Flypapers for Coral and Other Planktonic Larvae

New materials incorporate morphogens for applications in research, restoration, aquaculture, and medicine

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Many animals in the sea reproduce to yield vast numbers of minute, weakly swimming larvae that remain arrested in development while they are dispersed in the plankton. In many species, the larvae settle randomly and then continue to develop, but in other species the larvae emerge from developmental arrest only after settling in a particular microhabitat that is suitable for their postmetamorphic growth and survival to reproduction. What cues might indicate to such larvae that they have reached the appropriate environment? We and other investigators have found that these larvae recognize specific chemical signals from the environment that induce settlement from the plankton and metamorphosis to the adult form. This requirement helps ensure that the larvae settle and metamorphose selectively in the most suitable microhabitats.

Larval recognition of the required morphogens (signal molecules inducing metamorphosis), in the species we have investigated, is mediated by highly specific chemosensory receptors that control downstream signal transduction pathways closely related to those responsible for information processing in human brain and other cells specialized for signal recognition. We have developed an artificial substrate containing the purified, immobilized morphogenic cue recognized by certain coral larvae. This morphogen-containing artificial surface acts like a larval "flypaper" because it induces substratum-specific settlement and metamorphosis of the coral larvae in the laboratory and in the natural reef environment. This material is proving useful for the experimental resolution of factors controlling larval settlement, metamorphosis, and recruitment in the ocean.

Experiments now in progress are aimed at extending this technology to other species and to the use of larval flypapers as tools for coral reef monitoring, management, and reseeding. Substrata containing morphogenic signal molecules immobilized on their surfaces may serve similar purposes for other ecologically and commercially valuable species, and they may offer potential applications for aquaculture and human medicine as well.

Larval settlement and metamorphosis in some marine invertebrates are controlled by sensory recognition of exogenous chemical signals

Signal recognition controls recruitment

Laboratory studies have shown settlement and metamorphosis in a variety of marine invertebrates to be controlled by larval sensory recognition of, and responsiveness to, exogenous chemical signals and other environmental stimuli (reviewed in Bonar et al. 1990, Fitt et al. 1987, Hadfield 1978, 1986, Hadfield and Pennington 1990, Morse 1985, 1990, 1992, Pawlik 1992, Rittschof 1993). In some cases this sensory recognition is required to activate the genetically programmed sequence of behavioral and developmental processes that had been arrested in the dispersive larval stage (Degnan and Morse 1993, 1995, Morse 1990, 1992, Morse et al. 1979, 1980a), whereas in others, negative cues deter settlement and metamorphosis on inappropriate substrata (Holmstrom et al. 1992, Johnson and Strathmann 1989, Woodin 1991). Although for some species larval settlement from the plankton, attachment to a surface, and the induction of new gene expression leading to cellular differentiation and metamorphosis clearly are induced by the same cue molecule, it is not clear whether a single induction event is sufficient to induce this entire sequence or whether multiple parallel inductions are involved at different levels (e.g., be-

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havioral and developmental; A. Morse 1994, Morse 1992). A number of researchers are now beginning to learn how larval recognition of these chemical cues interacts with other factors, such as the hydrodynamic flow field and larval delivery, to control settlement from the plankton, metamorphosis, and recruitment (e.g., Butman and Grassle 1992, Grassle and Butman 1992, Pawlik and Butman 1993, Turner et al. 1994, Wethey 1986, Zimmer-Faust and Tamburri 1994).

Although the small larvae of most benthic marine invertebrates are essentially passive with respect to the large-scale advective processes that deliver larvae to potential settlement sites (Sammarco and Heron 1994), in some species the irreversible commitment to attachment and metamorphosis is tightly controlled by sensory information processed by the larvae and not solely by the hydrodynamics of larval delivery and retention (compare Jensen and Morse 1990, Mullineaux and Butman 1991, Pawlik and Butman 1993, Pawlik et al. 1991, Turner et al. 1994). Using artificial substrata with and without a sticky coating, Walters (1992), for example, showed that barnacle and bryozoan larvae actively select microhabitats for metamorphosis that are different from the initial sites of hydrodynamic delivery. Pawlik et al. (1991) and Mullineaux and Butman (1991), in experiments analyzing the behavior of larvae in hydrodynamic flumes, came to similar conclusions for other species. Zimmer-Faust and his colleagues have used innovative tracking experiments to show that oyster larvae can follow plumes of soluble peptides to settlement sites near conspecifics (Turner et al. 1994, Zimmer-Faust and Tamburri 1994). For some species, and under certain conditions, field work has demonstrated that the substratum specificity of larval settlement and metamorphosis is an important determinant of the distribution of recruits (i.e., survivors of various postmetamorphic size and age classes) in the natural environment (Connell 1985, Crisp 1974, Hadfield 1978, 1986, Highsmith 1982, Jensen and Morse 1984, 1990, Keough and Downes 1982, A. Morse 1992, 1994, A. Morse and Morse 1984, Morse et al. 1980b, 1988, Raimondi 1988, 1990, 1991, Sebens 1983).

