FINAL TECHNICAL REPORT

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GRANT TITLE: Molecular Mechanisms of Chemosensory Receptors, Signal Transducers, and the Activation of Gene Expression Controlling Establishment of a Marine Symbiosis

PERIOD OF PERFORMANCE: 1 August 1987 - 31 October 1990

OBJECTIVE:

(1) To characterize the molecular mechanisms by which marine invertebrate larval chemosensory receptors and their associated signal transducers regulate surface attachment and metamorphosis of the larvae in response to chemical signals from the environment; and (2) To characterize the molecular mechanisms regulating the activation of specific gene expression in the developmentally arrested marine invertebrate larva, in response to chemical inducers of metamorphosis.

TECHNICAL ACCOMPLISHMENTS:

We have found that metamorphosis in larvae of the marine mollusc, *Haliotis rufescens* (red abalone), is controlled by exogenous chemical signals recognized and transduced by two convergent chemosensory pathways: a *morphogenetic pathway* activated by a GABA-mimetic peptide morphogen encountered by the larvae on surfaces of recruiting (host) algae, and a *regulatory pathway* stimulated by lysine in seawater. The system displays both habituation, acting at the level of the morphogenetic pathway receptors (Trapido-Rosenthal and Morse, *PNAS*, 1986), and facilitation, mediated by the regulatory pathway (Baxter and Morse, *PNAS* 1987; 1991). Output and sensitivity of the morphogenetic pathway are amplified as much as 100-fold by stimulation of the regulatory pathway (Morse and Morse, *Oceanus*, 1988; Morse, 1990, 1991). We also have shown that settlement and metamorphosis of larvae of the gregarious, cementing polychaete, *Phragmatopoma californica*, a significant macrofouler, is controlled by molecular mechanisms similar to those found for *Haliotis*, and that these operate in the natural ocean environment, as well as in the laboratory (Jensen and Morse, 1989, 1990; Jensen et al., 1991).

Recent experiments *in vivo* demonstrated that the regulatory pathway in *Haliotis* larvae is controlled by chemosensory receptors and signal transducers distinct from those of the morphogenetic pathway, and that the lysine receptors of this regulatory pathway activate a sequential G protein-(phospholipase) diacylglycerol-protein kinase C signal transduction cascade (Baxter and Morse, *PNAS*, 1987). This system of dual-control, in which the integration of two different kinds of chemosensory signals from the environment modulates the settlement behavior of the *Haliotis* larvae, provides a capacity for fine-tuning larval responsiveness to exogenous chemical signals that may have adaptive significance in enhancing the site-specificity of larval settlement and metamorphosis in potentially favorable habitats (Morse, 1990, 1991).

The *Haliotis* larvae have provided a uniquely tractable experimental model system with which the receptors and signal transducers of the two controlling chemosensory pathways, and their

mechanisms of action, have been analyzed. In work completed this past year, we have: purified cilia containing these elements from the larval epithelium, and used these cilia to resolve and further analyze the lysine receptors and signal transducers of the regulatory pathway *in vitro* (Baxter and Morse, 1990); amplified, cloned and sequenced cDNAs corresponding to two novel G proteins (receptor-regulated signal transducers) from mRNA purified from the isolated cilia (Wodicka and Morse, 1991); and completed the sequence analysis of the cloned cDNA (and corresponding protein structure) of a novel serine protease that is expressed specifically in the intestine, and functions as a digestive enzyme, enabling utilization of exogenous food in the post-metamorphic recruit (Groppe and Morse, 1989, 1991).

Cilia purified from the *Haliotis* larval epithelium were found to contain the lysine receptors and signal transducers previously found *in vivo* to control the amplifier (Baxter and Morse, 1991). These elements were found to retain their functional coupling in the isolated cilia, exhibiting *in vitro* the sequential activation of a G protein and diacylglycerol-stimulated protein kinase C triggered by the binding of lysine to the lysine receptor. The purified cilia constitute an open, membrane-associated chemosensory system that has proved ideal for *in vitro* analyses.

