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Chapter 10
Molecular mechanisms controlling metamorphosis and recruitment in abalone larvae

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ABSTRACT: Abalone larvae have proved especially useful for experimentally resolving the molecular mechanisms controlling the settlement and metamorphosis of marine invertebrate larvae in response to chemical signals from the environment. Recent studies *in vitro* have confirmed the existence of two separate families of chemosensory receptors and signal transduction pathways that process such chemical information from the environment to control metamorphosis in abalone larvae. A variety of chemical stimuli have been found that act at the chemosensory receptors controlling these two pathways, while other chemical stimuli have been found to induce larval settlement, or enhance the sensitivity of the larvae to settlement inducers, by interacting directly with the signal transducers. The receptors and signal transducers of the regulatory (amplifier) pathway have been characterized *in vitro*, on cilia purified from the larval epithelium; results of these analyses confirm conclusions from earlier experiments *in vivo*. Properties of the receptors of the regulatory and morphogenetic pathways suggest how these may interact to control settlement in response to two different classes of chemical signals (in the water column and on surfaces) in the natural environment. At last count, 13 *Haliotis* species (including *H. discus hannai*) have been found to respond similarly to chemical inducers of settlement and metamorphosis. We have begun to clone genes expressed in the abalone early after metamorphosis, to facilitate analysis of the mechanisms controlling their developmental activation and their contribution to early growth.

RESUMEN: Las larvas de abulón son especialmente útiles para explicar experimentalmente los mecanismos moleculares que controlan el asentamiento y metomorfosis de las larvas marinas invertebradas como respuesta a señales químicas del medio ambiente. Recientes estudios *in vitro* han confirmado la existencia de dos familias distintas de receptores quimicosensores y senderos de transducción de señales que procesan dicha información química del medio ambiente para controlar las metamorfosis en las larvas de abulón. Se ha encontrado una variedad de estímulos químicos que actúan en los receptores quimicosensores que controlan estos dos senderos; asimismo, se ha visto que otros estímulos químicos inducen al asentamiento de las larvas, o aumentan la sensibilidad de las larvas a los inductores de asentamiento, a través de la interacción directa con los transductores de señales. Los receptores y los transductores de señales del sendero (amplificador) regulador han sido caracterizado *in vitro*, sobre cilios purificados del epitelio larval; los resultados de estos análisis confirman conclusiones de anteriores experimentos *in vivo*. Las propiedades de los receptores de los senderos reguladores y morfogenéticos indican cómo estos pueden actuar recíprocamente para controlar el asentamiento respondiendo a dos tipos diferentes de señales químicas (en la columna de agua y en las superficies) en el medio ambiente natural. En un recuento final, se han encontrado 13 especies de *Haliotis* (incluyendo la *H. discus hannai*) que responden en forma similar a los inductores químicos de asentamiento y metamorfosis. Hemos empezado a hacer clones de genes que se manifiestan en el abulón justo después de la metamorfosis, para facilitar el análisis de los mecanismos que controlan su activación durante el desarrollo y su contribución al crecimiento temprano.

INTRODUCTION

Larval metamorphosis in species of *Haliotis*, the abalone, provides a uniquely tractable system for the analysis of chemosensory and molecular mechanisms that control development and recruitment in marine organisms. The advantages of *Haliotis* for such studies include the large numbers of larvae that can be produced, with complete control of spawning; the relatively rapid and synchronous development of the larvae; the fact that the larvae are lecithotrophic; and the nearly absolute requirement of the larvae (when cultured under defined and controlled conditions) for an exogenous inducer of metamorphosis (Morse, 1990). This chapter presents new findings not previously published, reviews earlier work on the control of metamorphosis and recruitment in abalone larvae and discusses recent progress in the application of these findings.

