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Metamorphic processes in the soft corals *Heteroxenia fuscescens* and *Xenia umbellata*: The effect of protein kinase C activators and inhibitors

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Summary

During the life cycle of marine invertebrates, including corals with benthic and planktonic phases, embryogenesis leads to a free swimming larva which has to find a suitable habitat to settle and metamorphose. Initial settlement and induction of metamorphosis of benthic marine invertebrate larvae are generally determined by interaction of external biochemical and/or physical factors. In a preliminary study we showed that the phorbol ester 12-o-tetra-decanoyl-phorbol-13-acetate (TPA) induces metamorphosis in six Red Sea coral species. Phorbol esters are known to activate the enzyme protein kinase C (PKC) and therefore play an important role in studying the phosphatidylinositol signal (PI) transduction system. The potency of several phorbol esters to induce metamorphosis in planulae of the Red Sea soft coral species *Heteroxenia fuscescens* and *Xenia umbellata* was examined to find further indications for the involvement of PKC in anthozoan metamorphosis. All tested phorbol esters, 12-o-tetra-decanoyl-phorbol-13-acetate, phorbol-12,13-didecanoate, 12-retinoyl-phorbol-13-acetate and phorbol-12,13-dibutyrate triggered metamorphosis in planulae of both species in a concentration-dependent way. The maximum levels of metamorphosis achieved varied from 30% to 100%, depending on both the species tested and the phorbol ester applied, using concentrations ranging from 10^{-7} to 10^{-9} mol/l except for 12-retinoyl-phorbol-13-acetate. In addition, the response of planula fragments to artificial inducers was studied. Our results indicate that the different cnidarian taxa not only respond to a variety of metamorphic cues, but also possess divergent mechanisms for reception and transmission of metamorphic inducers. Phloretin, an inhibitor of mammalian PKC, completely blocked phorbol ester-induced metamorphosis in *Heteroxenia fuscescens* planulae. Data which indicate the presence of PKC-related enzymes as the biochemical base for metamorphosis are now documented for species of the cnidarian classes Hydrozoa, Scyphozoa and Anthozoa. Based on our results, and the work of other authors, we therefore suggest that the phosphatidylinositol-signaling pathway might represent the general mechanism which regulates metamorphosis in cnidarians.

Key words: Red Sea, Octocorallia, planulae, metamorphosis, signal transduction

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Introduction

Coral reefs represent one of the most complex and productive ecosystems, where the scleractinian corals are the main reef builders. Octocorals possess internal sclerites as their skeleton and therefore do not contribute to the expansion of reefs. Soft corals (Octocorallia: Alcyonacea) are regarded as the second most important benthic organisms on many Indo-Pacific reefs (Benayahu and Loya, 1981; Dai, 1990, 1993; Reinicke, 1995). During the life cycle of marine invertebrates including octocorals, embryogenesis leads to the development of free swimming larvae which have to find a suitable habitat for settlement and subsequent metamorphosis (Chia and Bickell, 1978; Pennington and Hadfield, 1989). Adult colonies of octocorals are able to reproduce asexually (Lasker, 1988), yet sexual reproduction is necessary for the development of new genotypes and dispersion.

Initial settlement and induction of metamorphosis of benthic marine invertebrate larvae are generally determined by interaction of external biochemical and/or physical factors (Pawlik, 1992). Studies have dealt with characterization of environmental signals leading to settlement and metamorphosis of the larvae. Natural inducers of metamorphosis can be associated with conspecific individuals, prey species or microbial films (review by Rodriguez et al., 1993). The use of artificial inducers is helpful in studying induction of settlement and associated signal transduction mechanisms involved in larval responses (Morse, 1985; Yool et al., 1986; Hofmann and Brand, 1987; Bonar et al., 1990).

Several studies have shown that certain chemicals such as various cations, peptides and neurotransmitters are able to act as artificial inducers. The cation Li^+ induces metamorphosis in *Hydractinia echinata* (Spindler and Müller, 1972), K^+ in *Crepidula fornicata* (Eyster and Pechenik, 1988; Pechenik and Gee, 1993), NH_4^+ in *Crassostrea gigas* (Coon et al., 1990b) and Cs^+ in *Mitrocomella polydiademata* (Freeman and Ridgway, 1990). The hexapeptide carbobenzoxy-(z)-GPGGPA induces settlement and metamorphosis in the scyphozoan *Cassiopea andromeda* (Hofmann and Brand, 1987). In addition, exogenous neurotransmitters such as DOPA (3,4-dihydroxyphenylalanine) and GABA (γ-aminobutyric acid) can elicit a metamorphic response in invertebrate larvae (Morse, 1985; Coon et al., 1985). Yet, none of the above-mentioned studies found a common artificial inducer, which is generally effective in all tested species.

Müller (1985) showed for the first time that a tumor-promoting phorbol ester can induce metamorphosis of the hydroid *Hydractinia echinata*. Further studies demonstrated that metamorphosis can be triggered artificially by different phorbol esters in various members of Cnidaria: Hydrozoa (Freeman and Ridgway, 1990), Scyphozoa (Bischoff et al., 1991) and Anthozoa (Henning et al., 1996). The phorbol ester TPA induces metamorphosis in all these taxa.

TPA and other tumor-promoting phorbol esters are important in studying the signal transduction system of the phosphatidylinositol (PI)-cycle (Castagna, 1987). After binding of external ligands to surface receptors and activation of phospholipase C by G-proteins, phosphatidylinositol-(4,5-)bisphosphate (PIP_2) is hydrolyzed to (1,4,5-)inositoltrisphosphate (IP_3) and diacylglycerol (DG). Activation of protein kinase C (PKC) by DG and Ca^{2+}, which is mobilized from intracellular stores by IP_3, leads to phosphorylation of proteins (Nishizuka, 1992; Divecha and Irvine, 1995). Tumor-promoting phorbol esters are able to activate PKCs, the key enzyme family of this signaling pathway, by binding to the DG binding site.

