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# Checkpoints in the life-cycle of *Cassiopea* spp.: control of metagenesis and metamorphosis in a tropical jellyfish#

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**ABSTRACT** **Experimental data reveal that most, if not all, major events in the metagenetic life-cycle of *Cassiopea* spp. at these checkpoints depend on the interaction with specific biotic and physical cues. For medusa formation within a permissive temperature range by monodisk strobilation of the polyp, the presence of endosymbiotic dinoflagellates is indispensable. The priming effect of the algal symbionts is not primarily coupled with photosynthetic activity, but was found to be enhanced in the light. Budding of larva-like propagules by the polyp, however, is independent from such zooxanthellae. On the other hand the budding rate is influenced by various rearing conditions. Exogenous chemical cues control settlement and metamorphosis into scyphopolyps of both sexually produced planula larvae and asexual propagules. In laboratory experiments two classes of metamorphosis inducing compounds have been detected: a family of oligopeptides, featuring a proline-residue next to the carboxyterminal amino acid, and several phorbol esters. Using the peptide $^{14}$C-DNS-GPGGPA, induction of metamorphosis has been shown to be receptor-mediated. Furthermore, activation of protein kinase C, a key enzyme within the inositolphospholipid-signalling pathway appears to be involved in initiating metamorphosis. In mangrove habitats of *Cassiopea* spp. planula larvae specifically settle and metamorphose on submerged, deteriorating mangrove leaves from which biologically active fractions have been isolated. The chemical characterisation and comparison of these compounds from the natural environment with the properties and mode of action of oligopeptide inducers is in progress.**

KEY WORDS: *Cnidaria, Cassiopea, strobilation, metamorphosis, signal-transduction*

## Introduction

Invertebrate species from marine benthic communities, such as coral reef environments, show in most cases life-histories with indirect development which include one or several larval stages. Recruitment of larvae in these species plays an essential role in maintaining populations, in repopulating of depleted or damaged habitats, and in dispersing of the species. The majority of species of hydrozoa, scyphozoa and cubozoa alternate between a sessile, asexually propagating polyp form and a typically pelagic, sexually reproducing medusa, rendering life-cycles of these species even more complex. Furthermore, a number of cases exist where the polyp exhibits several modes of asexual reproduction (e.g. *Aurelia aurita*, see Berrill, 1949, for a classical review) and/or in which medusae vegetatively produce medusae in addition to gamete formation and subsequent sexual reproduction (e.g. *Rathkea octopunctata*, Werner, 1958). Ultimately, larvae resulting from sexual reproduction and larva-like asexual propagules, such as frustules or swimming buds, terminate their motile pelagic or bentho-pelagic phase, settle and transform into the sessile polyp form. A large body of evidence from both field observations and laboratory work indicates that each transition between stages within an individual cnidarian life-history has to be considered as a *checkpoint* at which specific physical, chemical, or biological factors elicit (or inhibit) a particular pattern of development. Our paper aims at contributing findings related to control of development in species of the tropical scyphozoan genus *Cassiopea*. We demonstrate that most events in the metagenetic life-cycle, if not all, are not under autonomous control of the animal proper but at these checkpoints depend on the presence of and interaction with specific biotic and physical cues. We consider the indispensability of endosymbiotic dinoflagellates and the role of ambient temperature for initiation of medusa formation, and we report on cellular mechanisms and environmental conditions involved in budding of the polyp. Particular interest is attributed to induction of larval settlement and metamorphosis by synthetic peptides, phorbol esters and a putative natural inducer from the mangrove habitat. We summarize evidence for receptor-mediated chemical induction of metamorphosis and present results supporting the hypothesis that the activation of protein kinase C (PKC) is involved in transducing external signals into morphogenetic responses.

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#Dedicated to the memory of Prof. Menachem Rahat, The Hebrew University of Jerusalem, who inspiringly participated in this research for many years.

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## Life-history of *Cassiopea* species