INDUCERS OF SETTLEMENT AND THE MORPHOGENETIC PATHWAY

A wide variety of biotic and chemical substances have been found or have been proposed capable of inducing the settlement and metamorphosis of abalone larvae. The biotic materials include crustose coralline red algae (Crofts, 1929; Morse D. *et al.*, 1979a,b, 1980c; Morse A. *et al.*, 1984; Morse & Morse, 1984a; Morse D. 1988), various microalgae and bacteria (Morse D. *et al.*, 1979b, Morse A. *et al.*, 1984; Akashige *et al.*, 1981; Leighton, 1985) and abalone mucus (Seki & Kanono, 1981; Akashige *et al.*, 1981; Slattery, 1987). While each of these findings has led to the development of useful improvements in the technology for cultivation of abalone, only the crustose red algae thus far have been demonstrated, in quantitative and controlled analyses, to enhance substratum-specific recruitment of *Haliotis* spp. in the natural environment. Such results have been obtained for *H. discus hannai* (Saito, 1981), *H. laevigata* and *H. scalaris* (Shepherd & Turner, 1985); *H. cracherodii* (Douros, 1985) and *H. rubra* (Prince *et al.*, 1987). The results of Douros and Prince *et al.* also appear to exclude any significant contribution to recruitment from conspecific (e.g. mucus) cues in the natural environment, as both studies found that settlement preference for microhabitats was independent of conspecific density.

It has not been determined whether the settlement-inducing molecules found at the surfaces of crustose red algae are encoded in the red algal genome or in the genomes of associated microorganisms. Recently, however, Aileen Morse (unpub. obs) found that 33 different strains of bacteria isolated from samples of crustose red algae all failed to induce settlement or metamorphosis of abalone larvae.

The precise identity of the inductive chemical from the crustose red algae still eludes us. We do know that this inducer is a molecule first found associated with a colourless protein from the algae; biochemical fractionation resolves this activity into a smaller fraction that is associated with a peptide which contains several unusual amino acid residues (Morse A. *et al.*, 1984; Morse & Morse, 1984a; Morse A., 1988). While other (i.e. foliose) red algae (and the biochemically and evolutionarily related cyanobacteria) also contain closely related inducing molecules intracellularly, these algae and bacteria, while intact, fail to induce the settlement

and metamorphosis of abalone larvae, in contrast to the induction caused by intact crustose red algae (Morse A. *et al.*, 1984; Morse & Morse 1984a,b). This difference was shown to be due to the unique availability of the inducing molecules at the surfaces of the crustose red algae (Morse & Morse, 1984a). The inducers may be transported to the surfaces of the crustose coralline red algae by the high secretory and exfoliative activities of these algae.

The algal inducer, in both its protein-associated and peptide-associated forms, has proved to be GABA-mimetic. That is, the molecule (purified 20 000-fold from the recruiting alga) mimics the action of the simple amino acid neurotransmitter, gamma-aminobutyric acid (GABA), in binding strongly, specifically and saturably to GABA receptors purified from mammalian brain (Morse A., 1985; Morse D., 1985; Morse D., 1990; A.N.C. Morse & H.G. Trapido-Rosenthal, unpub. obs.). Apparently, similar receptors on the abalone larvae mediate the recognition of the algal inducer, causing settlement and metamorphosis (Trapido-Rosenthal and Morse D., 1986a; A.N.C. Morse & H.G. Trapido-Rosenthal, unpub. obs.).

Several lines of evidence suggest that the externally accessible receptors on the abalone larvae that can be labelled with radioactive GABA analogues are those which mediate the induction of metamorphosis (Trapido-Rosenthal & Morse, 1986a; Morse D., 1990). The fact that the natural inducer of larval settlement and metamorphosis purified from a naturally recruiting crustose coralline algal host, *Lithothamnium californicum*, competes with radioactive GABA analogues for binding to these receptors is the strongest evidence suggesting that the receptors that recognize the natural algal inducer are the same receptors that also recognize GABA and its analogues in the induction of settlement and metamorphosis by these compounds (Trapido-Rosenthal & Morse, 1986a; Morse D. 1990; A.N.C. Morse & H.G. Trapido-Rosenthal, unpub. obs.). In addition:

(1) the affinity of these larval receptors for various GABA analogues predicts the effectiveness of the analogues as inducers of settlement and metamorphosis;
(2) the receptors appear on the larvae as a function of time of development and their appearance precedes (and apparently is required for) the development of larval competence to respond to both algal and GABA inducers; and
(3) precocious exposure of larvae to GABA 'down regulates' or habituates the larvae, blocking the subsequent developmental increase in the number of externally accessible receptors and rendering the larvae 'blind' to induction by GABA (although they are fully inducible by compounds that act at the level of signal-transduction downstream from the receptor; see below).

At the present, more is known about the nature of these receptors than about their location. This is because it has not yet proved possible to label these receptors with sufficient specificity to ensure that only the receptors controlling metamorphosis have been tagged, as would be required for their unequivocal direct visualization, whereas biochemical methods do permit resolution and analysis of the receptor-binding properties under these conditions (Trapido-Rosenthal & Morse, 1986a). A number of observations indirectly suggest that the receptors controlling metamorphosis may be external, although GABA also is known to be

taken up by *Haliotis* larvae (Trapido-Rosenthal & Morse, 1986a; Jaeckle & Manahan, 1989). Thus, the following are all consistent with the suggestion that the receptors controlling metamorphosis are peripheral and possibly external:

(1) the finding that the most efficient inducer is the macromolecular GABA-mimetic (Morse A., 1985);
(2) the fact that baclofen (a GABA analogue) is a better inducer than GABA itself, yet is taken up (internalized) by *Haliotis* larvae less efficiently than GABA (Trapido-Rosenthal & Morse, 1986a); and
(3) the observation that the receptors specifically labelled with baclofen are shed from the larvae at the time during metamorphosis when cilia and other external larval structures are shed (Trapido-Rosenthal & Morse, 1986a).

Experimental evidence reviewed elsewhere (Morse D., 1990) has led to the suggestion, as a simplified working hypothesis, of a pathway of signal transduction events that may follow recognition of the exogenous morphogenetic stimulus by abalone larvae. This evidence suggests that binding of the exogenous inducing molecule by specialized chemosensory receptors results in the sequential activation of a receptor- and membrane-associated adenyl cyclase, synthesis of cyclic AMP, activation of a cyclic AMP- and calcium-regulated protein kinase (likely to be protein kinase A), phosphorylation of an endogenous protein, opening of chloride (or other anion) channels in the chemosensory cell membrane and an efflux of chloride (or other anion), believed to result in an excitatory depolarization, or firing, of the chemosensory cell. In this way, the morphogenetic chemical signal from the environment may be transduced to an electrochemical signal that can be propagated by the larval nervous system. Evidence leading to this simplified working hypothesis has been presented and discussed in detail previously (Morse *et al.*, 1980a; Baloun & Morse, 1984; Morse D., 1985, 1990).

Consistent with the above, a large number of chemicals known to affect intracellular cyclic AMP and calcium levels, protein phosphorylation, cell membranes, membrane ion channels, ion translocation and ionic depolarization of cell membranes have been found also to induce settlement and metamorphosis of abalone larvae (Table 10.1). These include cyclic AMP analogues, forskolin, substituted xanthines such as isobutylmethylxanthine and theophylline, calcium ionophores and chelators, an elevated external concentration of potassium ion (which conveniently depolarizes externally accessible, electrically excitable membranes), a low external chloride concentration, specific and non-specific ion-channel openers (such as ivermectin and organic solvents, respectively) and free arachidonic and palmitoleic acids (Morse *et al.*, 1980a; Baloun & Morse, 1984; Baxter & Morse, 1987; Morse D., 1990; Jensen *et al.* 1990).