In recent years it has been found that regulation of metabolism, gene expression, cell growth and cell division are based on related signal transduction mechanisms first found in vertebrates, and later in invertebrates, bacteria and plants (Shibanaka et al., 1993; De Petrocellis et al.; 1993; Chasan, 1995; Johnson et al., 1996; Zhang, 1996).

The PI-cycle is involved in biological mechanisms leading to metamorphosis in planulae of *H. echinata* (Leitz and Müller, 1987; Leitz and Klingmann, 1990; Schneider and Leitz, 1994). In addition, the involvement of PKC in metamorphosis of the scyphozoan *Cassiopea* has been suggested by Fleck and Bischoff (1993). The PKC signal transduction system was found also to be involved in larval metamorphosis of the barnacle *Balanus amphitrite* (Yamamoto et al., 1995).

Information on settlement and metamorphic events in coral planulae has remained mainly descriptive (Fadlallah, 1983; Krupp, 1983; Benayahu and Loya, 1984), yet knowledge of reproduction in corals has increased in recent years (i.e., Harrison and Wallace, 1990; Benayahu et al., 1990; Benayahu, 1991; Tanner 1996). The only study on induction of settlement and metamorphosis among octocorals is of the temperate species *Alcyonium siderium* (Sebens, 1983). In the stony coral *Agaricia humilis*, Morse et al. (1988) demonstrated that a sulfated polysaccharide isolated from red crustose algae can cue metamorphosis.

In a previous study we have shown that TPA is capable to induce metamorphosis in planulae of five Red Sea soft coral species and in a stony coral (Henning et al., 1996). Using combined PKC activator-

inhibitor experiments, the current study examines whether, like in hydrozoans and scyphozoans, a PKC-related enzyme is also involved in the induction of metamorphosis of anthozoans. In addition, the response of planula fragments to artificial inducers was studied. The obtained results indicate that a PKC-related enzyme may also be present in the two common Red Sea soft coral species *Heteroxenia fuscescens* and *Xenia umbellata*.

Materials and Methods

Collection of colonies and planulae

Mature colonies of the brooding soft corals *H. fuscescens* and *X. umbellata* were collected by SCUBA diving at depths of 5–12 m from the reef in front of the Marine Biological Laboratory at Eilat (Red Sea, Israel) during 1992–1995. Planulae of these species were obtained following the method described by Henning et al. (1996). The obtained planulae were rinsed 3–4 times in natural Millipore-filtered seawater (0.2 μm pore size), and later transferred into filtered seawater to which 100 μg/ml of each of the following antibiotics were added: penicillin-G potassium salt, neomycin sulfate and streptomycin sulfate (ACS: antibiotic-containing seawater).

Bioassays

All solutions were freshly prepared prior to each experiment. A bioassay was conducted on three replicates per tested concentration and controls, each with 10 or 20 planulae (see Results). All experiments were carried out in sterile 2 ml 24-well cell culture plates and run at 25 °C for up to 7 days and monitored daily. Results were recorded after 4 days of incubation. Planulae were considered to be undergoing metamorphosis when they developed an attachment disk, an oral opening and tentacles. The mean percentage of metamorphosis for a given concentration was calculated based on two identical experiments for each treatment. The data were analyzed by one-way analysis of variance (ANOVA) followed by the DUNCAN test to determine significant differences between random samples ($\alpha = 5\%$).

Phorbol esters

TPA (12-o-tetra-decanoyl-phorbol-13-acetate), PDD (phorbol-12,13-didecanoate), PDBu (phorbol-12,13-dibutyrate) and RPA (12-o-retinoyl-phorbol-13-acetate) (Serva) were predissolved in methanol. The solutions were further diluted with ACS for final concentrations of 10^{-6} mol/l to 10^{-15} mol/l. Methanol effects were tested by dissolving it in amounts equivalent to its final concentrations in each of the phorbol ester experiments.

Sectioning of planulae

Planulae were cut transversely into two parts of equal length by using a tungsten needle to assay the capability of larval fragments to respond to the artificial inducers. Basal and apical fragments of *H. fuscescens* and *X. umbellata* were immediately incubated separately in 8.1×10^{-8} mol/l TPA or $7.4. \times 10^{-8}$ mol/l PDD. Basal and apical fragments as well as intact planulae incubated in ACS served as control, and intact planulae maintained in phorbol ester solutions served as additional controls.

Protein kinase inhibitors

The protein kinase inhibitors sphingosine, psychosine and phloretin (Sigma) were predissolved in methanol and further diluted with ACS for a final concentration of 10^{-3} mol/l to 10^{-3} mol/l. For these experiments, TPA at 8.1×10^{-8} mol/l was used to induce metamorphosis. For all bioassays, inhibitor and inducer solutions were applied simultaneously to the planulae. Control experiments were carried out using TPA, ACS, and inhibitors alone.

Results

Release, settlement and metamorphosis of planulae

Larvae of cnidarians and of other taxa of marine invertebrates that pass through a true pelagic phase in many cases exhibit a particular settling behavior prior to further metamorphic responses to environmental cues. Planulae of *H. fuscescens* do not belong to this type of larvae. They are extruded from the parental colony and passively translocated onto solid substrata, due to the lack of ciliation appropriate for active swimming. The normal sequence of metamorphic events includes: attachment of the larvae through glandular secretions, contraction, assuming a spherical or ellipsoidal shape, onset of polyp morphogenesis. There is no specific settlement behavior of the larvae which are unable to perform significant active locomotion. These observations have been confirmed recently by Ben-David Zaslow and Benayahu (1996). Planulae of *X. umbellata* are smaller than those of *H. fuscescens* and already carry zooxanthellae. Their

release, the passive translocation, and the general pattern of settlement and metamorphosis are the same as described above.