Receptors on the isolated cilia bind the facilitating amino acid, lysine, in a specific, reversible and rapidly saturable manner. This specific binding on the cilia is increased $>10^3$ over that shown by the intact larvae, indicating significant purification. Binding to these lysine receptors is completely independent of sodium, suggesting that it is not occurring at sodium-dependent amino acid transport sites, and thus may represent binding to true chemosensory receptors. The affinities of the receptor for a series of lysine analogs is directly related to the effectiveness of these compounds as facilitators of the larval response to inducers of metamorphosis. Binding of lysine to these receptors on the isolated cilia activates a tightly coupled, receptor-dependent G protein. This activation is reflected by the increased labeling of the G protein α-subunit enzymatic ADP-ribosylation catalyzed by cholera toxin. There is a tight coupling between the lysine receptor and its associated G protein. Direct activation of the G protein in the cilia, with either GppNHp or cholera toxin and NAD, causes a reciprocal inactivation of binding affinity for lysine. The K_d of the high-affinity lysine receptor is increased by these treatments ca. 15-fold, while the B_{max} remains unchanged. This observation suggests that the lysine receptor may be a member of the rhodopsin-like, G protein-coupled transmembrane receptor superfamily, as other members of this group typically display such reciprocal inactivation by their associated G proteins. The resulting prediction that the lysine receptor may contain domains homologous to the transmembrane helical regions and cytosolic regulatory C-terminal segment highly conserved in this family will be tested directly by cDNA sequence analysis, in work planned for the coming year.

Activation *in vitro* of the ciliary lysine receptor, and of the G protein it controls, in turn activates a protein kinase C (PKC) (presumably by stimulating release of the PKC-activating second messenger, diacylglycerol, and calcium ion). This activation leads to the increased phosphorylation of an endogenous 130 kDa protein substrate in the isolated cilia. The lysine receptors, G protein and phosphorylated target protein have been labeled *in vitro*, and the latter two resolved electrophoretically. This is one of the first eukaryotic chemosensory pathways in which the receptors, G protein, PKC and target phosphoprotein, and their sequential control, all have been functionally demonstrated and resolved *in vitro*.

To further resolve the mechanisms by which the chemosensory and neuronal receptors, transducers and pathways are integrated to control behavior in these small larvae, analyses using the tools of molecular genetic dissection are required (Morse, 1990). As a first step toward that objective, we have purified mRNA from the isolated cilia described above, and from this mRNA, we have synthesized, amplified, cloned and sequenced the cDNAs corresponding to two novel Gα signal transducing proteins (Wodicka and Morse, 1991). These sequences were amplified from the cilia cDNA by PCR (polymerase chain reaction), using degenerate synthetic oligonucleotide primers corresponding to the highly conserved G and G' guanosine nucleotide binding domains common to all G proteins; degeneracy of the primers was reduced to reflect codon frequencies we recently determined for *Haliotis*

mRNAs (Groppe and Morse, 1989, 1991). From the unique sequences of the Gα cDNAs, we then synthesized non-degenerate primers which allowed us to amplify the corresponding genes from *Haliotis* genomic DNA. The genomic sequences that we thus far have characterized correspond exactly to their cDNAs, and contain the intron at the same position between the G and G' domains as found in mammalian G protein genes. Proof that these sequences were from *Haliotis* mRNA, and not from any possible contaminants, was confirmed by the failure of the unique ciliary sequences to direct amplification of any homologous sequences in control samples of bacterial, protozoan or fish DNA, under conditions in which the perfectly homologous sequences were detected and amplified from *Haliotis* genomic (sperm) DNA. The two Gα sequences we obtained from the larval cilia differ significantly from one another; Gα1 proves to be virtually identical to Gq, a new class of G proteins recently shown to regulate phospholipase C in mammalian brain. Gα1 (Gq) displays pronounced differences from G_i, G_s, G_o and G_{olf} sequences characterized from other systems, whereas the larval cilia Gα2 sequence is significantly more closely related to G_i and G_o (from *Drosophila* and rat) than it is to the G_s and G_{olf} from these species. Significantly, Gq is known to activate PLC; this is the activity also deduced from our studies of the transduction pathway in the larval cilia.

We have completed the cloning and sequence analysis of a novel intestinal serine protease (digestive enzyme) from *Haliotis* (Groppe and Morse, 1989, 1991). Northern hybridization analyses demonstrate that the gene coding for this enzyme is expressed rapidly following the induction of metamorphosis. Our recent results include: confirmation of the preliminary structural analyses of the protein; verification of its unique substrate specificity and very high turnover number; the finding that the molluscan serine protease represents a previously unsuspected intermediate in the phylogeny of the structure and activity of the serine protease family; and confirmation of our earlier prediction of a novel sequence in the pre-proenzyme at which autocatalytic processing occurs to release the mature enzyme from its inactive zymogen.

