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

Günter Plickert
Zoologisches Institut, Universität, Im Neuenheimer Feld 230, D-6900 Heidelberg, Federal Republic of Germany

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 dislocating structures and thus appear to interfere with or are even part of the pattern-controlling mechanism. Both factors 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 developmental 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 during metamorphosis and regeneration of polyps within a colony. They are capable of dislocating structures and thus appear to interfere with or are even part of the pattern-controlling mechanism. Both factors are of low molecular size (about 500 daltons), hydrophilic and probably not peptides.

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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 LH 20 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
Cs +

larva

stolon tip
tentacle bud

primary polyp

hypostome
tentacle
gastric region
basal disc
stolon tip
stolon

0 4 12 18 30 Time (h)

Fig. 1. Metamorphosis of *Hydractinia echinata*. Planula larvae of *Hydractinia* can be stimulated to undergo metamorphosis by pulse treatment with CsCl (56 mM, 3–4 h; Müller and Buchal 1973). The axial pattern of the primary polyp first becomes visible through the appearance of tentacle buds and stolon tips, 18 h after application of Cs +

preparation of about 10 kg *Eudendrium* material, wet weight; 1 × 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 × 100 cm, V = 1700 ml; 2.6 × 145 cm, V = 750 ml; 1 × 30 cm, V = 20 ml (all P2, 200–400 mesh); and 1 × 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.

Colonial 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 χ² 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.