Induction of metamorphosis by phorbol esters

The control experiments indicated that ACS alone or methanol at concentrations ranging from 1% to 10^{-4}% did not affect the planulae, which did not metamorphose. All tested phorbol esters, TPA, PDD, RPA and PDBu induced metamorphosis in planulae of *H. fuscescens* and *X. umbellata* in a concentration dependent way (Fig. 1). The maximum levels of metamorphosis achieved varied from 30% to 100% after 4 days of incubation, depending on both the species tested and the phorbol ester applied at concentrations ranging from 10^{-7} to 10^{-9} mol/l, except for RPA. RPA was most effective at higher concentrations: maximum percentages of metamorphosis were achieved in both species at a concentration of 7.3×10^{-6} mol/l (Fig. 1c), and no larvae metamorphosed at concentrations $<7.3 \times 10^{-8}$ mol/l. Significantly different responses between the two species were found in all phorbol esters tested. TPA induced 100% metamorphosis in *H. fuscescens* planulae at 8.1×10^{-8} mol/l and 8.1×10^{-9} mol/l (Henning et al., 1996); and, therefore, it is a highly potent artificial trigger for this species but not for *X. umbellata* with a maximum of 30% metamorphosed planulae at 8.1×10^{-8} mol/l (Fig. 1a). For planulae of *X. umbellata*, PDD represents the most effective inducer, resulting in 73.3% metamorphosis (Fig. 1b).

In *X. umbellata*, metamorphosis was triggered by both PDBu and TPA over a wide range of the tested concentrations (Fig. 1a, d). The data were analyzed using ANOVA. Maximum percentages of metamorphosis were obtained at 8.1×10^{-8} mol/l TPA and at 9.9×10^{-7} mol/l PDBu. In *H. fuscescens*, PDBu-induced metamorphosis was restricted to concentrations ranging from 9.9×10^{-8} mol/l to 9.9×10^{-10} mol/l (Fig. 1d). All primary polyps produced after induction of metamorphosis looked normal (Fig. 2a, b), showed no deformations (Fig. 2c) and resembled the polyps metamorphosed on natural substratum (Benayahu et al., 1989).

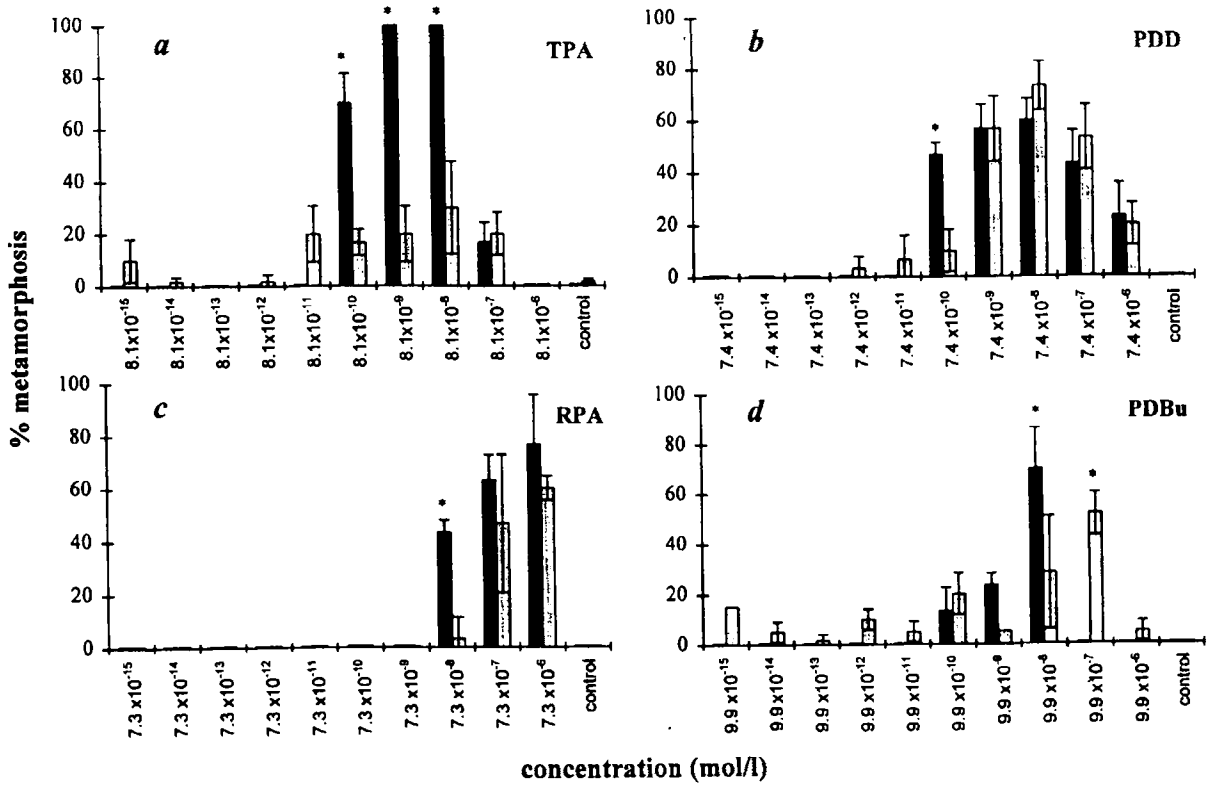

Fig. 1. Induction of metamorphosis in *Heteroxenia fuscescens* (■) and *Xenia umbellata* (□) by different phorbol esters. Means ±SD from two replicate experiments with 3×10 planulae (*H. fuscescens*) or 3×20 planulae (*X. umbellata*) each. Data in Fig. 1 compiled from Henning et al., 1996. Asterisk indicates significantly different responses between the two species.

Fig. 2. Metamorphosed polyps of the studied species. a: Primary polyps of *Heteroxenia fuscescens* induced by TPA. b:Primary polyps of *Xenia umbellata* induced by PDD. c: Primary polyps of *Heteroxenia fuscescens* developed on natural substratum. Arrows indicate tentacle pinnules. Scale bars: 1 mm.

