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Ecological regulation of development: induction of marine invertebrate metamorphosis

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ABSTRACT In the marine environment a wide range of invertebrates have a pelagobenthic lifecycle that includes planktonic larval and benthic adult phases. Transition between these morphologically and ecologically distinct phases typically occurs when the developmentally competent larva comes into contact with a species-specific environmental cue. This cue acts as a morphogenetic signal that induces the completion of the postlarval/juvenile/adult developmental program at metamorphosis. The development of competence often occurs hours to days after the larva is morphologically mature. In the non-feeding – lecithotrophic – larvae of the ascidian *Herdmania curvata* and the gastropod mollusc *Haliotis asinina*, gene expression patterns in pre-competent and competent stages are markedly different, reflecting the different developmental states of these larval stages. For example, the expression of Hemps, an EGF-like signalling peptide required for the induction of *Herdmania* metamorphosis, increases in competent larvae. Induction of settlement and metamorphosis results in further changes in developmental gene expression, which apparently is necessary for the complete transformation of the larval body plan into the adult form.

KEY WORDS: *ascidian, competence, Haliotis, Herdmania, Reneira*

Introduction

Nearly all metazoan phyla consist of marine species that inhabit the benthos. Many of these animals have a biphasic life cycle that includes a pelagic larva that is microscopic and morphologically distinct from the adult form (Fig. 1). Metamorphosis of the larva into the juvenile/adult typically occurs in concert with or directly following settlement out of the water column. In some marine invertebrates the morphological changes that occur at metamorphosis are pronounced, with a majority of the adult body plan being derived from undifferentiated cells that have been set aside in the larva (e.g. sea urchins; Peterson *et al.*, 1997). In other cases, metamorphosis occurs through a complex set of morphogenetic events that include both a reorganisation of existing larval tissues and differentiation of primordia (e.g. gastropods; Degnan and Morse, 1995; Page and Pedersen, 1998). Larval cells can also transdifferentiate into another type at metamorphosis (e.g. sponges; Leys and Degnan, 2002).

The antiquity of this pelagobenthic life cycle can be inferred by its wide phylogenetic distribution, with species in most pre-bilaterian and bilaterian phyla having pelagic larval and benthic adult phases. While the morphogenetic capacity to direct the development of distinct larval and adult body plans appears to have been present in the first metazoans, the origin and evolution of this life cycle remains an area of debate (e.g. see Haszprunar *et al.*, 1995; Peterson *et al.*, 1997; Nielsen, 1998).

In most cases, the transition from pelagic larva to benthic juvenile is contingent upon contact with an inductive environmental cue (reviewed in Burke, 1983; Morse, 1990; Pawlik, 1992; Rodriguez *et al.*, 1993; Wieczorek and Todd, 1998). As such, the later developmental program (i.e. metamorphosis) in the pelagobenthic life cycle is regulated by and contingent upon specific environmental morphogenetic signals. Detection of an appropriate signal, which is via a species-specific sensory system, typically results in behavioural and morphogenetic changes. This ability to discriminate and respond to signals associated with different benthic substrata apparently ensures that larvae settle in a habitat that is suitable for juvenile growth and survival. Inductive morphogenetic cues are often associated with conspecifics, food sources (e.g.

Abbreviations used in this paper: CEC, columnar epithelial cells; FSW, filtered sea water; PL, postlarvae.

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prey, algae, microbial films or suspensions), or particular benthic substrata (reviewed in Hadfield, 1998; Zimmer and Butman, 2000). Settlement in an unfavourable habitat would significantly reduce the chances of an individual successfully recruiting into a population.

Bioactive chemicals released by or on the surfaces of a wide range of benthic organisms also can inhibit settlement and metamorphosis (reviewed in Hay, 1996). In biodiverse marine ecosystems, such as coral reefs, a larva swimming near the benthos may experience a complex cocktail of chemicals over a small spatial scale. The ability of a competent larva to initiate and complete metamorphosis can be affected by the chemicals that it had previously contacted (e.g. Degnan and Johnson, 1999; Green *et al*., 2002).

We are interested in how marine invertebrate larvae develop the ability to respond to inductive cues (i.e. acquire competence) and how the benthos impacts on larval and postlarval developmental programs. Here we review recent studies that merge developmental gene expression studies with the analysis of settlement and metamorphosis, focussing on data from three disparate groups of marine invertebrates: ascidians, gastropod molluscs and sponges.