The jellyfish *Cassiopea andromeda*, first described as *Medusa andromeda* by Forskal from Thor at the Red Sea in 1775 and later on transferred to the genus *Cassiopea* by Eschscholtz in 1829 (see Gohar and Eisawy, 1960a, for references) differs in its habits considerably from other genera of the typically holopelagic scyphomedusae. Living preferably in shallow water in tropical lagoons and mangrove areas, the medusae, densely packed with the symbiotic dinoflagellate *Symbiodinium microadriaticum*, dwells exumbrella down on sandy or muddy bottoms, thereby exposing the typical rhizostome oral appendages and the slowly contracting bell rim (Fig. 1). This appearance has led to the common name "upside-down jellyfish". Gohar and Eisawy (1960a,b) provided detailed accounts on morphology, taxonomy and development of *C. andromeda*. Apart from this species occurring in the Red Sea and the Indopacific, Bigelow (1898, 1900) described a species from Jamaica, *C. xamachana*, which is common in the Caribbean and occurs sometimes in enormous numbers in many areas of the Florida Keys. Morphologically indistinguishable from each other, also with regard to the cnidome (Hofmann and Jensch, unpublished observation), Gohar and Eisawy (1960a) suggested *C. xamachana* not to represent a separate species but only a geographic variation of *C. andromeda*. A third species, *C. frondosa*, also occurs in the Caribbean, but in most areas appears to be less common than *C. xamachana*.

The sex-ratio of *C. andromeda* in a small population near Eilat (Red Sea) was found to be 1:1 (Hofmann, Fitt, Rahat, unpublished observations). Gamete release and fertilization has not yet been observed under natural conditions, however female medusae are regularly found with masses of eggs or embryos enveloped in mucus and wrapped around the bases of sex specific vesicles in the center of the oral disk. This simple mode of brood protection lasts until ciliated planula larvae hatch from the egg envelopes. Larvae, still devoid of endosymbiotic dinoflagellates, show morphological and physiological polarity and swim with the blunt end ahead. The somewhat thigmotactic type of locomotion has sometimes been interpreted as "searching behavior". As already noted by Gohar and Eisawy (1960b) planulae may settle within hours or days on "suitable substrate", attach irreversibly at their blunt anterior end, and metamorphose into the benthic polyp stage. Only after formation of the oral opening the developing polyp can acquire the algal symbionts. The fully developed scyphopolyp (Fig. 2) may start forming a medusa by monodisc strobilation. Due to a progressive constriction of the calyx (Fig. 2) the upper, tentacle bearing portion becomes separated and is transformed into a single medusa-anlage, the ephyra ,within about one week. The remaining basal polyp regenerates the lost tentacular and hypostomal region, resumes feeding and may strobilate again at intervals ranging from three to several weeks (Ludwig, 1969; Hofmann *et al.*, 1978).

As an alternate mode of asexual reproduction polyps form spindle-shaped, larva-like, ciliated buds at one or several perradial sites at the lower part of the calyx (Bigelow, 1900; Gohar and Eisawy, 1960b; Ludwig, 1969; Curtis and Cowden, 1971; Hofmann *et al.*, 1978; van Lieshout and Martin, 1992) (Fig. 3).When fully differentiated (bud stage 5, Hofmann and Gottlieb, 1991, Fig. 1b) buds separate and swim off, the former distal end in front. Like the planula larvae buds may settle on "suitable substratum", irreversibly attach with the anterior pole and then metamorphose into scyphopolyps (Fig. 4) This type of larva-like swimming bud is exclusively found in species of the order rhizostomea. As Bigelow (1900) already pointed out it provides an important mode of asexual propagation of the polyp generation and concomitantly yields an increase of the potential to strobilate sexually reproducing medusae. These observations on development apply to both *C. andromeda* and *C. xamachana*.

Polyps of *Cassiopea* species can easily be cultured in the laboratory on a simple brine shrimp diet, starting either from planula larvae or from buds which can be chemically induced to metamorphose (see below). Symbiotic polyps may strobilate medusae which can be grown to larger size by skilled hands. However, sexual reproduction of medusae reared in the aquarium has been achieved only by Zahn (1985) in the Aquazoo, Düsseldorf (FRG). The results reviewed in the following sections have been obtained almost exclusively in the laboratory, based on cultured animals, or on larvae sampled in the field from brooding medusae. Recruitment of larvae and development of polyps in the natural mangrove habitat (Fig. 5) will be briefly considered.

## Control of strobilation

Though the polyp generation can be propagated asexually by budding, polyp formation from sexually produced larvae is the dominant pathway. Thus control of formation of the gamete producing medusae is a crucial checkpoint. Ludwig (1969) found that those *C. andromeda* polyps bearing symbiotic dinoflagellates could undergo medusa formation whereas aposymbiotic individuals were never observed to strobilate. Hofmann and Kremer (1981) confirmed and extended these findings, and Fitt (1984) arrived at the same conclusion working with *C. xamachana*. Rahat and Adar (1981), however, did not obtain the same results in *C. andromeda*.