The case of the free fatty acids, arachidonic and palmitoleic acids, is particularly interesting. These compounds, first erroneously suggested to be the natural and species-specific inducers of settlement and metamorphosis of the tube-building polychaete, *Phragmatopoma californica*, (Pawlik, 1986) are widely known in other systems as potent disrupters of membrane structure (Orly & Schramm, 1975; Chan *et al.*, 1983; Baba *et al.*, 1984; Kim & Clapham, 1989; Ordway *et al.*, 1989), with activities including the resultant activation of membrane-associated adenyl cyclase (causing an increase in cyclic AMP synthesis) and potassium

Table 10.1 Chemical inducers of settlement and metamorphosis for *Haliotis* spp. and the cellular levels at which they apparently act, see text for explanation.

Probable sensory receptor stimuli
Algal, microbial GABA-mimetics
GABA, GABA analogues
Mucus
Cyclic AMP effectors
db-cAMP
Forskolin
Isobutylmethylxanthine, theophylline
Fatty acids
Ca^{++} Effectors
A23187 ionophore
Chelators
Membrane depolarizers
High external K^+
Low external Cl^-
Ivermectin
Solvents

db-cAMP = dibutyryl-3', 5'-cyclic AMP.
Forskolin = activator of enzyme that synthesizes cAMP.
Isobutylmethylxanthine and theophylline = inhibitors of enzyme that degrades cAMP.
Ivermectin = chloride ion channel opener.

channel-mediated transmembrane ion flux. Apparently as a consequence of one or more of these activities, arachidonic and palmitoleic acids induce settlement (plantigrade attachment) and metamorphosis of abalone larvae, at concentrations even lower than those required to induce these changes in *Phragmatopoma* (Jensen *et al.*, 1990). Recent evidence shows that these fatty acids are in fact not taxon-specific or substratum-specific natural inducers, but instead are widely active artifacts of the method of extraction (Jensen *et al.*, 1990). Like forskolin, isobutylmethylxanthine and excess potassium ion (Yool *et al.*, 1986; Jensen & Morse, 1990; Morse D., 1990; see Table 10.1 and below), their activity on both abalone and polychaete larvae apparently reflects action(s) at sites physiologically downstream from (or parallel to) the actions of the natural, substratum-specific inducing molecules (Jensen *et al.*, 1990).

GENERALITY OF RESULTS

As found by a number of researchers in several different countries, the results discussed above are applicable to several abalone species. At the last count, 13 different species of temperate and tropical *Haliotis* have been found in which the larvae are induced to settle (attach to substratum), metamorphose and commence normal post-metamorphic growth in response to crustose coralline red algae and in response to GABA (reviewed in Morse D., 1990). Akashige *et al.* (1981)

reported that *H. discus hannai* failed to show such results in response to GABA and that after induction, evidence of toxicity was seen. However, examination of the experimental conditions employed in the research laboratories of Akashige and colleagues and of the author revealed several significant differences which apparently account for the different results observed. The principal differences appear to include the degree and manner of bacterial prophylaxis. Indeed, in the results of Morse *et al.* (1979b, Table 10.1), when GABA was used with larval cultures containing bacteria without antibiotics, high mortality and failure of metamorphosis similar to those seen by Akashige *et al.* (1981) were observed; in these same experiments, the inclusion of antibiotics prevented the mortality and ensured a high success rate of GABA-induced metamorphosis (Table 10.1). Uncontrolled bacterial growth in the larval cultures can thus alter the results obtained, perhaps as a consequence of bacterial metabolism and its products (Morse 1984, 1990).