Ascidians: a Role for EGF Signalling in Competence and Metamorphosis

Most ascidians have life histories that include a free-swimming lecithotrophic larval stage. Compared to most marine invertebrate larvae, the cellular composition of the ascidian larvae and the fate of larval cells at metamorphosis are extremely well documented (Cloney, 1982; Satoh, 1994; Hirano and Nishida, 1997, 2000). This detailed knowledge greatly facilitates analysis of the effect of environmental factors on settlement and metamorphosis. We are analysing the interactions between endogenous developmental programs and the environment in the tropical ascidian *Herdmania curvata*. This ascidian inhabits the undersides of boulders and ledges on coral reefs (Degnan, 2001). Because of the relatively high rates of spontaneous settlement and metamorphosis displayed by *H. curvata* larvae maintained in 0.2 μm filtered sea water (Degnan *et al*., 1997b), we have been able to identify and characterise both inductive and inhibitory cues (Fig. 2; Degnan *et al*., 1997b; Degnan and Johnson, 1999; Degnan, 2001; Green *et al*., 2002).

Using KCl-elevated seawater or a natural inducer of settlement and metamorphosis, associated with the bryozoan *Margaretta triplex*, we have demonstrated that *H. curvata* requires approximately three hours after hatching to develop competence to settle and metamorphose (Degnan *et al*., 1997b). It appears that induction of metamorphosis occurs by activation of papillae sensory neurons that directly stimulate anterior secretory cells to release a factor that initiates metamorphosis (Degnan *et al*., 1997b; Eri *et al*., 1999).

During the period between hatching and obtaining competence there are marked changes in the prevalence of a large number of transcripts, with a majority of the mRNAs decreasing in abundance (Hinman, 2000; Hinman and Degnan, 2000; Hinman *et al*., 2000; L. Bebell, R. Woods, M. Lavin and B. Degnan, unpublished). During this period overall transcriptional activity appears to be low (Green *et al*., 2002), although the development of competence does require new gene expression (Eri *et al*., 1999; Davidson and Swalla, 2001; Green *et al*., 2002). Axial patterning genes *Hox1, Hox5*, *Pax2/5/8*, *Otx*, *Cdx* and *Evx* are expressed during ascidian embryogenesis and metamorphosis. In the tailbud embryo, these are expressed in a spatially restricted manner along the anteroposterior axis of the nervous system (Katsuyama *et al*., 1995; Hinman, 2000; Hinman and Degnan, 2000; Hinman *et al*., 2000). Examination of *Otx* (*Hec-Otx*) and *Cdx* (*Hec-Cdx*) homeobox genes reveals that there is a repression of neuroectodermal expression and an activation of pharyngeal and gut expression during metamorphosis (Hinman and Degnan, 2001). During the development of competence transcript abundance of these and other transcription factors decreases (Fig. 3), reflecting the differences in developmental state in these two larval stages. Within the first few days of metamorphosis, postlarval expression of these regulatory genes begins