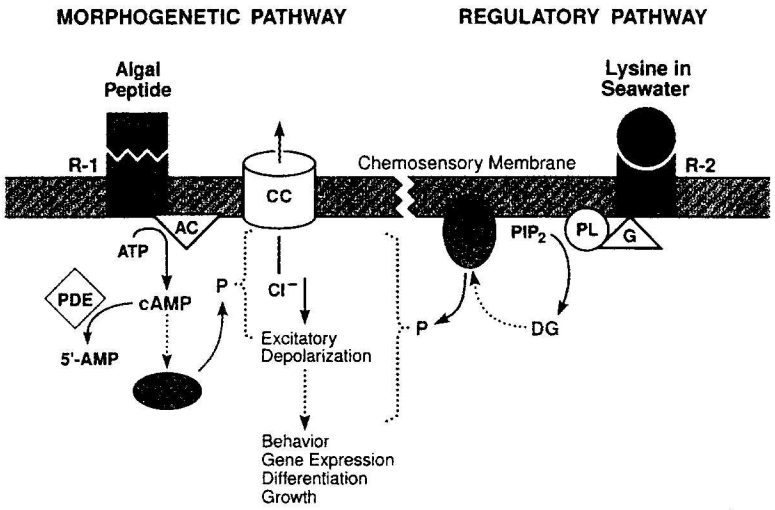

Figure 1. Two convergent chemosensory signal transduction pathways proposed to control substratum-specific settlement and metamorphosis of *Haliotis rufescens* (red abalone) larvae in response to two different kinds of chemical signals from the environment—one found on the surfaces of certain algae, the other dissolved in seawater. The morphogenetic inducer recognized by these larvae is a GABA-mimetic oligopeptide (a small peptide whose structure and function resemble those of the neurotransmitter γ-aminobutyric acid) produced by the algae on which the larvae are induced to settle. Binding of this signal molecule to a specific chemosensory receptor (R-1) activates a membrane-associated adenylate cyclase (AC) that catalyzes the production of the intracellular second messenger cyclic AMP (cAMP). cAMP and calcium ions then activate the protein kinase A (PKA)-catalyzed phosphorylation of a protein that opens a chloride channel (CC) in the chemosensory membrane, leading to the efflux of chloride ions. The result of this controlled efflux is an excitatory depolarization of the sensory neuron, causing the cell to fire (i.e., to generate an action potential). In this way, the chemical signal from the environment is transduced to an electrochemical signal that can be propagated by the larval nervous system, leading to the behavioral changes, gene activation, cellular differentiation, and proliferation that result in metamorphosis. (PDE = cAMP phosphodiesterase, an enzyme that degrades cAMP and limits the persistence and strength of the morphogenetic signal.) In response to threshold concentrations of lysine in seawater (which may serve as an indicator of nutrient-rich areas), the sensitivity of the larvae to low concentrations of the required algal morphogen is increased as much as 100-fold. This amplification, by a dissolved amino acid, of larval responsiveness to the algal cue is mediated by a separate signal transduction pathway controlled by a chemosensory receptor specific for lysine (R-2); binding of lysine activates a receptor-associated signal transducing G protein (G) that in turn activates a phospholipase (PL), causing it to produce the second messenger diacylglycerol (DG). This messenger in turn activates a diacylglycerol- and calcium-dependent protein kinase (PKC) that phosphorylates a protein required for amplification. The interaction between these two chemosensory signal transduction pathways, which allow the larvae to read two different chemical features of the environment, may provide them with a capacity for fine tuning the selection of microhabitats that are favorable for metamorphosis and subsequent growth (Baxter and Morse 1987, 1990, Morse 1990, 1992, 1993, Trapido-Rosenthal and Morse 1986a, 1986b, Wodicka and Morse 1990.)