Deleterious effects of phorbol esters on planulae

Effects caused by high concentrations of all tested phorbol esters on planulae of the two studied species are summarized in Tables 1 and 2. More toxic effects of phorbol esters occurred in *H. fuscescens* than in *X. umbellata.*

High RPA concentrations did not cause any visible toxic effects. High concentrations of PDD-solutions resulted in deformed planulae or primary polyps of *H. fuscescens* but not of *X. umbellata.* Concentrations of 9.9×10^{-6} and 10^{-7} mol/l PDBu were 100% lethal for *H. fuscescens*, but not for *X. umbellata.* TPA caused visible toxic effects in both species; all planulae died in 8.1×10^{-6} mol/l TPA; 8.1×10^{-7} mol/l was still toxic for the majority of them.

Responses of dissected planulae to phorbol esters

TPA induced 100% metamorphosis of intact planulae in *H. fuscescens* whereas PDD caused 73.3% metamorphosis of intact planulae in *X. umbellata.* Intact planulae of neither species underwent metamorphosis when kept in ACS Transversely dissected planulae of *H. fuscescens* and *X. umbellata* incubated in ACS developed into larvae of reduced size within 6 days of treatment, but never metamorphosed nor showed partial polyp morphogenesis. Both basal and apical parts of planulae of *X. umbellata* metamorphosed in response to the inducer PDD, similarly to the intact control planulae (Table 3). In *H. fuscescens*, however, basal fragments incubated in TPA developed into larvae of reduced size without undergoing metamorphosis, whereas apical parts of dissected planulae metamorphosed into small primary polyps (Table 4).

Inhibition of phorbol ester induced metamorphosis

The controls for each experiment showed that planulae kept in ACS did not metamorphose, whereas 8.1 × 10-8 mol/l TPA induced metamorphosis in all planulae. Experiments with sphingosine alone caused 100% lysis of planulae at a concentration of 1.7×10^{-3} mol/l. No visible toxic effects occurred at lower

Table 1. Effects of different phorbol esters on planulae of *Heteroxenia fuscescens*

Phorbolester	Concentration (mol/l)	Dead planulae (%)	Planulae (%)	Metamorphosis (%)
PDBu	9.9×10^{-6}	100	0	0
	9.9×10^{-7}	100	0	0
TPA[a]	8.1×10^{-6}	100	0	0
	8.1×10^{-7}	63.3	20	16.7
PDD	7.4×10^{-6}	0	76.7[b]	23.3[b]
	7.4×10^{-7}	0	56.7[b]	43.3[b]
RPA	7.3×10^{-6}	0	23.3	76.7
	7.3×10^{-7}	0	36.7	63.3

[a]Data of TPA treatment compiled from Henning et al., 1996.
[b]Indicates deformed planulae or primary polyps. n=2; 3 × 10 planulae for each concentration.

Table 2. Effects of different phorbol esters on planulae of *Xenia umbellata*.

Phorbolester	Concentration (mol/l)	Dead planulae (%)	Planulae (%)	Metamorphosis (%)
TPA[a]	8.1×10^{-6}	100	0	0
	8.1×10^{-7}	80	0	20
PDBu	9.9×10^{-6}	0	95	5
	9.9×10^{-7}	0	48.3	51.7
PDD	7.4×10^{-6}	0	80	20
	7.4×10^{-7}	0	46.7	53.3[b]
RPA	7.3×10^{-6}	0	40	60
	7.3×10^{-7}	0	53.3	46.7

[a]Data of TPA treatment compiled from Henning et al., 1996.
n=2; 3 × 10 planulae for each concentration.

concentrations. Incubation of *H. fuscescens* planulae with the inducer, 8.1×10^{-8} mol/l TPA, and sphingosine at different concentrations, resulted in concentration-dependent inhibition of the phorbol ester-induced metamorphosis (Fig. 3). Sphingosine at a concentration of 1.7×10^{-4} mol/l inhibited metamorphosis in 65% of the planulae ($p < 0.05$). Psychosine at concentrations of 10^{-4} mol/l and 10^{-5} mol/l caused 100% lysis of planulae in experiments without TPA. Toxic effects were observed at a concentration of 10^{-6} mol/l. Metamorphosis in *H. fuscescens* could be inhibited partly by psychosine at concentrations of 10^{-7} mol/l and 10^{-8} mol/l. (Fig. 3). In experiments with phloretin alone, 70–80% mortality of planulae was observed at a concentration of 9.1×10^{-4} mol/l, and no visible toxic effects occurred at lower concentrations. Metamorphosis of *H. fuscescens* planulae was prevented completely when the PKC inhibitor phloretin at a concentration of 9.1×10^{-5} mol/l was applied together with TPA (Fig. 3).

Discussion

The course of larval metamorphosis of a marine invertebrate can be documented by exposing the larvae to a suitable substratum which provides natural metamorphic cues (Pechenik and Gee, 1993). As long as natural inducers of metamorphosis are not identified, external artificial inducers which can be readily obtained and whose concentration can be precisely controlled are helpful in studying induction of settlement and metamorphosis (Morse, 1985; Yool et al., 1986; Hofmann and Brand, 1987; Eyster and Pechenik, 1988; Pennington and Hadfield, 1989; Coon, 1990). In addition, experiments with artificial inducers known to interfere with known intracellular pathways may reveal signal transduction mechanisms involved in larval responses (Rodriguez et al., 1993; Leitz, 1993; Schneider and Leitz, 1994).