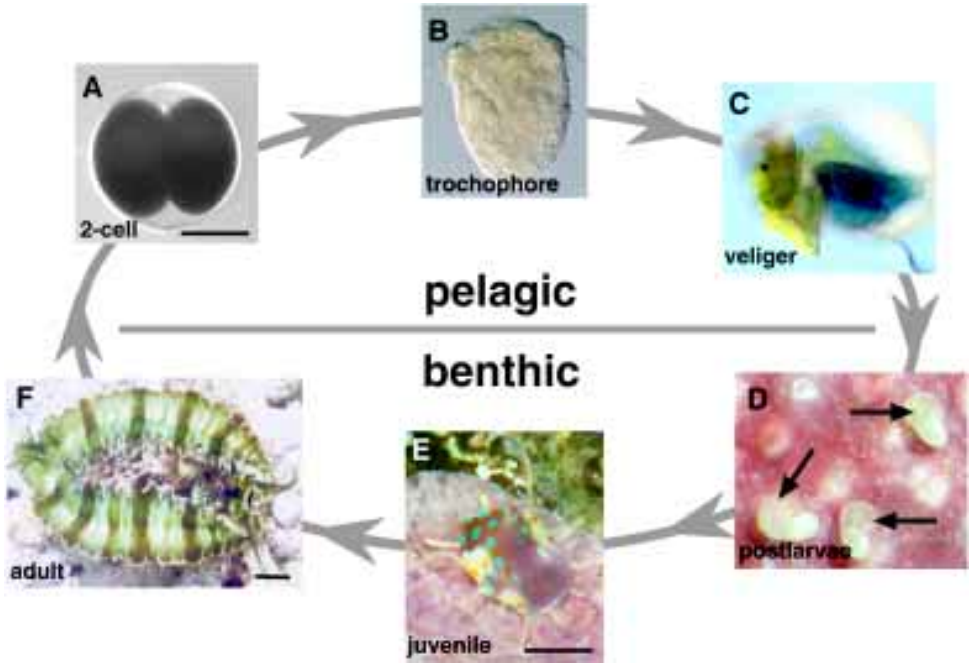

Fig. 1. The pelagobenthic lifecycle of the tropical abalone *Haliotis asinina*. *Female and male* H. asinina *synchronously spawn their gametes into the surrounding sea regularly during the summer (Counihan* et al., *2001).* **(A)** *External fertilization occurs and development ensues; 2-cell embryo.* **(B)** *Trochophore larvae hatch and swim upwards, dispersing via the ocean currents.* **(C)** *After further development, competent veliger larvae swim downwards and probe the benthos.* **(D)** *Upon contact with the appropriate species of nongeniculate coralline algae, they settle and initiate metamorphosis; arrows point to newly settled* H. asinina. **(E)** *The benthic juvenile abalone grow and eventually mature.* **(F)** *Adult* H. asinina. *Scale bar in A, 0.1 mm; in E,F, 10 mm.*

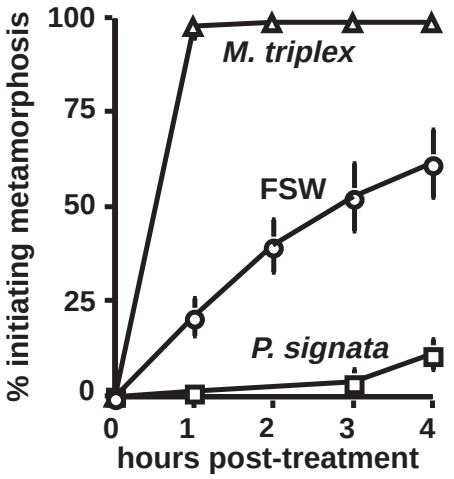

Fig. 2. Benthic invertebrates can induce or inhibit larval settlement. *Exudates from an erect ectoproct,* Margaretta triplex, *and an encrusting ectoproct,* Pleurocodonellina signata, *induce and inhibit, respectively, metamorphosis of competent larvae of the ascidian* Herdmania curvata. *When cultured in 0.2 μm filtered sea water (FSW),* H. curvata larvae *spontaneously settle. See Degnan* et al. *(1997b) for methodological details.*

in concert with the formation of adult tissues from larval primordia (Hinman and Degnan, 2000, 2001). These data suggest that there are distinct embryological and postlarval developmental programs in *Herdmania*.

The EGF Pathway appears to play a Central Role in Ascidian Metamorphosis

Genes encoding EGF-like signaling peptides (*Hemps* and *Ci META1*) and a factor that appears to potentiate EGF secretion (*cornichon*) have been shown to be differentially expressed during larval development and metamorphosis of three ascidians - *Herdmania*, *Ciona* and *Boltenia* (Arnold *et al.*, 1997a; Eri *et al.*, 1999; Davidson and Swalla, 2001; Nakayama *et al.*, 2001). The expression of these genes increases between hatching and the acquisition of competence, strongly suggesting that EGF signaling plays a key role in ascidian metamorphosis. Functional analysis of Hemps also supports this conclusion (Eri *et al.*, 1999). *Hemps* encodes a novel protein which contains four EGF-like repeats, three novel cysteine-rich repeats and a putative secretion signal sequence (Arnold *et al.*, 1997a). Northern blot analysis and immunoblotting demonstrates that *Hemps* transcripts and protein accumulate whilst larvae are developing competence (i.e. after hatching) and then again during the first few hours of metamorphosis (Fig. 4; Eri *et al.*, 1999). *Hemps* mRNA and protein is localized to the papillae and anterior epidermis of competent tadpole larvae (Fig. 4), the region previously shown to be required for induction of metamorphosis (Degnan *et al.*, 1997b). Larvae cultured in the presence of anti-Hemps antibodies do not undergo metamorphosis, although they still retract their papillae (i.e. they undergo the very first stage of settlement and metamorphosis). Incubation with recombinant Hemps protein causes competent larvae to metamorphose at rates significantly faster than the spontaneous rate (Eri *et al.*, 1999).

The Hemps signalling system appears to activate a cascade of gene expression, starting within three hours of induction (Eri *et al.*, 1999; Green *et al.*, 2002). Microarray analysis of gene expression in larvae treated with the neutralizing anti-Hemps antibody reveals that expression of hundreds of genes are affected within the first 3 hours of Hemps-induced metamorphosis (R. Woods, B. Degnan and M. Lavin, unpublished). This morphogenetic event results in global changes in the patterns of expression of regulatory genes (Fig. 3; Hinman and Degnan, 2001) and eventually leads to the destruction of redundant larval tissues and formation of the adult body plan.