Searching for the physiological role of the algal symbionts, which were found to be indispensable for strobilation, photosynthetic $^{14}C$-carbon fixation has been studied extensively by Hofmann and Kremer (1981), as well as $^{14}C$-incorporation in the dark and in DCMU-treated polyps. A total of 5-10% of net algal photosynthates appears to be released *in vivo* to the host, which is presumably much less than required for nutrition of the host animal. Since polyps perform strobilation even if photosynthesis and concomitantly photosynthate supply are completely quenched by the inhibitor DCMU, it does not seem justified to assume that some single major photosynthate (e.g. glucose or glycerol) simply acts as an inducer of strobilation. The specific contributions by the symbionts to the host must hence be traced among those products which are formed also *without* the primary participation of photosynthesis. It is intriguing, however, that the rate of strobilation is considerably enhanced in polyps with the normal complement of symbionts and exposed to light. Thus strobilation is not definitively triggered but significantly supported by algal photosynthesis, indicating that induction of strobilation must be based on a more complex system of regulation which cannot be specified at present.

Temperature has been shown in laboratory experiments to be an environmental factor involved in strobilation control in *C.*

*andromeda* by Hofmann *et al.*, (1978) and Rahat and Adar (1981). Whereas symbiotic polyps maintained at a constant temperature of 20°C did not strobilate, raising of the ambient temperature up to 25° or 30°C led to medusa formation after a lag phase ranging from one to several weeks. After regenerating the basal polyp, they could strobilate again. A laboratory strain of *C. xamachana* currently produces ephyrae at variable intervals at a constant temperature of 23±1°C (Hofmann *et al.*, unpublished observations).

## Control of bud formation

Strobilation and budding of polyps are generally alternative modes of asexual reproduction, however polyps may exceptionally start strobilating while a bud has not yet been completed; or a bud may emerge before the ephyra has been released, and before the basal polyp has completed regeneration. Contrary to buds of most other cnidarians, the spindle-shaped buds in *Cassiopea* spp. under normal conditions do not reach the juvenile polyp stage while still attached to the parent (Fig. 3). Buds are released as larva-like, motile stages which remain in morphogenetic stasis unless induced to metamorphose (Bigelow, 1898, 1900; Curtis and Cowden, 1971; Hofmann *et al.*, 1978). Bigelow (1900) noted continuity of bud and parent tissue and suggested that every part of the young is formed by the corresponding part of the polyp. More recent observations supported this view. Light- and electronmicroscopic investigations (Hofmann and Honegger, 1990) showed that bud formation in *C. andromeda* involves no significant changes in the composition of the epithelia. The ectoderm consists of three cell types, the endoderm of two. Nerve elements, septal muscles, amoebocytes, and symbionts are apparently derived from the parent during budding. Two types of nematocytes were detected in the ectoderm and one in the endoderm, in both the buds and the polyp (Hofmann and Jensch, unpublished observations). Using intracellular vital staining, Hofmann and Gottlieb (1991) investigated epithelial recruitment and dynamics during bud formation in the ectoderm. They found the region of cell recruitment to encircle the budding site asymmetrically with the aboral side contributing much less than the oral and lateral sides. Furthermore, epithelial cell flow observed in bud formation was recognized as part of a permanent apicobasal displacement of ectodermal cells along the scyphistoma.

During tissue dislocation directed to the bud, cells leave the parent polyp and hence must be replaced. As expected, BrdU/antiBrdU immunofluorescence labeling and the $^{3}$H-thymidine incorporation method revealed high labelling indices in the polyps calyx and a conspicuous pattern of labelled cells in the budding area, indicating high proliferative activity. On the other hand, continuous incubation of polyps for 10 days with the mitosis inhibitor colchicine at concentrations from $2.5 \times 10^{-4}$ M to $1.25 \times 10^{-3}$ M specifically influenced budding. In polyps treated when exhibiting visible sign of budding (see Hofmann and Gottlieb, 1991, Fig. 1b), buds completely developed and separated from the parents, sometimes with minor delay. However only 34 to 40% of those polyps releasing a bud, began thereafter to form a second bud. Such buds were defective and either did not separate, or did not reach the full size, or underwent regression at an early stage respectively. If non-budding individuals were submitted to colchicine treatment, only 2-10% of the polyps produced and released one bud (Hofmann and Reckenfelderbäumer, unpubl. observ.)


**Fig. 1. *Cassiopea xamachana*: brooding female, diameter 15 cm, in it its typical upside-down position in the habitat.** *Mangrove area, Grassy Key (Florida, USA).*

A number of rearing conditions were found to influence the budding rate, e.g. the frequency of feeding, the number of individuals kept per culture dish, the size and age of the polyps (Hofmann *et al.*, 1978). Furthermore, the size of the buds was found to be positively correlated with the size of the parent polyps (Hofmann and Gottlieb, 1991). At temperatures significantly above 20°C, bud formation was 'competing' with strobilation in symbiotic polyps: periods of strobilation were observed to be followed by phases of budding (Hofmann *et al.*, 1978; Rahat and Adar, 1981). With regard to the endosymbiotic dinoflagellates, Ludwig (1969) and all subsequent investigators agreed that bud formation, in contrast to strobilation, is *not* dependent on the presence of endosymbiotic algae in the polyp.