SIGNIFICANCE:

Our results demonstrate that the purified larval cilia provide a model system uniquely suited for *in vitro* resolution of the chemosensory receptors and signal transducers controlling metamorphosis in planktonic marine invertebrate larvae. Our discovery that functional mRNA can be purified from these cilia in quantities sufficient to establish a cDNA library extends the tractability of the *Haliotis* larval system to analyses of the chemosensory elements at the cDNA and protein sequence level. This is the first discovery of functional mRNA in cilia from any system, and itself is likely to open new areas of research in many laboratories. Results of these studies are providing the first elucidation of the detailed molecular mechanisms by which chemosensory receptors and transducers regulate larval settlement behavior and metamorphosis. Results of the continuing cDNA investigations should provide insights into the basic mechanisms of action (and the evolution) of the chemoreceptors and their associated signal-transducers in the molluscan larvae. The mechanisms by which these control responsiveness to stimuli, physiological and behavioral processes, and the activation of gene expression and development, can be expected to be applicable to a wide variety of other sensory, neuronal, hormonal and developmental systems as well. These results may help identify new targets and strategies for the prevention of larval settlement attachment and biofouling through non-polluting means.

INVENTIONS:

The chemosensory receptor for lysine and its membrane associated signal transducers might prove useful for the development of novel molecular sensors. The cloned protease may have industrial usefulness, particularly in the digestion of protein contaminants in preparations of polysaccharides and related biopolymers.

No patents have been filed.

PUBLICATIONS AND REPORTS:

Publications and Manuscripts:

1. Groppe, J. and D.E. Morse. 1989. Molecular cloning of novel serine protease cDNAs from abalone. In: Current Topics in Marine Biotechnology, ed. by S. Miyachi, I. Karube and Y. Ishida. Japan. Soc. Mar. Biotechnol., Tokyo:285-288.

2. Jensen, R.A. and D.E. Morse. 1989. The bioadhesive of *Phragmatopoma californica* tubes: a silk-like cement containing L-DOPA. *J. Comp. Physiol. B* 158:317-324.

3. Morse, D.E. 1990. Recent progress in larval settlement and metamorphosis: Closing the gaps between molecular biology and ecology. *Bull. Mar. Sci.* 46:465-483.

4. Jensen, R.A. and D.E. Morse. 1990. Chemically induced metamorphosis of polychaete larvae in both the laboratory and ocean environment. *J. Chem. Ecol.* 16:911-930.

5. Jensen, R.A., D.E. Morse, R.L. Petty and N. Hooker. 1990. Artificial induction of larval metamorphosis by free fatty acids. *J. Exp. Mar. Biol. Ecol.* 67:55-71.

6. Wodicka, L. and D.E. Morse. 1991. cDNA sequences reveal two novel Gα signal transducing proteins from larval cilia. *Biol. Bull.* (in press)

7. Morse, D.E. 1991. Molecular mechanisms controlling larval metamorphosis in abalone larvae. In: Abalone, ed. by M. Tegner and S. Shepherd. (in press).

8. Morse, D.E. and A.N.C. Morse. 1991. What are planktonic larvae "looking" for? A molecular view of the morphogens, receptors, and signal transducers controlling site-specific settlement and metamorphosis of animals in the ocean. *American Scientist* (invited paper; in press).

9. Morse, D.E. 1991. Morphogens, signal molecules and other non-toxic bioactive substances that play a role in structuring interactions and distributions in the marine environment. In: *Marine Bioactive Substances*, ed. by M.-F. Thompson, R. Sarojini and R. Nagabhushanam, Oxford Press, Delhi (in press).

10. Morse, A.N.C. 1991. GABA-mimetic peptides from marine algae and cyanobacteria as potential diagnostic and therapeutic agents. In: *Marine Bioactive Substances*, ed. by M.-F. Thompson, R. Sarojini and R. Nagabhushanam, Oxford Press, Delhi (in press).

11. Jensen, R.A. 1991. Bioadhesion and morphogenesis in a marine polychaete. In: *Marine Bioactive Substances*, ed. by M.-F. Thompson, R. Sarojini and R. Nagabhushanam, Oxford Press, Delhi (in press).

Submitted:

1. Baxter, G. and D.E. Morse. 1991. Chemosensory signal transduction in cilia from molluscan larvae: Coupling of lysine receptors, G protein and protein kinase C. *J. Biol. Chem.*

2. Johnson, C.R., D.G. Muir, A.L. Reysenbach, A.N.C. Morse and D.C. Sutton. 1991. Characteristic bacteria associated with surfaces of coralline algae: A role in inducing settlement and metamorphosis of abalone larvae? *Mar. Ecol. Prog. Ser.* (in press).