Molluscs: Environmental Morphogenetic Signals and Larval Developmental Gene Expression

The range and specificity of settlement cues that induce metamorphosis in molluscan larvae appear to be as varied as the species that respond to them. Some species may be induced by a general biofilm of bacteria and microalgae (Taylor *et al.*, 1998) or detritus (Stoner *et al.*, 1996), while others will only respond to a molecule produced by a specific plant or animal (e.g. Hadfield and Pennington, 1990). In contrast, other species will eventually settle and metamorphose in the absence of any apparent cue (Pechenik and Eyster, 1989; Inestrosa *et al.*, 1993). Assays designed to test the inductive characteristics of various synthetic compounds, molecules and chemicals and to elucidate the chemical nature of environmental inducers have allowed inferences to be made about

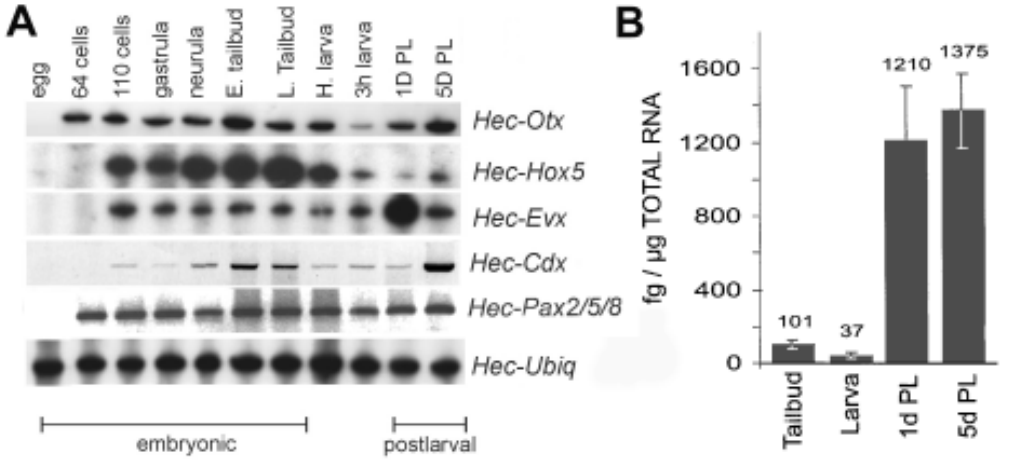

Fig. 3. Developmental expression of homeobox, *Pax* and *ubiquitin* genes in *Herdmania curvata*. (A) *RT-PCR analysis of transcript abundance. Between hatching (H. larva) and the development of competence (3 h larva) transcript abundance changes, with* Hec-Otx, -Hox5 *and* –Pax2/5/8 *transcripts decreasing and* Hec-Evx *transcripts increasing.* **(B)** *Quantitative analysis of* Hec-Otx *transcript prevalence during development shows a significant increase in abundance during metamorphosis (1 and 5 day PL). PL, postlarval stage.*

natural cues (reviewed in Burke, 1983; Morse, 1990; Rodriguez, 1993; Wieczorek and Todd, 1998).

Advances in our understanding of the induction and regulation of molluscan metamorphosis have been most significant in those species that require specific signals to be induced to metamorphose. Abalone – haliotid vetigastropods - are well suited to this type of research as they are fecund and their lecithotrophic larvae are specific in their requirements for induction of metamorphosis, allowing quantitative and molecular analysis of the factors that affect settlement and metamorphosis. Most species that have been investigated are induced to settle and metamorphosis by a signal associated with the surface of specific species of coralline algae (see Morse, 1990). The neurotransmitter γ-aminobutyric acid (GABA) mimics this factor (Morse, 1990) and has allowed a more detailed analysis of the signalling and morphogenetic events that underly these processes (e.g. Trapido-Rosenthal and Morse, 1986). A facilitatory pathway, activated by diamino acids through a G protein coupled receptor, can increase the sensitivity of *H. rufescens* larvae to GABA and its analogues by 100 fold (Trapido-Rosenthal and Morse, 1985; Baxter and Morse, 1992). An advantage for the facilitation of metamorphosis by diamino acids has been suggested to involve selection by competent larvae of nutrient rich waters (containing higher levels of dissolved organic matter) where future food (macroalgae) would flourish.