## Chemical control of metamorphosis induction in buds and planula larvae

When Curtis and Cowden (1971) took a first look on metamorphosis under laboratory conditions they observed that buds of *C. xamachana* settled on small pieces of algae and occasionally on brine shrimp eggs and developed into polyps. However there was no settlement of buds and planulae on clean surfaces,

in seawater containing antibiotics (ABS) and under strictly axenic conditions (Curtis and Cowden, 1971; Hofmann *et al.,* 1978; Fitt *et al.,* 1987; Hofmann and Henning, 1991). These findings suggested that marine bacteria might be involved in the production of compounds that induce metamorphosis of *Cassiopea* spp. Several authors isolated biologically active fractions from the supernatant of suspension culture medium of growing marine bacteria belonging to the genus *Vibrio* . The inducing factors had apparent molecular weights ranging from 1000-10000 Da, they were relatively heat-stable and lost their activity after acid hydrolysis (Hofmann *et al.,* 1978; Neumann, 1979; Neumann *et al.,* 1980). Hofmann and Brand (1987) showed that *Vibrio alginolyticus* bacteria produced peptides by digestion of type I bovine collagen which induced metamorphosis in buds of *C. andromeda.* Enzymatic cleavage of the milk protein casein also yielded biologically active fractions (Hofmann *et al.,* 1984; Fitt and Hofmann, 1985).

Complete proteolysis of the helical domain of native collagen is known to yield peptides consisting of the amino acid sequence GXX (X= A, K, P, Hyp). Several synthetic oligopeptides which had a primary structure similar to that of collagen cleavage products were found to induce metamorphosis in buds and planula larvae of *Cassiopea* spp. Furthermore some β-casomorphins representing peptides derived from β-casein proved to be biologically active (Fitt and Hofmann, 1985; Hofmann and Brand, 1987; Fleck and Hofmann, 1990; Fleck and Bischoff, 1993; Fleck, 1994; Fleck, unpublished observations).

All metamorphosis-inducing oligopeptides share one common feature: they are characterized by a preterminal proline at the carboxyl end (Table 1). However since several peptides(e.g. YPF, Hofmann and Brand, 1987), were found to be biologically inactive the position of proline next to the carboxyterminal amino acid is an essential but not a sufficient prerequisite of an active compound. It is also of special interest that the tripeptide GHypA containing hydroxyproline, which possesses only one hydroxyl group more than proline, was found to be ineffective in triggering metamorphosis, whereas GPA was effective (Fleck, 1994). Biological activity is also dependent on the amino acid composition and on the length of the peptide. In structurally closely related oligopeptides the efficiency increases with the extension of the amino acid chain. The carboxyterminal sequence GPA ,which is a typical product of total proteolysis of collagen, was found to be among the most effective peptides. Compared to buds planula larvae usually required lower concentrations of the inducers to undergo metamorphosis (Table 1).

Studies focusing on the chemical modification of metamorphosis-triggering peptides showed that coupling of ligands with increasing hydrophobic character to the amino-terminus enhanced the biological activity (Fleck and Hofmann, 1990; Fleck and Bischoff, 1993; Fleck, 1994). Carbobenzoxy (Z)-peptides initiated 100 % metamorphosis within 24 h at lower concentrations than the corresponding unsubstituted oligopeptides (Table 1). Binding of the more hydrophobic dansyl (DNS) group led to a further increase of the activity. Peptides coupled to dabsylchloride (DABS) representing the most hydrophobic substitutive proved to be the most efficient synthetic inducers of metamorphosis in *Cassiopea.*

Hofmann and Brand (1987) reported that β-casomorphin 1-5 lost its biological activity when blocked by a carboxyterminal $NH_2$-group. Experiments conducted by Fleck (1994) with the tetrapeptide DNS-GGPA-$NH_2$ confirmed that substitution of the carboxyl terminus results in deactivation. Although applied 50 times higher than the minimal concentration required to induce 100 % metamorphosis within 24 h with DNS-GGPA, the corresponding amide derivative did not trigger settlement in buds of *C. andromeda.*

Since minor modifications of proline as the preterminal amino acid at the carboxyl end and blocking of the free carboxyl terminus resulted in the loss of the biological activity, Fleck (1994) assumed that proline and the free carboxyl end of the terminal amino acid are the site of the oligopeptides that possibly interact with specific receptors in the cell membrane of buds and planulae.