We have studied in detail four species (from three different phyla) in which the stringency (degree of dependence) and specificity of the larval requirement for an exogenous inducer of settlement and metamorphosis are high (Morse 1990). In these species—the gastropod mollusc *Haliotis rufescens* (red abalone), the gregarious polychaete *Phragmatopoma californica*, and the scleractinian (stony) corals *Agaricia humilis* and *Agaricia tenuifolia*—stringency and specificity do not deteriorate with age in the laboratory, in contrast to the behavior exhibited by the less stringent larvae of many species, for example barnacles (Jensen and Morse 1984,

Morse and Morse 1991, Morse et al. 1979, 1980a, 1988). This capacity to delay metamorphosis in the absence of a required inducer may enhance both dispersal and substratum specificity of the final distribution of recruits. Indeed, the distributions of recruits of these species in the natural environment reflect closely the requirements for settlement and metamorphosis identified in the laboratory (Jensen and Morse 1984, 1990, A. Morse 1992, 1994, Morse 1990, 1992, A. Morse and Morse 1984, Morse et al. 1980b, 1988).

As illustrated in Figure 1, the chemosensory signal recognition and processing mechanisms controlling metamorphosis of abalone larvae in response to both algal substratum-associated and waterborne chemical cues are complex (Baxter and Morse 1987, 1992, Morse 1992, 1993, Trapido-Rosenthal and Morse 1986a, b, Wodicka and Morse 1991). The complexity of these mechanisms, and their sensitivity to multiple levels of regulation, may confer on the larvae of some species a capacity for fine scale discrimination of (and regulated responsiveness to) chemical features of the environment that had not been anticipated just a few years ago (Morse 1993).

Although the larvae of many temperate species of several phyla have now been shown to exhibit cue-dependent, substratum-specific settlement and metamorphosis, some scientists believed that these species might represent exceptions to a more general rule and that in marine ecosystems with the greatest species diversity, such as tropical coral reefs, recruitment might instead be a lottery-like process in which larval settlement is random and chaotic, as it may be for some reef-associated fish (Sale 1978). In these ecosystems, it was thought, settlement might depend simply on the unpredictable appearance of available space and on the delivery of larvae of one species or another in the plankton, with little if any control by chemosensory recognition of substratum-specific, morphogenic signal molecules. But it became evident that among coral species, differences in larval behavior could significantly affect the distributions of adults (Bak and Engel 1979, Carlon and Olson 1993, Gay and Andrews 1994, Morse and Morse 1991, Morse et al. 1988, 1994, Sammarco 1994). The distances that larvae travel, and the depths they select, proved to be important determinants of the species-specific patterns of dispersal and recruitment (Carlon and Olson 1993, Gay and Andrews 1994, Sammarco 1994). Detection of chemical cues controlling the selection of settlement sites also plays a role in determining these patterns for coral.

A morphogenic signal molecule for coral larvae

In the group of Caribbean corals that we have studied, larval settlement, metamorphosis, and recruitment are determined strictly by contact-dependent chemosensory recognition of specific signal molecules uniquely available on the surfaces of specific encrusting red algae. Larvae of the common shallow-water corals *A. humilis* and *A. tenuifolia* (members of the group commonly known as lettuce and ribbon corals) settle and metamorphose specifically in response to a nondiffusible morphogenic cue on the surfaces of *Hydrolithon boergesenii* and certain other crustose coralline red algae that grow as thin crusts on rocks and dead corals (Morse and Morse 1991, Morse et al. 1988, 1994). This process is ecologically significant because recruitment of these corals and their closely related congenerics dominates patterns of scleractinian recruitment throughout much of the Caribbean (Bak and Engel 1979, Dustan 1977, Hughes 1985, Hughes and Jackson 1985, Rogers et al. 1984, Rylaarsdam 1983, Smith 1992, van Moorsel 1989).

The requirement of the *Agaricia* larvae for this cue is stringent; in the absence of this signal, the larvae fail to metamorphose and eventually die (Morse and Morse 1991, Morse et al. 1988, 1994). Moreover, this signal appears to be specific to a small number of algae; the many other encrusting red algae that look similar to the naked eye, and account for the greatest coverage of surface area in the reef environment, lack the inductive molecule and do not induce the larvae to settle or metamorphose. These observations, together with our finding that in the field, young *A. humilis* recruits are found almost exclusively on inductive species of crustose coralline red algae, suggest that the larval requirement for this inducer may contribute, in part, to the spatial pattern of recruitment of this coral in the natural environment. Because these coral larvae retain both the stringency and the specificity of their requirement for more than 30 days, this requirement of the larvae is likely to contribute both to the dispersion of the species (e.g., from island to island) and to the maintenance of substratum specificity of recruitment.