Induction of metamorphosis by phorbol esters

The results of this study show that all tested phorbol esters induce metamorphosis in both *H. fuscescens* and *X. umbellata* planulae, with maximum levels of metamorphosis achieved varying from 30% to 100%, depending on both the tested species and the phorbol ester applied after 4 days of incubation at concentrations of 10^{-6}–10^{-9} mol/l. The most effective inducers

Table 3. The fate of isolated basal and apical parts of planulae of *Xenia umbellata* induced by 7.4×10^{-8} mol/l PDD

	Basal parts			Apical parts			Intact planulae			Dissected planulae in ACS		
	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6
Incomplete parts, %	100	20 ± 8.2	0	50 ± 8.2	0	0	—	—	—	70 ± 10	16.7 ± 15.3	0
Regenerated, size-reduced planulae, %	0	40 ± 8.2	26.7 ± 9.4	36.7 ± 4.7	40 ± 8.2	0	—	—	—	30 ± 10	83.3 ± 15.3	100
Metamorphosed planulae, %	0	40 ± 8.2	73.3 ± 9.4	13.3 ± 9.4	60 ± 8.2	100	50 ± 8.2	100	100	0	0	0

Means ± SD; $n = 3$, 10 planulae in each set.

Table 4. The fate of isolated basal and apical parts of planulae of *Heteroxenia fuscescens* induced by 8.1×10^{-8} mol/l TPA

	Basal parts			Apical parts			Intact planulae			Dissected planulae in ACS		
	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6
Incomplete parts, %	100	20 ± 8.2	0	6.7 ± 4.7	0	0	—	—	—	83.3 ± 5.8	10 ± 10	0
Regenerated, size-reduced planulae, %	0	40 ± 8.2	26.7 ± 9.4	80 ± 8.2	26.7 ± 9.4	26.7 ± 9.4	—	—	—	16.7 ± 5.8	90 ± 10	100
Metamorphosed planulae, %	0	40 ± 8.2	73.3 ± 9.4	13.3 ± 9.4	73.3 ± 9.4	73.3 ± 9.4	20 ± 8.2	60 ± 9.4	73.3 ± 9.4	0	0	0

Means ± SD; $n = 3$, 10 planulae in each set.

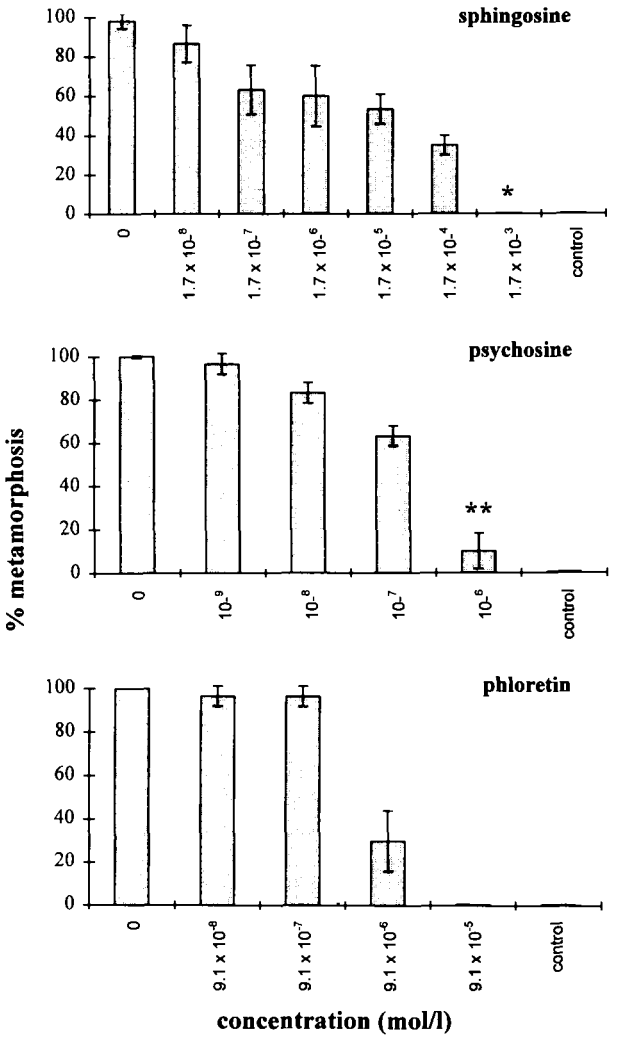

Fig. 3. Inhibition of TPA-induced metamorphosis of *Heteroxenia fuscescens* by PKC inhibitors (means ±SD, n=3, 10 planulae in each treatment). Asterisk indicates complete lysis of planulae and a double asterisk deformed polyps.

for *H. fuscescens* are TPA yielding 100% metamorphosis and PDD for *X. umbellata* inducing 73% metamorphosis. Consequently, phorbol esters can be used as artificial inducers of metamorphosis in these soft corals; it would be of interest to find additional artificial inducers with the capacity to engage with alternative sections of the signaling pathway.

The observation that two species react differently to the same phorbol ester could indicate the presence of several PKC isoforms. At least 11 such isoforms have been identified so far, and its has been shown that this enzyme family includes PKCs resistant to PKC activators, such as TPA (Hug and Sarre, 1993; Simpson et al., 1996). Different phorbol esters caused a variety of responses in a given species. Freeman and Ridgway (1990) and Fleck and Bischoff (1993) also described differences in the response of planulae to phorbol esters with maximum percentages of metamorphosis ranging from a few percentages to 100%. In our study, the course of metamorphosis of both species was identical after natural and phorbol ester-induced metamorphosis (Fig. 2), yet it is interesting to note that in some cases, settlement was dismissed, although the primary polyps developed attachment disks. Similar observations have been made by Hofmann and Brand (1987): buds of the scyphozoan *Cassiopea andromeda* were able to metamorphose without prior attachment to a substratum hanging upside down from the surface pellicle. Yamamoto et al. (1995) noticed that larvae of the barnacle *Balanus amphitrite* metamorphosed to juvenile form without settling to substrata when exposed to phorbol esters at higher concentrations. However, anthozoan primary polyps not settling on suitable substratum in the field will con-tribute neither to reproduction nor to expansion of the population.