For a long period of time peptides represented the only known triggers of settlement in *Cassiopea.* None of the inducers of planulae metamorphosis in the well explored hydrozoan *Hydractinia echinata* (for review see Berking, 1991) or known to act on other invertebrate larvae were effective (Fitt *et al.,* 1987; Hofmann and Brand, 1987). Ammonia induced only partial metamorphosis in buds of *C. andromeda* (Berking and Schüle, 1987).

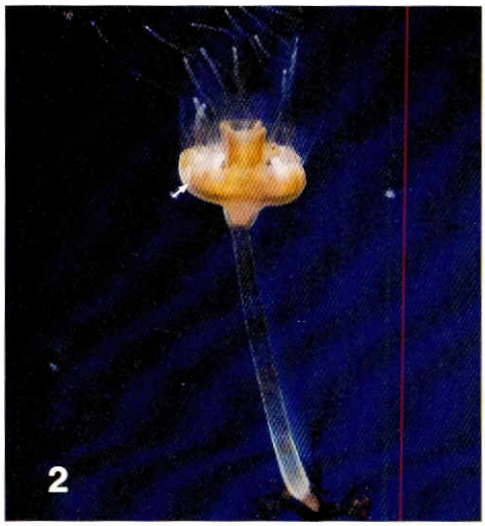
Tumor-promoting phorbol esters represent a new class of biologically active compounds which include effective inducers of metamorphosis in *Cassiopea* (Bischoff *et al.,* 1991; Fleck and Bischoff, 1993). 12-tetra-decanoyl-phorbol-13-acetate (TPA), 12-retinoyl-phorbol-13-acetate (RPA), phorbol-12, 13-didecanoate (PDD) and phorbol-12, 13-dibutyrate (PDBu) effected settlement in buds and planula larvae of *Cassiopea* spp. (Table 2).

Phorbol esters are the first group of inducers which are not only effective in triggering metamorphosis in *Cassiopea* but are also known to induce polyp formation in planula larvae of the hydroid species *Hydractinia echinata* (Müller, 1985) and *Mitrocomella polydiademata* (Freeman and Ridgway, 1990), as well as in the soft coral *Heteroxenia fuscescens* (Henning *et al.,* 1991).

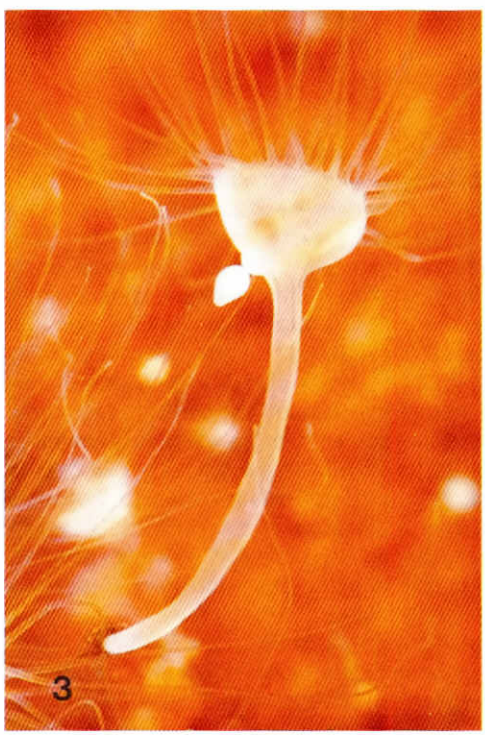
Larvae of a number of marine invertebrate species may acquire competence to respond to metamorphic cues only upon a period of life in the water column (see Pawlik, 1992, for a recent review). In *Cassiopea* spp. however planula larvae are already competent when hatching from or being squeezed off the egg envelopes. Buds are competent before release from the parent, but only after a constriction is seen between the polyp and the bud (Fitt *et al.,* unpublished observations; Hofmann and Fitt, unpublished observations).