The settlement-inducing molecule that the coral larvae recognize is associated with the insoluble calcified cell walls of the recruiting algae or their microbial associates (Morse et al. 1988). This insolubility posed an obstacle to purification of the molecule. To overcome this barrier, we partially hydrolyzed the inductive cell walls with purified enzymes that cut polysaccharides (the principal polymers of the cell walls) at specific chemical bonds. This treatment solubilized a fragment of the inducer while at the same time revealing part of its structure. By using enzymes to map the essential structural features of the inductive molecule in much the same way that specific restriction enzymes now are used to map the DNA molecules of the genes, we learned that the inductive portion of the cell wall polymer is a complex sulfated oligosaccharide (Morse and Morse 1991).

Although selective enzymatic hydrolysis of the inductive cell wall polymer liberates a small, soluble fragment of the molecule that is recognized by the chemosensory receptors of the coral larva and induces metamorphosis, this fragment proved too unstable to permit further purification. We resolved this problem by gently dissolving the calcified limestonelike matrix of the algal cell walls to release the inductive polymer in a soluble form that is larger and more stable than the fragment released by hydrolysis

(Morse et al. 1994). The procedure solubilized the large polymeric morphogen in high yield and in a form that can then be further purified by chromatography. (These results also showed that the morphogen is a component of the calcified walls of the recruiting alga itself, rather than a product of any contaminating microorganisms that are uncalcified.) When added in solution, the purified signal molecule rapidly induces the swimming larvae to attach to virtually any available surface (including clean glass or plastic) and quickly metamorphose (Figure 2). Therefore, it is an exogenous molecular signal, rather than some textural or other feature of the settlement surface, that is the principal requirement for induction of metamorphosis of these larvae.

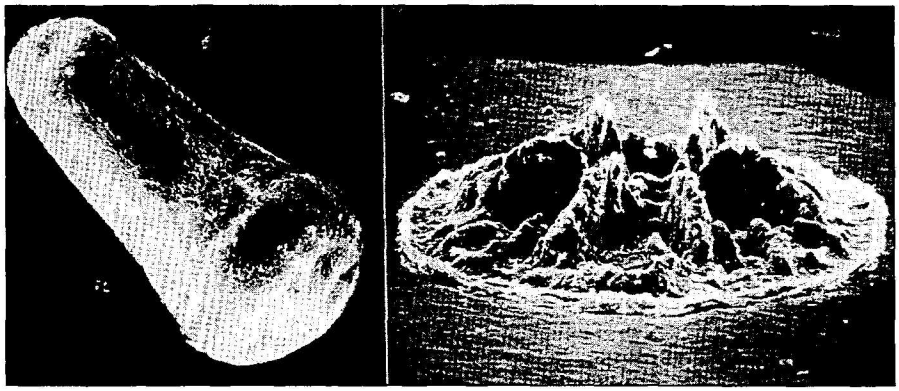

Figure 2. (Left) Swimming planktonic larva of the Caribbean coral, *Agaricia humilis*; note thousands of small cilia, responsible for propulsion, covering the larval surface. In the absence of added morphogen or alga, there is no settlement or metamorphosis. (Right) Attachment and metamorphosis of the larva induced by the soluble morphogen (signal molecule) purified from the recruiting alga; endoskeleton of postmetamorphic, differentiated juvenile shown attached to polystyrene two days after induction by the purified signal molecule. Scanning electron micrographic images are not to scale; larva is 0.7 mm and postmetamorphic juvenile is 1.5 mm. (From Morse and Morse 1991.)