Toxic effects on planulae and primary polyps were observed when planulae were exposed to high concentrations of phorbol esters, as previously described by Müller (1985), Freeman and Ridgway (1990) and Fleck and Bischoff (1993).

Effects of phorbol esters on dissected planulae

Cnidarian planulae undergo metamorphosis only after receiving an external stimulus. Our dissection experiments, the first approach using microsurgery in these two species, were designed to test the capacity of autonomous development of anterior and posterior fragments of larvae and the ability of such fragments to respond to an artificial inducer. From the latter series of experiments we expected to obtain evidence as to which part of the larva bears cells recognizing the chemical cue. Our results show that in transversely dissected larvae apical as well as basal planula parts of *X. umbellata* are able to metamorphose in response to PDD and are therefore not irreversibly determined along their longitudinal axis. On the other hand, parts of *H. fuscescens* planulae did not possess equivalent developmental potencies. Only apical fragments metamorphosed into small primary polyps when exposed to the inducer TPA, whereas basal fragments developed into larvae of reduced size. Planula fragments of both species did not undergo complete or even partial polyp morphogenesis in the untreated controls. These findings differ from previous results obtained for *Cassiopea andromeda* (Neumann, 1977) and for *Hydractinia echinata* (Schwoerer-Böhning et al., 1990). In these species, only basal fragments were able

to metamorphose into primary polyps indicating that external stimuli, including artificial inducers, act on the basal part of the planula and are subsequently transmitted to the apical part. In that case, only part of the larval organism responds to the exogenous inducer.

Our observations give partial information on mechanisms of reception and transmission of inducing cues in these soft coral species. In *X. umbellata*, basal and apical planula portions metamorphosed into small primary polyps, indicating that the whole larval organism responded to the external signal. Apical fragments reacted faster to the stimulus than basal fragments, suggesting a slightly higher reception potency located in this part of the planula. Surprisingly, in *H. fuscescens*, only apical and never basal planula fragments metamorphosed into primary polyps when incubated in TPA. Although TPA represents an artificial inducer and analogous experiments with natural inducers of metamorphosis have not been conducted, we infer that the different cnidarian taxa not only respond to a variety of metamorphic cues, but also possess different mechanisms for reception and transmission of metamorphic inducers. To study the various modes, it should be worthwhile to record the development of isolated basal and apical parts of larvae in other species of marine Cnidaria.

Inhibition of phorbol ester induced metamorphosis

In recent years, evidence has been gathered that the PI-cycle represents the biochemical base for triggering metamorphosis in the hydroid *Hydractinia echinata* (Leitz and Müller, 1987; Leitz and Klingmann, 1990; Leitz, 1993) and in the scyphozoans *Cassiopea andromeda* and *C. xamachana* (Fleck and Bischoff,1993; Fleck, 1994). In hydrozoans as well as in scyphozoans, the first indication that PI-signaling might be involved in metamorphosis was derived from experiments with activators and inhibitors aiming at PKC (for review see Fleck, 1997). In *Cassiopea* spp., both phorbol ester-induced metamorphosis and peptide-induced metamorphosis by Z-GPGGPA could be completely blocked by the PKC inhibitor psychosine. In *Hydractinia echinata*, K252a inhibited Cs^+-induced metamorphosis as well as diacylglycerol-induced metamorphosis. However, none of these inhibitors is absolutely specific for PKC. The compounds used on our study were selected according to published work on larval metamorphosis in hydrozoans (Freeman and Ridgway, 1990; Leitz and Klingmann, 1990; Schneider and Leitz, 1994) and scyphozoans (Fleck and Bischoff 1993; Fleck 1994). All of them have been applied essentially in *in vivo* experiments.

In other inhibitor experiments performed in our laboratory on propagules of *Cassiopea* spp., chelerythrine proved to be highly toxic and H7 showed some inhibitory activity only at concentrations which had already deleterious effects on the pseudoplanules (Fleck, 1994). Therefore, these compounds were not chosen for our experiments on soft coral larvae. H7 is considered as highly nonspecific (Nixon, 1997). Even H9 may act on protein kinase A and protein kinase G in addition to PKC. There remains a general problem of the use of inhibitors that very specific agents, such as pseudosubstrates blocking the substrate binding site of PKC, are not amenable in our *in vivo* experiments because they are toxic or do not penetrate the cell membrane (unpublished observations on *Cassiopea* spp. in our laboratory). Direct evidence for kinase activity in cnidarians was found by Schneider and Leitz (1994) in *Hydractinia echinata*. They characterized the enzyme as being similar to mammalian PKC with a molecular weight of about 70 kD. Since tumor-promoting phorbol esters induce metamorphosis in *H. fuscescens* and *X. umbellata* planulae, a PKC-related enzyme might be present also in these species, leading to suggest that the PI-cycle may be involved in initiating the transformation of the planula into the benthic living coral polyp. Our results show that the PKC inhibitor phloretin completely inhibits phorbol ester-induced metamorphosis in *H. fuscescens*. Freeman and Ridgway (1990) reported similar results for the hydrozoan *Mitrocomella polydiademata*. Induction of metamorphosis by phorbol esters was blocked when phloretin was applied together with the inducer. In *H. fuscescens* sphingosine, another inhibitor of PKC, also had a potent inhibiting effect on the induction of metamorphosis by TPA. The results for psychosine, which has been described to be a very effective inhibitor of metamorphosis in *Cassiopea* spp. (Fleck and Bischoff, 1993), are difficult to interpret since toxic or inhibitory effects occurred simultaneously.

The current results suggest that among Anthozoa, the PI-signaling system is also involved in transduction of the external signal to undergo metamorphosis into an internal one which causes the morphogenetic response. Further studies should be carried out to confirm this hypothesis.

Marine bacteria are most probably natural inducers of metamorphosis in *H. fuscescens* and *X. umbellata* (Henning, 1996). Therefore, if the natural inducer indirectly activates a PKC-related enzyme by binding to membrane receptor molecules, a PKC inhibitor like

phloretin should also inhibit induction of natural metamorphosis in these soft corals. To characterize and isolate a natural inducing substance is also important since this substance may assist in locating membrane receptor molecules in the planulae.