## Induction of metamorphosis by oligopeptides is receptor-mediated

To test the hypothesis of receptor-mediated induction of metamorphosis Fleck (1994) conducted an *in vivo* binding assay with a radioactively labeled inducing peptide according to the method established by Trapido-Rosenthal and Morse (1986) for the mollusc *Haliotis rufescens.* Fleck (1994) demonstrated specific and saturable binding of the hexapeptide $^{14}$C-DNS-GPGGPA in buds of *C. andromeda.* Analysis of the specific binding data by employing a Scatchard plot revealed a dissociation constant $K_D$ in the order of 7 μM. The total number of receptors was calcu-







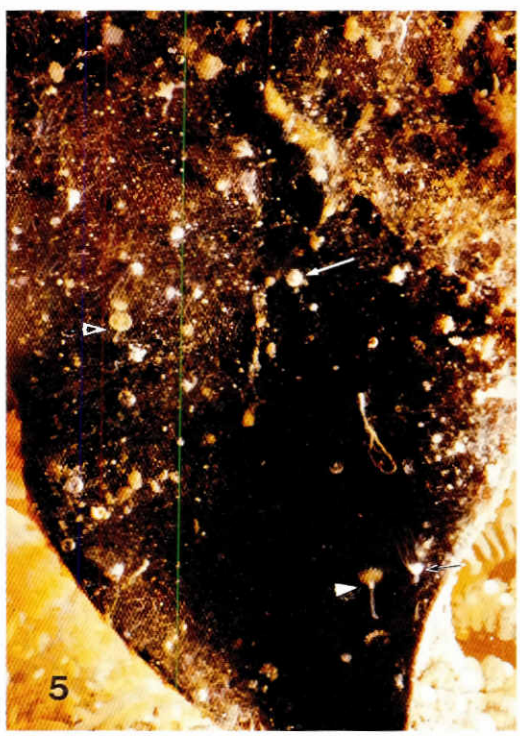




**Fig. 2. *C. andromeda*:** *full-grown polyp at an early stage of strobilation. Note the constriction of the calyx, regression of tentacles and formation of rhopalial and interrhopalial lappets (arrowhead). Upper diameter about 2.5 mm.*

**Fig. 3. *C. andromeda*:** *full-grown polyp with bud emerging from the lower part of the calyx. The bud is fully differentiated (stage 5) and about to be released. Bud length about 500 µm.*

**Fig. 4. *C. andromeda*:** *two buds attached to brine shrimp cysts at different stages of metamorphosis into polyps. Note the segregation into a foot, stalk and calyx portion. Size about 500-700 µm.*

**Fig. 5. *C. xamachana*:** *submerged, degrading leaf of the red mangrove, Rhizophora mangle, underside with recently settled planula larvae which metamorphosed into polyps (arrows) and strobilating scyphistomae (arrowheads). Mangrove area, Grassy Key (Florida, USA).*

lated to be approximately $1x10^{10}$ per bud under saturation conditions.

Several findings indicate that receptors for biologically active oligopeptides might be located on the basal pole of the buds which represents the prospective pedal disk of the polyp. When young buds of *Cassiopea* were cut into two halves only the basal part underwent settlement and metamorphosis into a small polyp in the presence of an inducer. The oral half always formed a polyp head when exposed to a biologically active peptide or kept in ABS (Neumann, 1980; Fleck, unpubl. observ.). Furthermore the terminal knob-like structure at the basal pole differs morphologically from the remaining parts of the bud (Hofmann and Honegger, 1990; van Lieshout and Martin, 1992) suggesting a particular role related to interaction with the metamorphic cues.

## Possible control of morphogenetic responses to metamorphic inducers by protein kinase C

One popular strategy for the transmission of signals from external ligands into the cell is the activation of protein kinase A by the second messenger cAMP. When Wolk *et al.* (1985) found that cholera toxin and thyrotropine effected settlement of buds and planulae of *C. andromeda* it was supposed that cAMP might be involved in the process of signal transduction underlying induction of metamorphosis. However Fitt *et al.* (1987) demonstrated that changes of cAMP during metamorphosis induced by pancreatic casein hydrolysate or thyrotropine were associated with the metamorphic development but not obviously with the triggering mechanism.

Another well-known pathway for the transduction of signals consists in the activation of protein kinase C (PKC). This enzyme takes part in the regulation of cell growth and differentiation by phosphorylating several important substrates (for a recent review see Hug and Sarre, 1993). PKC is activated by increased amounts of diacylglycerol in membranes. Diacylglycerol is formed by hydrolysis of (inositol) phospholipids which is induced by coupling of a ligand to a receptor in the cell membrane. In mammalian cells phorbol esters can replace diacylglycerol by activating PKC directly (Castagna *et al.*, 1982).