The solubilized polymeric cue possesses both a negatively charged, hydrophilic tail (containing the negatively charged sulfate groups) and a hydrophobic head group; it is thus described as amphipathic. We have exploited this useful property to concentrate the morphogen by adsorption of the hydrophobic head groups to a hydrophobic chromatography resin and then to partially purify the inductive molecule by hydrophobic-interaction chromatography (Morse et al. 1994). Because of its negatively charged sulfate groups (functionally, the other end of the molecule from the hydrophobic head), the morphogen can then be further purified by ion-exchange chromatography. Final purification is achieved by gel filtration chromatography, which resolves molecules on the basis of their apparent molecular size. The purified cue molecule is extremely potent and specific in its action; larvae of coral species that are not induced to settle on the specific crustose red alga from which the molecule was purified are not induced to settle or metamorphose by the purified molecule. This finding shows that induction is mediated by specific receptor recognition rather than by direct activation of a more ubiquitous component of a downstream signal transduction pathway (e.g., a protein kinase). Structural analyses by stereospecific enzymatic dissections, biochemical analyses, and proton nuclear magnetic resonance have confirmed and extended our earlier results, indicating that the purified inducer molecule is a highly sulfated lipoglycosaminoglycan (a kind of complex polysaccharide) containing repeating β(1,4)–linked N-acetyl-lactosamine sulfate units and a lipidlike aliphatic side chain (Morse and Morse 1991, Morse et al. 1994). This structure explains the amphipathic properties of the molecule; the many sulfated sugars provide the negatively charged hydrophilic tail, whereas the lipid is the strongly hydrophobic head.

Morphogen-based flypaper for Caribbean corals

We have further exploited the strong hydrophobic property of the purified signal molecule to couple it to small (50-micron) inert acrylic beads of a hydrophobic interaction chromatography resin that we then cemented to plastic surfaces. The result is a prototype morphogen-based chemical flypaper for *A. humilis* larvae (Figure 3) that is proving useful for recruitment studies in the field.

The purified and immobilized morphogenic cue retains its full activity and remains tightly bound to the inert acrylic beads in seawater (Morse et al. 1994). Activity of this purified and immobilized cue proved to be identical in the ocean and the laboratory (when tested with *A. humilis* larvae produced in the laboratory). The metamorphosis-inducing activity does not leach off into the seawater because the high salinity keeps the amphipathic signal molecule tightly bound to the hydrophobic surface.

When tested with larvae in Nylon mesh microcosm containers in the field, the response of the larvae to the purified inducer on these artificial surfaces was identical to their response to the natural inductive alga: all of the larvae settled and metamorphosed. By contrast, in matched controls in the absence of the inducer or the inductive alga, settlement was nil (Morse et al. 1994). To our knowledge, this purified morphogen is the first highly purified natural inducer of larval settlement and metamorphosis to be incorporated into an artificial substratum suitable for testing factors that control recruitment in the ocean. (Although artificial substrata were used for studies of settlement and recruitment of the polychaete, *P. californica* [Jensen and Morse 1984, 1990, Pawlik 1986, 1988], the cues employed in those earlier studies did not satisfy these criteria [Jensen et al. 1990].) This uniform, nonsticky morphogen-based recruiting surface has the great advantage of eliminating the complexities and variability of the natural algal substrata that usually confound the ef-

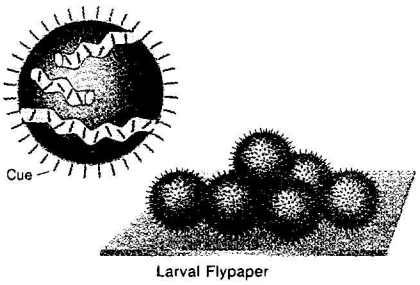

Figure 3. Prototype larval flypaper, illustrated schematically. The purified signal molecule (cue) is shown attached to spherical beads of hydrophobic chromatography resin, which is then cemented to a plastic surface. This material induces substratum-specific settlement and metamorphosis of *Agaricia humilis* coral larvae in the ocean and in the laboratory (Morse et al. 1994). Binding of inducer to narrow channels inside the beads, and inefficient contribution by the spherical beads to surface area coverage of the plastic sheet, provided targets for improvement, as shown in Figure 4.

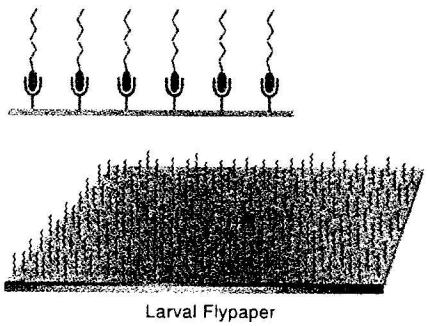

Figure 4. Improved larval flypaper. The purified signal molecule is shown attached to adaptor molecules that are coupled to an inert, nonporous surface. The hydrophobic head groups of the signal molecule are bound by hydrophobic pockets on the adaptors. The result is a uniform deployment of the inducer of larval settlement over the surface. Activity is shown in Figure 5.

fects of chemical morphogen with those of heterogeneity of texture and shape, presence or absence of cryptic animal predators or allelopathic competitors, and other chemical and biological interactions. Moreover, it allows researchers to experimentally control substratum location and orientation.