That crustose coralline red algae and GABA are each capable of efficiently inducing normal settlement, attachment and metamorphosis of *H. discus hannai* larvae under conditions similar to those originally described by Morse *et al.* (1979a,b, 1980a,b) is shown in Figs 10.1 and 10.2. It was observed that larvae of *H. discus hannai* cultivated and analysed at 22°C in filtered seawater containing antibiotic became fully competent to attach and metamorphose in response to 1 mM GABA at approximately 70 hours post fertilization (Fig. 10.1). This was

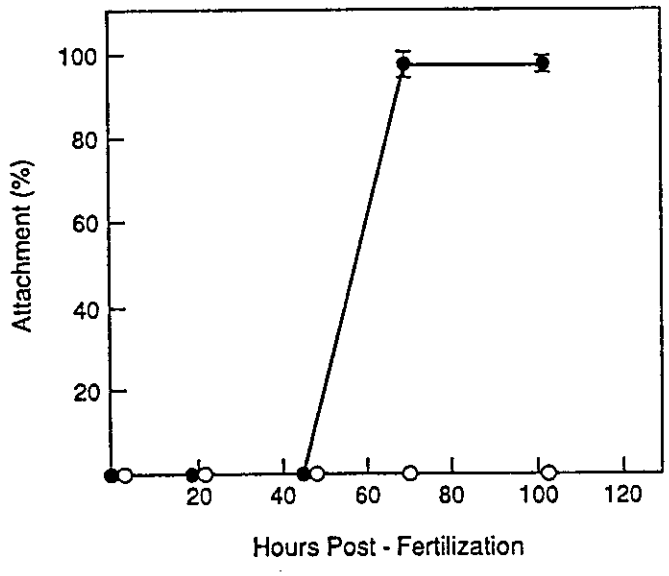

Fig. 10.1 Development of competence of *Haliotis discus hannai* to metamorphose in response to stimuli. Adult Ezo abalone were induced to spawn with hydrogen peroxide (Morse *et al.*, 1977): the gametes were fertilized and larvae reared at low density (≤1/ml) in seawater (1 μm-filtered, containing 100 ppm penicillin G) changed every 12 hours. Larvae were tested for competence as a function of time following fertilization, by exposure of duplicate samples of 100 ± 25 larvae incubated in 15 ml fresh seawater (1 μm-filtered, 100 ppm penicillin G; 2.4 cm depth), either with no additions (open circles) or with 10^{-3} M GABA (filled circles). Results (mean ± range) were scored 30 mins after the start of incubation. Attached refers to plantigrade attachment. All operations were at 22°C.

observed to be the same time required for the same population to become competent to respond to induction of attachment and metamorphosis by crustose coralline red algae (data not shown). As in the case of *H. rufescens* and other species tested, although the high concentration (1 mM) of GABA used in Fig. 10.1 is useful for rapidly (i.e. in 30 minutes) detecting the development of competence in *H. discus hannai*, this high concentration is toxic on prolonged incubation (cf Morse 1979a, 1980a,b); a lower concentration is needed to induce metamorphosis with minimal mortality. Both the concentration dependence and the concentration optimum for GABA-induced metamorphosis of competent *H. discus hannai* (Fig. 10.2) are similar to those observed for *H. rufescens* (Morse *et al.*, 1980b) and most other *Haliotis* species tested (cf Morse, 1984). At the optimal concentration (ca 10^{-6} mol/l) of GABA, 86% ± 2% (range) of the larvae were induced to undergo normal metamorphosis, with negligible mortality. Metamorphosis induced by parallel samples of crustose coralline algae was 79% ± 4% (range), while zero-addition and rock controls yielded 0% ± 0% metamorphosis. Preliminary results of these experiments with *H. discus hannai*, conducted in collaboration with Professor Zhang Fusui at the Institute of Oceanology in Qingdao, were reported elsewhere (Morse, 1984, 1990).