Since phorbol esters initiate metamorphosis in buds and planulae of *Cassiopea* spp. (Bischoff *et al.*, 1991; Fleck and Bischoff, 1993), it is thought that PKC is involved in the signal transduction process leading to settlement and polyp differentiation. Experiments conducted with inhibitors of PKC provided further support for this hypothesis. Several PKC blockers inhibited metamorphosis in buds of *Cassiopea* spp. induced by TPA and PDD (Bischoff *et al.*, 1991; Fleck and Bischoff, 1993). Psychosine (Hannun and Bell, 1987) proved to be the most effective inhibitor. The findings that psychosine also completely prevented settlement of buds and planula larvae by biologically active oligopeptides was of immense importance for the development of the hypothesis of the cellular and biochemical mechanism of metamorphosis induction in *Cassiopea* (Bischoff *et al.*, 1991; Fleck and Bischoff, 1993; Fleck, 1994).

TABLE 1

**DATA BASE OF SYNTHETIC BIOLOGICALLY ACTIVE OLIGOPEPTIDES IN *CASSIOPEA* SPP.**

| | *Cassiopea andromeda* buds | *Cassiopea xamachana* buds | *Cassiopea xamachana* planula larvae |
|---|---|---|---|
| GPGGPA | $1.1x10^{-4}$ | $6.6x10^{-5}$ | $4.4x10^{-5}$ |
| GGPA | $8.3x10^{-4}$ | $8.3x10^{-4}$ | $3.3x10^{-4}$ |
| GPA | $8.2x10^{-3}$ | $8.2x10^{-3}$ | $8.2x10^{-3}$ |
| APA | $2.0x10^{-2}$ | n.d. | n.d. |
| APG | $4.1x10^{-2}$ | $2.1x10^{-2}$ | $> 1.6x10^{-2}$ |
| PA | $> 5.4 \times 10^{-2}$ | $5.4x10^{-2}$ | $> 5.4x10^{-2}$ |
| YPFPGPI (ß-casomorphin 1-7) | $1.9x10^{-4}$ | $6.3x10^{-5}$ | $3.8x10^{-5}$ |
| YPFPG (ß-casomorphin 1-5) | $5.7x10^{-4}$ | $4.3x10^{-4}$ | $1.4x10^{-4}$ |
| Z-GPGGPA | $1.3x10^{-5}$ | $1.3x10^{-5}$ | $3.4x10^{-6}$ |
| Z-GPA | $2.1x10^{-4}$ | $2.1x10^{-4}$ | $8.0x10^{-5}$ |
| Z-GPFPL | $3.8x10^{-4}$ | $3.8x10^{-4}$ | $3.8x10^{-4}$ |
| Z-APG | $1.3x10^{-3}$ | $6.6x10^{-4}$ | $6.6x10^{-4}$ |
| Z-PA | $1.6x10^{-3}$ | n.d. | n.d. |
| DNS-GPGGPA | $7.3x10^{-6}$ | $7.3x10^{-6}$ | $2.9x10^{-6}$ |
| DNS-GPA | $1.0x10^{-4}$ | $1.0x10^{-4}$ | $2.1x10^{-5}$ |
| DNS-GGPA | $1.5x10^{-4}$ | $1.5x10^{-4}$ | $1.5x10^{-4}$ |
| DNS-APG | $1.0x10^{-3}$ | $1.0x10^{-3}$ | $5.2x10^{-4}$ |
| DNS-PA | $1.2x10^{-3}$ | $6.0x10^{-4}$ | $> 2.4x10^{-3}$ |
| DABS-GPGGPA | $3.4x10^{-6}$ | $2.7x10^{-6}$ | $2.0x10^{-6}$ |
| DABS-GPA* | $9.4x10^{-5}$ | $3.8x10^{-5}$ | $3.8x10^{-5}$ |
| DABS-GGPA* | $1.4x10^{-4}$ | $8.5x10^{-5}$ | $1.4x10^{-4}$ |

Numbers indicate minimal concentrations of the peptides required to induce 100 % metamorphosis within 24 h in buds and planula larvae. Concentrations are given in mol/l. n.d., no data. *, only partly soluble in ABS.

TABLE 2

**INDUCTION OF METAMORPHOSIS IN BUDS AND PLANULA LARVAE OF *CASSIOPEA* SPP. BY TUMOR-PROMOTING PHORBOL ESTERS**

| | phorbol ester | highest percentage of metamorphosis | phorbol ester concentration [mol/l] |
|---|---|---|---|
| *Cassiopea andromeda*, buds | TPA | 98 | $5.0x10^{-6}$ |
| | RPA | 90 | $2.5x10^{-5}$ |
| | PDD | 85 | $2.5x10^{-5}$ |
| | PDBu | 100 | $1.0x10^{-4}$ |
| *Cassiopea xamachana*, buds | TPA | 96 | $5.0x10^{-5}$ |
| | RPA | 96 | $2.5x10^{-5}$ |
| | PDD | 94 | $1.0x10^{-5}$ |
| | PDBu | 58 | $1.0x10^{-4}$ |
| *Cassiopea xamachana*, planulae | TPA | 80 | $1.0x10^{-5}$ |