We believe that these and similar cue-containing surfaces are likely to be especially useful for experiments designed to identify the physical and biological factors that control the settlement and metamorphosis of larvae and the recruitment of corals and other valuable resource species in the ocean. The results of our experiments with *A. humilis* larvae in microcosms demonstrate that there is no difference in the response of competent larvae to inductive surfaces over a range of depths from 3 m to 30 m; nearly all settle when they contact the inductive surface, and they do so only on inducer-containing surfaces.[1] Hence, settlement on the morphogen-containing surfaces in the open reef environment should provide accurate estimates of the abundance and delivery of cue-responsive larvae and thus help explain the depth distributions of the Agariciid corals. Studies such as these should allow researchers to use larval flypaper-type surfaces as tools to resolve the contributions of the abundances, delivery, and behavior of larvae in the plankton, larval recognition of surface-associated signal molecules, and of other features of the environment, to the control of recruitment of many species in the natural environment.

Improvements in larval flypaper technology

To develop an experimental tool that can be placed in the open to analyze the availability and behavior of larvae in the plankton, it is necessary to increase the scale of production significantly. Large surface areas of inductive substrate are likely to be needed if the material is to be useful for the induction of settlement and metamorphosis of so-called wild larvae out of the plankton. We estimate that the efficiency and scale of production must be increased by an order of magnitude. Because all attempts thus far to substitute a small molecule for the complex natural cue for *Agaricia* have been unsuccessful, this increased scale of production must depend on improvements in surface deployment of the purified natural cue. Recent experiments suggest that this goal

[1]P. Raimondi and A. Morse, 1996, manuscript in preparation. Marine Science Institute, University of California, Santa Barbara, CA.

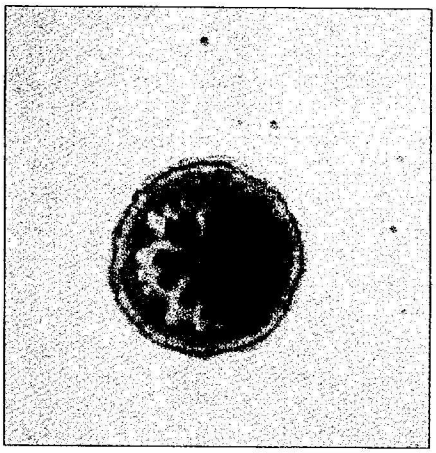

Figure 5. Two-day-old coral (*Agaricia humilis*) induced to settle as a larva and to metamorphose on an improved inductive surface prepared by coupling the morphogenic signal molecules to a glass disc, as diagrammed in Figure 4. The radial white pattern of the coral skeleton (1.5 mm diameter) developed rapidly after metamorphosis. There was no settlement or metamorphosis on otherwise identical surfaces that lacked the signal molecule. (Photograph courtesy of Larry Friesen.)

is feasible.

We made the first prototype inductive substratum (Figure 3) using a commercially available hydrophobic chromatography resin. This resin is manufactured as porous spherical beads containing a dense network of minute pores and channels designed to maximize the internal surface area available for adsorption, thus maximizing chromatographic efficiency. The large internal surface area within these pores (only 0.0001 mm in diameter) is not accessible to the much larger coral larvae (approximately 1 mm in diameter); thus, only a fraction of the purified inducer molecules adsorbed to these porous beads was actually on the outer surface, accessible to the larvae. The spherical beads also contribute inefficiently to surface coverage of a recruitment panel (compare Figure 3). By substituting nonporous flat surfaces of hydrophobic polymer for the highly porous acrylic beads, it should be possible to cover large surfaces with a uniform, monomolecular layer of the purified inducer, thus ensuring maximal exposure of all of the cue molecules to the coral larvae in the

environment. Using surface chemistry techniques employed in the production of new semiconductors and optoelectronic materials (Wudl et al. 1991), we are investigating the synthesis of hydrophobic surfaces suitable for coupling the purified inducer to plastic or glass (Figure 4). This approach is proving feasible (Figure 5) and should allow us to produce sufficient quantities of inductive surfaces for new and interesting applications. The versatility of the new coupling technologies (Wudl et al. 1991) should also make it possible to adapt other chemical linkages and to produce similar surface-coupled inducers for other species as well.