At least two commercially successful abalone cultivation companies in California (Ab Lab, Inc, Port Hueneme, CA and The Cultured Abalone, Inc, Santa Barbara) are now using GABA to induce larval settlement and metamorphosis, verifying

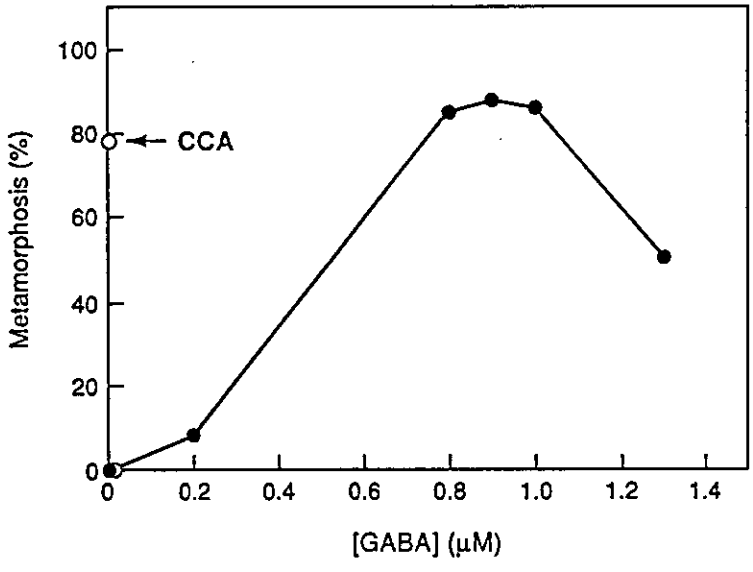

Fig. 10.2 Induction of metamorphosis of *Haliotis discus hannai*. Experiment as in Fig. 10.1, except that larvae were 80 hours post fertilization, the concentration of GABA varied from 0 to 1.3×10^{-6} M, and results were scored after 48 hours. Metamorphosis refers to abscission of velum and growth of flared, ribbed shell. Mortality in all treatments ≤ 4%. All operations were at 22°C. CCA = crustose coralline red algae on rock, collected with parallel samples of rock without CCA (yielding zero metamorphosis; lower open circle) from same intertidal. CCA and rock samples were incubated in 250 ml volumes containing same numbers of larvae and same concentration of penicillin as in smaller incubations.

the usefulness of the method for production purposes. The author's laboratory has been using the method, with high survival of the resulting progeny, for more than 10 years. Other production facilities are also finding other sources of inducers, particularly abalone mucus, especially useful for this purpose (Chapter 44; cf Seki & Kan-no, 1981; Uki, 1989). It is likely that each type of inducer will be found to have its own specific set of costs and benefits associated with its use (Morse 1984; Uki, 1989).

Elements involved in the post-receptor pathway of signal transduction suggested to control settlement and metamorphosis in the red abalone, *Haliotis rufescens*, (Table 10.1) have also been implicated in similar processes in the larvae of several other species and phyla (Baloun & Morse, 1984; Yool *et al.*, 1986; Jensen & Morse, 1990; Morse D., 1990). While specific differences exist between the pathways implicated in *H. rufescens* and those in the non-haliotid species, the ability to induce metamorphosis efficiently by direct depolarization with excess potassium is proving widely useful for studies of morphogenesis in a number of marine invertebrates (Yool *et al.*, 1986; Morse D., 1990).

FACILITATION AND THE REGULATORY PATHWAY

A second pathway of chemical signal recognition and transduction that regulates the metamorphic response of abalone larvae has recently been found. Lysine and related diamino acids dissolved in seawater can amplify the sensitivity of the larvae to the required morphogenetic signal by as much as 100-fold, although these amino acids by themselves do not induce metamorphosis (Trapido-Rosenthal & Morse, 1985, 1986b; Baxter & Morse, 1987 and unpublished data; Morse D., 1988, 1990). This amplification may increase the propensity of larvae to settle in potentially favourable areas, and may possibly contribute to some of the observed spatial and temporal variation in abalone recruitment. The regulatory lysine signal is recognized by binding to high-affinity chemosensory receptors (Baxter & Morse, 1987) found on specialized cilia purified from the larval epithelium (Baxter & Morse, unpub. obs.). This lysine receptor binding signal is transduced by a receptor-associated G protein, which in turn leads to the activation of a diacylglycerol-stimulated, calcium-stimulated protein kinase C (PKC) that phosphorylates a specific target protein. These reactions, originally deduced from experiments *in vivo* (Baxter & Morse, 1987), have now been fully confirmed in molecular studies *in vitro*, in the isolated cilia (G. Baxter & D.E. Morse, unpub. obs.). The mechanism by which this pathway amplifies the sensitivity or responsiveness to the morphogenetic signal is under investigation.

