assayed on spacing control as outlined below. Samples of each fraction were dried and redissolved in sea-water or, for assays using Hydra, in spring-water. The osmotic value and pH of the solution were monitored and, if necessary, adjusted to normal values before application to the assay.

Metamorphosis assay. Larvae of Hydractinia echinata were induced to undergo metamorphosis by exposure to a solution of 56 mM CsCl in sea-water (Müller and Buchal 1973). The Cs + solution was replaced 3-4 h after application by normal culture medium (several times), and eventually by the test solution. Control animals were exposed to normal culture medium; 20-24 h later, metamorphosed animals were checked for pattern alterations, i.e. head and stolon formation and/or changes in the proportions of the polyp compared with control animals.

Regeneration assays. Effects of isolated factors on regenerative head or stolon tip (foot) formation were assayed on polyps of Ei. viridula and Hydra. The animals were cut at different body levels. Head-bearing distal and stolon-(foot-) bearing proximal pieces were exposed to the test solution immediately after isolation for various incubation times.

Budless Hydra polyps were used that had been fed for the last time 1 day before the experiment. Groups of 15–25 test pieces were incubated in 10 ml medium, which was replaced by normal medium 21 h after cutting. Head regeneration was judged to be finished when tentacle buds became visible. A foot was scored as regenerated when the animal was able to attach to the culture dish.

Polyps of Ei. viridula were removed from the colony 1 day prior to the experiment by cutting them just below the hydranth. Regeneration was observed until the regeneration frequency, i.e. the percentage of animals which accomplished regeneration, did not change further. Regenerated stolon tips were scored when typical morphology was displayed and tips became sticky as a result of periderm secretion. Head regeneration was evaluated on the basis of the appearance of tentacle buds.

Colony patterning assay. Simple colony units of Ei. viridula were used in order to evaluate effects on spacing control. Polyps of equal size were isolated from colonies and transferred to separate dishes, where they were allowed to attach to the dish and grow a stolon. One day before the elongating stolon was expected to form the first polyp bud, the test solution was added to the colony units. The effects of the isolated factors on spacing control were evaluated by measuring the interpolyp distances in treated and untreated colonies. Influence on tip function were assayed by measuring the velocity of stolon elongation.

Statistical analysis

The significance of experimental differences was calculated by the $\chi^2$ test or the Fisher-Yates test. Differences are significant ($P<0.05$) unless stated otherwise. In Figs. 5–9 mean values and standard deviations of regeneration frequencies in three parallel groups of animals are shown. In Fig. 10, the range of confidence was calculated by use of the standard deviation of the binomial distribution.