New applications of larval flypaper technology

Use of the larval flypaper for *A. humilis* has helped to validate the hypothesis that larval recognition of the purified and immobilized natural inducing molecule is responsible, in part, for substratum-specific settlement and recruitment of this coral in the natural environment. Improvements in this technology and its extension to other species may prove useful for several applications. Larval flypapers for Agariciid corals (and possibly other species as well) should provide practical tools for experiments resolving the roles of settlement cues, larval behavior, delivery and abundance of responsive larvae in the plankton, and other factors controlling the recruitment of species in the marine environment. Such materials might be especially useful in studies of larval recruitment to the unique deep sea hydrothermal vent communities, if inducers of vent species larvae can be purified and coupled to surfaces as described here. These materials may also serve as early warning devices for detecting and monitoring changes in the abundance of responsive larvae that reflect the impacts of environmental changes on reefs and other sensitive ecosystems in the ocean. Larval flypapers may also prove useful for the reseeding of corals and other species for environmental restoration, starting with larvae produced in onshore hatcheries and settled on inductive flypapers for outplanting to the natural environment. They may also improve the economic efficiency of cultivation of valuable marine resource species for which feeding requirements and growth rates make intensive on-land aquaculture economically impractical.

We are most interested in using larval flypapers containing immobilized morphogens prepared from closely similar algal species to investigate the specificity of morphogen recognition by the cognate larval receptors. This should allow us to investigate the possibility that differences in cue recognition may contribute to the differences in substratum specificity of recruitment among closely related sibling species of corals. These experiments would enable us to test our hypothesis that changes in larval specificity may serve as an axis for the rapid evolution of niche diversification and speciation (Morse et al. 1994), thereby contributing to the remarkably high degree of specialization and niche diversification now recognized in coral reef fauna (e.g., Knowlton and Jackson 1994).

We are also interested in the possibility that larval flypaper-type materials may prove useful in studies of the recruitment of some species of fish and perhaps also may enhance the activities of artificial reefs designed to enhance specific fisheries. Thus far there has been little success in the identification of factors controlling niche-specific settlement and metamorphosis of fish larvae from the plankton. Although the larvae of pelagic and midwater fish develop directly into the adult form without an apparent requirement for a substratum-specific morphogenic inducer, the larvae of some symbiotic or niche-specific fishes may possess such a requirement amenable to flypaper-like control.

Prospects for medical applications

The larval receptors and signal transducers that we have found controlling settlement and metamorphosis of planktonic abalone, polychaete, and coral larvae in response to signal molecules from the environment are functionally, structurally, and (in some cases) genetically homologous to counterparts that regulate neuronal and hormonal signal recognition, signal processing, differentiation, and proliferation in human cells (Baxter and Morse 1987, 1992, Morse 1985, 1990, 1993, Morse and Morse 1993, Morse et al. 1979, 1980a, b, Wodicka and Morse 1991). This homology, which in turn suggests evolutionary relatedness of the larval and mammalian receptors for these signals, led us to predict that the signal molecules that induce marine invertebrate larvae to settle and metamorphose may also bind with high specificity to the corresponding receptors from higher organisms and thus be potentially useful as new pharmaceuticals for human medicine.

Such cross-talk has now been seen in two widely divergent cases. One morphogen purified from coralline red algae also binds tightly and specifically to GABA (γ-aminobutyric acid) receptors purified from the mammalian brain (A. Morse 1988, Morse and Morse 1992). This algal oligopeptide inducer of abalone metamorphosis may thus prove useful for the development of new therapeutic and diagnostic agents for disorders of GABA receptors in the human brain, including epilepsies and various depressive and sleep disorders. The second instance of cross-talk involves the sulfated polysaccharide that the *Agaricia* coral larvae recognize on the surfaces of specific algae. This anionic polysaccharide is structurally similar to the anionic polysaccharide that forms an essential component of the signal molecule recognized on the lymph node endothelia by specific receptors on mammalian lymphocytes. These mammalian homing receptors regulate recruitment of the lymphocytes from the circulation, inducing attachment, proliferation, and differentiation of the white blood cells to produce antibodies in a process that is strikingly parallel to the recruitment, settlement, and metamorphosis of the coral larvae from the plankton (Figure 6). We discovered that the sulfated algal polysaccharide purified on the basis of its activity as a morphogen for *Agaricia* coral larvae strongly stimulates the proliferation of mammalian lympho-