This is the first eukaryotic chemosensory pathway in which the receptors, G protein, PKC and target phosphoprotein, and their sequential control, have all been functionally demonstrated and resolved *in vitro*. The regulatory receptor, G protein and phosphorylated target protein have been labelled and the latter two have been purified. Recently, Lisa Wodicka has purified, amplified and characterized the G protein mRNA and cDNA sequences from the isolated cilia (Wodicka & Morse, unpublished data). From these molecular studies, we are learning that the receptors and signal transducers controlling metamorphosis in abalone larvae are closely related to those controlling sensory, developmental and neuronal processes in other organisms.

INTERACTION BETWEEN THE MORPHOGENETIC AND REGULATORY PATHWAYS

From the studies discussed above, it is becoming clear that larval settlement, attachment and metamorphosis are regulated by the interaction between chemosensory pathways (and other biological and non-biological factors) with a degree of complexity far greater than previously anticipated. Table 10.2 summarizes the principal features of the two pathways controlling metamorphosis in response to external chemical stimuli. The interaction of these pathways appears to provide the larvae with a capacity for fine tuning the sensitivity of recognition of, and response to, chemical signals from the environment that is greater than was anticipated for marine invertebrate larvae just a few years ago.

Analyses of the binding properties of the chemosensory receptors controlling the two pathways are providing some clues about the way this complex process may work (Table 10.3). The morphogenetic responses (attachment and metamorphosis of the larvae) are normally dependent on the recognition of the algal GABA-mimetic molecules that are associated with the recruiting crustose red algal surface or other functionally similar signals (e.g. in mucus). This recognition is apparently mediated by highly specific GABA-type receptors (R_G) that have a very low affinity or weak binding capacity (indicated by the relatively high K_D value of 10^{-6}M) (Trapido-Rosenthal & Morse, 1986a). There is a large number

Table 10.2 Principal features of the two chemosensory pathways controlling attachment and metamorphosis of *Haliotis rufescens* larvae in response to chemical signals from the environment.

	Morphogenetic	Regulatory
Signal	Algal GABA-mimetic	DOM diamino acid
Receptor	GABA-type	Lysine-type
Transducers	Adenyl cyclase Cyclic AMP Ca^{++} Protein kinase Chloride channel	G Protein Phospholipase Diacylglycerol Ca^{++} Protein kinase
Output	Excitatory depolarization	Amplification of Morphogenetic Signal

Table 10.3 Interaction between chemosensory pathways controls attachment and metamorphosis of *Haliotis rufescens* larvae; values for affinity and number/larva of the GABA-type (R_G) and lysine-type (R_L) externally-accessible chemosensory receptors are approximate and taken from the data of Trapido-Rosenthal & Morse (1986) and Baxter & Morse (1987 and unpub. obs.), respectively, see text for explanation.

	R_G	R_L
Sensing medium	Surface	Water
Ligand	GABA-mimetic	Lysine
Affinity	Low (K_D 10^{-6}M)	Higher (K_D 10^{-7}M)
Number/larva	High (10^{10})	Low (10^{8})
Controls	Attachment	Sensitivity

of these receptors on the larvae, suggesting a kind of fail-safe function, in which a high concentration of the required inducing signal may normally be required to induce attachment.