Data which indicate the presence of PKC-related enzymes as the biochemical base for metamorphosis are now documented for species of three cnidarian classes (Leitz and Klingmann, 1990; Freeman and Ridgway, 1990; Fleck and Bischoff, 1993; this paper). Future research should be aimed at establishing the presence of enzymes of the PKC family in Scyphozoa and Anthozoa, to stand alongside the work on hydrozoans by Schneider and Leitz (1994), and to confirm our hypothesis that the PI-signaling pathway represents the general regulatory mechanism of metamorphosis among cnidarians.

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References

Benayahu, Y., Reproduction and developmental pathways of Red Sea Xeniidae (Octocorallia, Alcyonacea). Hydrobiologia, 216/217 (1991) 125–130.

Benayahu, Y. and Loya, Y., Competition for space among coral-reef sessile organisms at Eilat, Red Sea. Bull. Mar. Sci., 31 (1981) 514–522.

Benayahu, Y. and Loya, Y., Life history studies on the Red Sea soft coral *Xenia macrospiculata* Gohar, 1940. II. Planula shedding and post larval development. Biol. Bull. 166 (1984) 44–53.

Benayahu, Y., Achituv, Y. and Berner, T., Metamorphosis of an octocoral primary polyp and its infection by algal symbionts. Symbiosis, 7 (1989) 159–169.

Benayahu, Y., Weil, D. and Kleinman, M., Radiation of broadcasting and brooding patterns in coral reef alcyonaceans. In: Advances in Invertebrate Reproduction 5, Hoshi, M. and Yamashita, O. (eds.). Elsevier Science (Biomedical Division), Amsterdam, New York, 1990.

Ben-David Zaslow, R. and Benayahu, Y., Longevity, competence and energetic content in planulae of the soft coral *Heteroxenia fuscescens*. J. Exp. Mar. Biol. Ecol., 206 (1996) 55–68.

Bischoff, A., Fleck, J. and Hofmann, D.K., Phorbol esters induce metamorphosis in *Cassiopea andromeda* and *Cassiopea xamachana* (Cnidaria: Scyphozoa). Verh. Dtsch. Zool. Ges., 84 (1991) 484.

Bonar, D.B., Coon, S.L., Walch, M., Weiner, R.M. and Fitt., W.K., Control of oyster settlement and metamorphosis by endogenous and exogenous chemical cues. Bull. Mar. Sci., 46 (1990) 484–498.

Castagna, M., Phorbol esters as signal transducers and tumor promotors. Biol. Cell, 59 (1987) 3–14.

Chasan, R., Signaling a role for phospholipid-derived compounds in plants. Plant Cell, 7 (1995) 1971–1974.

Chia, F.S. and Bickell, L., Mechanisms of larval attachment and the induction of settlement and metamorphosis in coelenterates: a review. In: Settlement and Metamorphosis of Marine Invertebrate Larvae, Chia, F.S. and Rice, M.E. (eds.). Elsevier, New York, 1978, pp. 1–12.

Coon, S.L., Bonar, D.B. and Weiner, R.M., Induction of settlement and metamorphosis of the Pacific oyster, *Crassostrea gigas* (Thunberg) by L-DOPA and catecholamines. J. Exp. Mar. Biol. Ecol., 94 (1985) 211–221.

Coon, S.L., Walch, M., Fitt, W.K., Weiner, R.M. and Bonar, D.B., Ammonia induces settlement behavior in oyster larvae. Biol. Bull., 179 (1990b) 297–303.

Dai, C.-F., Interspecific competition in Taiwanese corals with special reference to interactions between alcyonaceans and scleractinians. Mar. Ecol. Prog. Ser., 60 (1990) 291–297.

Dai, C.-F., Patterns of coral distribution and benthic space partitioning on the fringing reefs of Southern Taiwan. Mar. Ecol., 14 (1993) 185–204.

De Petrocellis, L., Di Marzo, V. and Cimono, G., The possible involvement of protein kinase C and phospholipase A_2 in *Hydra* tentacle regeneration. Experientia, 49 (1993) 57–64.

Divecha, N. and Irvine, R.F., Phospholipid signaling. Cell, 80 (1995) 269–278.

Eyster, L.S. and Pechenik, J.A., Comparison of growth, respiration, and feeding of juvenile *Crepidula fornicata* (L.) following natural or KCl-triggered metamorphosis. J. Exp. Mar. Biol. Ecol., 118 (1988) 269–279.

Fadlallah, Y.H., Sexual reproduction, development and larval biology in scleractinian corals. Coral Reefs, 2 (1983) 129–150.

Fleck, J., Efficiency of modified inducing peptides, possible signal transduction mechanism and chemical fate of a biologically active peptide in *Cassiopea* spp. (Cnidaria: Scyphozoa). Ph.D. Thesis, Bochum, 1994 (in German, with English summary).

Fleck, J., Phosphatidylinositol (PI) signaling and subsequent events in metamorphosis induction of cnidarian larvae. Proc., 8th International Coral Reef Symposium, Panama, 2 (1997) 1225–1229.

Fleck, J. and Bischoff, A., Protein kinase C is possibly involved in chemical induction of metamorphosis in *Cassiopea* spp. (Cnidaria: Scyphozoa). Proc., 7th International Coral Reef Symposium, Guam, 1993, pp. 456–462.

Freeman, G. and Ridgway, E.B., Cellular and intracellular

pathways mediating the metamorphic stimulus in hydrozoan planulae. Roux's Arch. Dev. Biol., 199 (1990) 63–79.

Harrison, P.L. and Wallace, C.C., Reproduction, dispersal and recruitment of scleractinian corals. In: Ecosystems of the World 25: Coral Reefs, Dubinsky, Z. (ed.). Elsevier, New York, 1990, pp. 133–207.