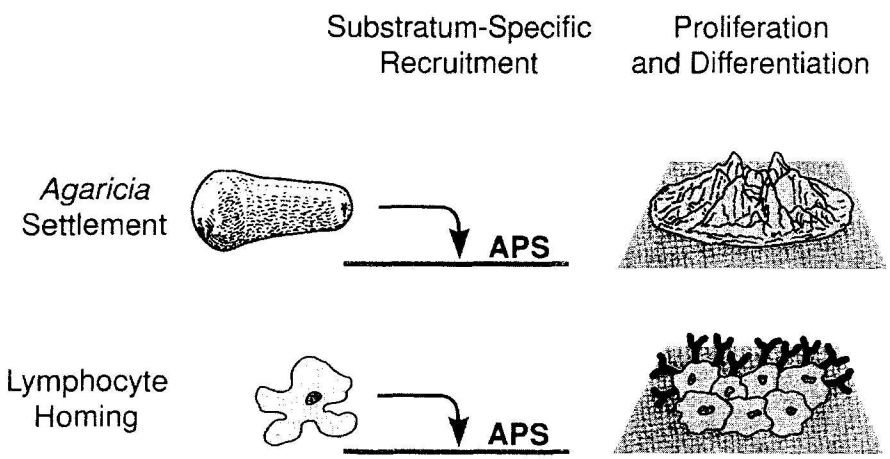

Figure 6. Parallels between the substratum-specific induction of recruitment of *Agaricia* coral larvae and mammalian lymphocyte homing induced by receptor-mediated chemosensory recognition of morphogenic signal molecules containing anionic polysaccharides (APS). Recognition of specific APS-containing signal molecules on the algal surface induces the coral larva to settle from the plankton, attach to the surface, and commence cellular proliferation and differentiation, resulting in metamorphosis. Recognition of specific APS-containing signal molecules on the capillary endothelium of the lymph node induces the lymphocyte to settle from the circulating blood, attach to the endothelium, invade the lymph node, and commence cellular proliferation and differentiation to produce antibodies (shown in blue).

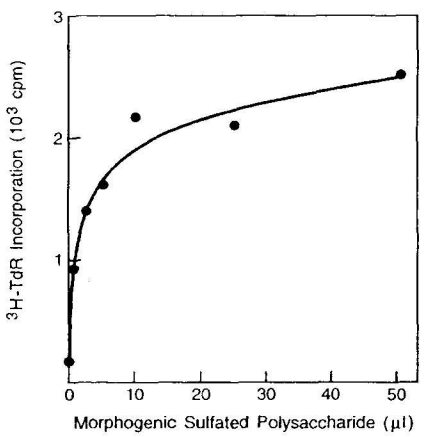

Figure 7. The sulfated polysaccharide inducer of *Agaricia humilis* metamorphosis induces proliferation of mouse lymphocytes in tissue culture. Cell division is monitored by the incorporation of tritiated thymidine (TdR) into replicating DNA.

cytes in tissue culture (Figure 7; Morse and Morse 1991). This finding suggests that a new family of pharmaceuticals based on this signal molecule might be useful as stimulators of the immune system to help combat cancers and a variety of viral and other infectious and immunodeficiency diseases. Coupling these agents to flypaper-like surfaces incorporated into circulatory shunts also might enable it to selectively remove specific subpopulations of lymphocytes from the body for therapeutic manipulation (including, for example, treatment with doses of agents that might be toxic to the whole body or genetic therapy with recombinant DNA) ex situ, before reintroduction to the patient's circulation.

Conclusions

As the morphogenic inducers recognized by the larvae of more marine species are identified and purified, as synthetic analogs of these molecules are synthesized, and as new coupling technologies are developed, extensions of larval flypaper technology should become commonplace. Research with morphogen-containing artificial surfaces can be expected to help resolve those factors controlling larval settlement, metamorphosis, and recruitment in the ocean. Practical applications in marine ecosystem monitoring, management, and reseeding can be extended from coral reefs to other ecosystems, and they may prove useful to commercial aquaculture and even to human medicine.

Acknowledgments

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