Values refer to an incubation time of 72 h

In mammalian cells many peptides are known to initiate hydrolysis of inositol phospholipids by binding to specific receptors in the cell membrane (for review see Castagna, 1987). Since metamorphosis in *Cassiopea* induced by oligopeptides was demonstrated to be receptor-mediated (Fleck, 1994) these compounds are thought to play a role corresponding to that reported for biologically active peptides in mammalian cells (Fleck and Bischoff, 1993; Fleck, 1994). According to this hypothesis metamorphosis-triggering oligopeptides in *Cassiopea* couple to specific receptors in the membranes of buds and planulae and thereby induce phospholipid breakdown. Diacylglycerol is formed and activates PKC which in turn regulates the processes leading to irreversible attachment, growth and differentiation to the polyp stage. However external application of diacylglycerol(s) which could replace phorbol esters in activating PKC were not effective in inducing metamorphosis in buds and planulae of *Cassiopea* (Fleck, unpublished observations).

Our hypothesis of involvement of PKC in initiation of metamorphosis in the scyphozoan *Cassiopea* fits well with the idea of several authors who previously discussed the role of PKC in signal transduction resulting in settlement and metamorphosis of larvae of hydrozoans (Freeman and Ridgway, 1990; Leitz, 1993, which also gives a summary of all previous findings in *Hydractinia*), anthozoans (Henning *et al.*, 1991), and molluscans (Baxter and Morse, 1992, also for a summary of previous data in *Haliotis*).

## Control of metamorphosis induction by compounds of the natural environment

All findings presented so far are based on experiments carried out under laboratory conditions and mostly on laboratory cultured animals. The polyp stage of *C. andromeda* have not been found in its habitat, the Red Sea, yet. However adult polyps of *C. xamachana* were discovered on submerged dark, degrading leaves of the red mangrove *(Rhizophora mangle)* (Fig. 5) in

shallow lagoons in the Florida Keys (USA) (Fitt, 1991; Fitt and Hofmann, unpublished observations). In the laboratory the leaves were settled by planulae derived from brooding female medusae of *C. xamachana* living in these mangrove areas (Fitt, 1991; Fitt and Fleck, unpublished observations). Larvae metamorphosed only rarely on recently fallen leaves (Fitt and Fleck, unpublished observations). The results of the biological tests indicated that degrading leaves from *Rhizophora mangle* possess a metamorphosis-inducing potency. In order to isolate biologically active factors crude homogenates were prepared from these leaves, the supernatant of the homogenate was further analyzed. After ultrafiltration and gel filtration a pool of metamorphosis-inducing compounds with a molecular weight ranging from 5000- 10000 Da was obtained (Fitt, 1991; Fitt and Fleck, unpublished observations). Bioassays conducted after HPLC of this pool with planula larvae of *C. xamachana* indicated that one single subfraction contained most of the biological activity (Fitt and Fleck, unpublished observations).

Since antibiotic treatment of degrading mangrove leaves resulted in a decrease of the rate of metamorphosed planulae it is likely that marine bacteria are involved in the release of the naturally occurring inducers (Fitt, 1991). Fungi might also take part in this process. The observations made in the natural environment of *Cassiopea* support the hypothesis stated by Hofmann and Brand (1987). These authors postulated that marine microorganisms release biologically active compounds by digesting substrates in the sea which can serve as metamorphic inducers for buds and planula larvae of *Cassiopea* spp.

## Perspectives

Future research on the complex topic of metamorphosis in *Cassiopea* will concentrate on the signal transduction mechanism and on natural inducers. *In vitro* proof of the existence of specific receptors for biologically active peptides as well as subsequent isolation and characterization of the type of receptor will give us valuable information about the involvement of PKC in the signal transmission process. We also continue looking for potent activators and inhibitors of PKC to get further support for our hypothesis of the biochemical and cytological pathway in metamorphosis induction.

Little is known about the exact chemical structure of natural triggers of metamorphosis in marine invertebrate larvae. Inducers of only four species have been characterized yet (for review see Pawlik, 1992). Purification and characterization of the biologically active compounds derived from degrading mangrove leaves in the habitat of *C. xamachana* will not only contribute to the poorly explored field of naturally occurring metamorphic inducers but will give us the chance to show that our hypotheses based on intensive laboratory research really do apply to processes proceeding in nature.

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