Henning, G., Induction of larval metamorphosis in selected soft coral species (Cnidaria: Alcyonaria): Role of biochemicals and of marine bacteria as inductors of metamorphosis. Ph.D. Thesis, Bochum, 1996 (in German with English summary and English index of figures and tables).

Henning, G., Hofmann, D.K. and Benayahu, Y., The phorbol ester TPA induces metamorphosis in Red Sea coral planulae (Cnidaria: Anthozoa). Experientia, 52 (1996) 744–749.

Hofmann, D.K. and Brand, U., Induction of metamorphosis in the symbiotic scyphozoan *Cassiopea andromeda*: Role of marine bacteria and of biochemicals. Symbiosis, 4 (1987) 99–116.

Hug, H. and Sarre, T.F., Protein kinase C isoenzymes: divergence in signal transduction? Biochem. J., 291 (1993) 329–343.

Johnson, L.N., Noble, M.E.M. and Owen, D.J., Active and inactive protein kinases: structural basis for regulation. Cell, 85 (1996) 149–158.

Krupp, D.A., Sexual reproduction and early development of the solitary coral *Fungia scutaria* (Anthozoa: Scleractinia). Coral Reefs, 2 (1983) 159–164.

Lasker, H.R., The incidence and the rate of vegetative propagation among coral reef alcyonarians. Proc., 6th International Coral Reef Symposium, Townsville, 2 (1988) 763–768.

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

Leitz, T. and Klingmann, G., Metamorphosis in *Hydractinia*: studies with activators and inhibitors aiming at protein kinase C and potassium channels. Roux's Arch. Dev. Biol., 199 (1990) 107–113.

Leitz, T. and Müller, W.A., 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 (1987) 82–89.

Morse, D.E., Neurotransmitter-mimetic inducers of larval settlement and metamorphosis. Bull. Mar. Sci., 37 (1985) 697–706.

Morse, D.E., Hooker, N., Morse, A.N.C. and Jensen. R.A., Control of larval metamorphosis and recruitment in sympatric agiriciid corals. J. Exp. Mar. Biol. Ecol., 116 (1988) 193–217.

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

Neumann, R., Polyp morphogenesis in a scyphozoan: evidence for a head inhibitor from the presumptive foot end in vegetative buds of *Cassiopea andromeda*. Wilhelm Roux Arch. EntwMech. Org., 183 (1977) 79–83.

Nishizuka, Y., Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science, 258 (1992) 607–614.

Nixon, J.S., The biology of protein kinase C inhibitors. In: Protein Kinase C, Parker P.J. and Dekker, L.V. (eds.). R.G. Landes, 1997.

Pawlik, J.R., Chemical ecology of the settlement of benthic marine invertebrates. Oceanogr. Mar. Biol. Rev., 30 (1992) 273–335.

Pechenik, J.A. and Gee, C.C., Onset of metamorphic competence in larvae of the gastropod *Crepidula fornicata* (L.), judged by a natural and an artificial cue. J. Exp. Mar. Biol. Ecol., 167 (1993) 59–72.

Pennington, J.T. and Hadfield, M.G., Larvae of a nudibranch mollusc (*Phestilla sibogae*) metamorphose when exposed to common organic solvents. Biol. Bull., 177 (1989) 350–355.

Reinicke, G.B., Xeniidae des Roten Meeres (Octocorallia, Alcyonacea). Essener Ökologische Schriften, Vol. 6. Wolf Graf von Westarp, Germany, 1995.

Rodriguez, S.R., Ojeda F.P. and Inestrosa, N.C., Settlement of benthic marine invertebrates. Mar. Ecol. Prog. Ser., 97 (1993) 193–207.

Schneider, T. and Leitz, T., Protein kinase C in hydrozoans: involvement in metamorphosis of *Hydractinia* and in pattern formation of *Hydra*. Roux's Arch. Dev. Biol., 203 (1994) 422–429.

Schwoerer-Böhning, B., Kroiher, M. and Müller, W.A., Signal transmission and covert prepattern in the metamorphosis of *Hydractinia echinata* (Hydrozoa). Roux's Arch. Dev. Biol., 198 (1990) 245–251.

Sebens, K.P., Settlement and metamorphosis of a temperate soft-coral larva (*Alcyonium siderium* Verrill): induction by crustose algae. Biol. Bull., 165 (1983) 286–304.

Shibanaka, Y., Hayashi, H., Takai, M. and Fujita, N., Eclosion hormone activates phosphatidylinositol hydrolysis in silkworm abdominal ganglia during adult metamorphosis. Eur. J. Biochem., 211 (1993) 427–430.

Simpson, J., Johnson, M.S., Clegg, R.A. and Mitchell, R., Separation of H7-resistant protein kinase C isoforms from gonadotroph-derived αT3-1 cells. Biochem. Biophys. Res. Com., 218 (1996) 720–725.

Spindler, K.D. and Müller, W.A., Induction of metamorphosis by bacteria and by a lithium-pulse in the larvae of *Hydractinia echinata* (Hydrozoa). Roux's Arch. Dev. Biol., 169 (1972) 271–280.

Tanner, J.E., Seasonality and lunar periodicity in the reproduction of pocilloporid corals. Coral Reefs, 15 (1996) 59–66.

Yamamota, H., Tachibana, A., Matsumara, K. and Fusetani, N., Protein kinase C (PKC) signal transduction system involved in larval metamorphosis of the barnacle, *Balanus amphitrite*. Zool. Sci., 12 (1995) 391–396.

Yool, A.J., Grau, S.M., Hadfield, M.G., Jensen, R.A., Markell, D.A. and Morse, D.E. Excess potassium induces larval metamorphosis in four marine invertebrate species. Biol. Bull., 170 (1986) 255–266.

Zhang, C.-C., Bacterial signaling involving eukaryotic-type protein kinases. Mol. Microbiol., 20 (1996) 9–15.