In contrast, the regulatory (or 'amplifier') pathway is apparently adapted to recognize relatively low concentrations of amino acid signals (specifically lysine and its analogues) dissolved in seawater. These receptors (R_L) have a higher affinity (lower K_D, as measured by Scatchard analyses), tuned to the concentration range over which lysine varies in dissolved organic material (Trapido-Rosenthal & Morse, 1985, 1986b), and there is a lower number of these receptors on the larvae (G. Baxter & D.E. Morse, 1987, and unpub. obs.; see Table 10.3). The output of this lysine-sensing pathway increases the threshold sensitivity of the abalone larvae to low concentrations of the required surface-associated inducer, thus potentially at least enhancing attachment in response to the detection of specific dissolved nutrients. Whether these two types of receptors and the two pathways they control operate within a single cell or in two chemosensory cells whose outputs are distally convergent is the object of our present investigations. It also remains to be demonstrated whether the operation of these two pathways may be of ecological or adaptive significance in the natural environment.

GENE ACTIVATION AND THE CONTROL OF POST-METAMORPHIC DEVELOPMENT AND GROWTH

Metamorphosis in abalone is the culmination of events following activation of differential gene expression and resulting cellular differentiation in the developmentally arrested larvae (Morse D., 1990). In *Haliotis rufescens*, metamorphosis is relatively slow, involving new shell synthesis (which requires mantle cell proliferation and conchiolin protein synthesis (Cariolou & Morse, 1988)) and conversion of the non-feeding larva to a feeding juvenile expressing newly-synthesized digestive enzymes (Spaulding, 1989; D. Spaulding & D.E. Morse, unpub. obs.). In this respect, abalone metamorphosis may differ somewhat from the more rapid transition observed in such species as the nudibranch *Phestilla sibogae*, which can quickly jettison its larval shell and transform other larval structures apparently without extensive new gene expression (Hadfield, 1978). To determine how the recognition of chemical signals from the environment and the chemosensory signal transduction processes described above can regulate the activation of differential gene expression in abalone larvae we have begun to characterize genes coding for cell-specific protein products that must be synthesized in unique cell lines early in the metamorphic process. Our objective is to trace the mechanisms of activation from the environmental signal receptor and transduction cascade to the promoter and enhancer sequences in the DNA controlling the stage-specific and cell-specific transcription of these genes.

Thus far, we have characterized three specific protein families that appear to be developmentally regulated in *Haliotis rufescens*. These include the calcium-binding conchiolin shell peptides (Cariolou & Morse, 1988), the aryl sulphatases (Spaulding, 1989; Spaulding & Morse, 1991), and a unique family of serine proteases (Groppe & Morse, 1989 and unpub. data). The latter two are digestive enzymes produced in specific tissues of the digestive tract, whereas conchiolin peptides are synthesized

specifically in the proliferating cells of the mantle epithelium following metamorphosis. In all three cases, we have found evidence for a multi-gene family; in the case of conchiolin and aryl sulphatase, strong evidence for gene switching at or following metamorphosis has been obtained.

Recently, Jay Groppe has developed techniques which have permitted the molecular cloning and sequence determination of the unique serine protease cDNAs and messengers (Groppe & Morse, 1989). Molecular hybridization studies detecting the protease messenger RNAs demonstrate that the genes for these proteases are very highly expressed, in a very tissue-specific pattern. In addition to providing unexpected information on the apparent importance of protein digestion in nutrition of the growing abalone, these genes and their enzyme products, as well as the genes for the other specialized proteins mentioned above, will serve as the focus for our studies of the mechanisms that control the developmental activation of differential gene expression in a cell-specific manner.

Several years ago, we found that mammalian insulin and growth hormone at low concentrations (10^{-7} M and 10^{-9} M, respectively) specifically and significantly accelerated the rate of early growth in post-metamorphic abalone (Morse, 1984). These observations, and the exciting recent observations of Kawauchi and his colleagues (unpublished data) in Japan, suggest that cell differentiation and early growth in *Haliotis* spp. may be regulated by endogenous neuroendocrine mechanisms homologous at the molecular level to those emerging from contemporary studies in mammals. The potential applications of these findings for accelerating the growth of abalone in cultivation have been discussed (Morse, 1984).

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