3. Spaulding, D. and D.E. Morse. 1991. Arylsulfatases from larval and adult *Haliotis rufescens*: evidence for gene switching during development. *J. Comp. Physiol. B.*

In Preparation:

1. Groppe, J.C. and D.E. Morse. 1991. Messenger RNA for a serine protease homologous to pancreatic enzymes is highly expressed in the intestine of the abalone, *Haliotis rufescens*. For: *J. Biol. Chem.*

2. Morse, A.N.C. and H. Trapido-Rosenthal. 1991. GABA-mimetic peptide ligand binding to chemosensory receptors from molluscan larvae and $GABA_A$ receptors from mammalian brain. For: *J. Neurochem.*

3. Groppe, J. and D.E. Morse. 1991. Detection of high molecular weight rRNA from the marine mollusc, *Haliotis rufescens*. For: *Nucleic Acids Res.*

4. Groppe, J. and D.E. Morse. 1991. A novel method for cloning of abundant, tissue-specific cDNAs. For: *J. Biol. Chem.*

Abstracts and Posters:

1. Groppe, J.C. and D.E. Morse. 1989. Cloning and identification of a molluscan serine protease cDNA. (Poster) Arrowhead Genetics Conference, Arrowhead, CA.

2. Morse, D.E. 1989. Morphogens, signal molecules, and other non-toxic bioactive substances that play a role in structuring interactions and distributions in the marine environment. (Abstract) Proc. Intl. Symp. Bioactive Substances from Marine Organisms, Goa, India.

3. Morse, A.N.C. 1989. GABA-mimetic peptides from marine algae and bacteria as potential diagnostic and therapeutic agents. (Abstract) Proc. Intl. Symp. Bioactive Substances from Marine Organisms, Goa, India.

4. Roell, M.K. and D.E. Morse. 1989. A rapid method for isolation of chloroplast DNA from the red alga, *Polysiphonia boldyii* , and hybridization with a cyanobacterial phycoerythrin gene. (Poster and Abstract) Northwest Algal Symposium, Seattle, WA.

5. Groppe, J.C. and D.E. Morse. 1989. Molecular cloning of novel serine protease cDNAs from abalone. (Abstract) Proc. First Intl. Symp. Marine Biotechnology, Tokyo.

6. Roell, M.K. and D.E. Morse. 1989. A rapid method for isolation and fractionation of nucleic acids from the red alga, *Polysiphonia boldyii*. (Poster and Abstract) Intl. Symp. Amer. Soc. Phycology, Toronto.

7. Morse, D.E. 1989. *In vitro* dissection of chemosensory pathways controlling larval metamorphosis. (Abstract) Proc. Symp. Amer. Soc. Zool., Boston.

8. Morse, D.E. 1989. Molecular mechanisms controlling metamorphosis and recruitment in abalone larvae. (Abstract) Proc. Intl. Symp. Abalone, La Paz.

9. Jensen, R.A. and D.E. Morse. 1989. Bioadhesion and chemically induced metamorphosis of polychaete larvae in the ocean and the laboratory. (Abstract) Proc. Symp. Amer. Zool., Boston.

10. Baxter, G. and D.E. Morse. 1989. The mechanism of signal transduction associated with chemoreception in larvae of the marine invertebrate, *Haliotis rufescens*. (Abstract) Proc. Symp. Western Soc. Naturalists, Tacoma.

11. Roell, M.K, and D.E. Morse. 1990. Cloning and nucleotide sequence of the phycoerythrin α and β subunit genes from *Polysiphonia boldyii* (Rhodophyta). (Abstract) Proc. Symp. Phycol. Soc. Amer., College Park.

12. Morse, D.E. 1990. Chemosensory and integrative mechanisms controlling settlement and metamorphosis of abalone larvae: Molecular dissection of the amplifier pathway. (Abstract) Proc. Symp. on Integrative Neurobiol., Amer. Malacol. Union, Woods Hole.

13. Morse, A.N.C. 1990. The role of algal metabolites, and the molecular mechanism of their action, in invertebrate larval recruitment. (Abstract) Proc. Intl. Symp. Plant-Animal Interactions in the Marine Benthos, Liverpool.

Reports:

1. Morse, D.E. 1989. Molecular Biology Program, Biological Sciences Division, ONR.

2. Gibor, A. and D.E. Morse. 1989. Genetic and cellular engineering of marine algae and bacteria. Annual Report to the Systemwide Biotechnology Program, University of California.

3. Morse, D.E. 1990. Molecular Biology Program, Biological Sciences Division, ONR.

STUDENTS/TRAINEES:

Total:	4
Female:	1
Minority:	0
Non-U.S.:	0