Another abalone, *Haliotis asinina*, appears to develop in a manner very similar, albeit faster, to *H. rufescens*. It also settles on a specific species of coralline algae. Unlike *H. rufescens*, this tropical abalone has a frequent and predictable spawning cycle (Counihan *et al.*, 2001). We have been investigating the relationship between localised gene expression, competence and metamorphosis in *Haliotis* in order to understand the interplay between the environment and endogenous developmental programs.

Larval Gene Expression in Haliotis

Features of *Haliotis* embryogenesis include spiral cleavage, mesentoblast formation and a trochophore larval stage, as is the case with many other spiralian lophotrochozoans (van den Biggelaar *et al.*, 1997). The *Haliotis* trochophore develops into a veliger larva that is an ensemble of larval and adult characters. For example the adult CNS forms directly from lineage–based embryogenesis and is maintained through metamorphosis. The *Hox* code is activated in the developing CNS in a manner akin to that observed in insects and vertebrates (V. Hinman and B. Degnan, unpublished). After ontogenetic torsion but before metamorphosis, most adult tissues and organs are essentially in their final position. At metamorphosis, larval structures, such as the velum and larval retractor muscle, are destroyed (Degnan and Morse, 1995; Degnan *et al.*, 1997a), and the juvenile/adult morphogenetic program is completed (Degnan *et al.*, 1995). These observations indicate that metamorphosis in *Haliotis* requires environmental morphogenetic signals.

The formation of non-larval structures prior to metamorphosis suggests a degree of 'settlement-preparation' on behalf of the larva. This 'anticipatory' developmental program is characterised by expression of genes during larval development whose products contribute to the formation of structures that will not be functionally deployed until after metamorphosis. Completion of this program is contingent upon contact with an inductive environmental cue. The longer this anticipatory program is running before the competent larva contacts a morphogenetic environmental cue, the more rapid

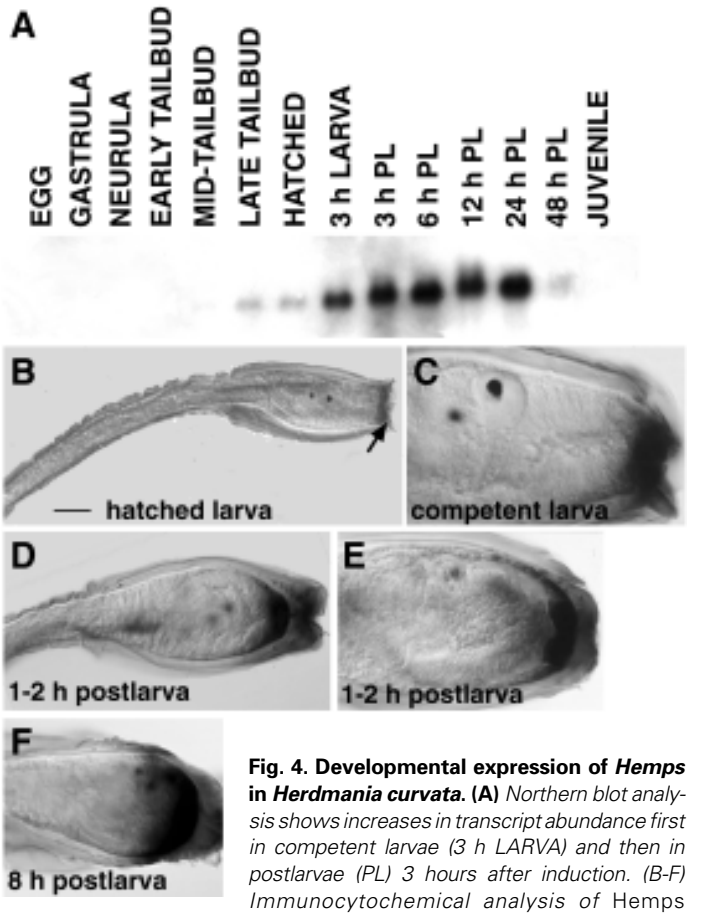

Fig. 4. Developmental expression of *Hemps* in *Herdmania curvata*. (A) *Northern blot analysis shows increases in transcript abundance first in competent larvae (3 h LARVA) and then in postlarvae (PL) 3 hours after induction. (B-F) Immunocytochemical analysis of* Hemps *localisation.* **(B)** *Hatched larva showing* Hemps *expression in palps (arrow); scale bar, 100 μm.* **(C)** *Trunk of competent larva with stronger expression in the palps.* **(D,E)** Hemps *is localised to the anterior epidermis during early metamorphosis (1-2 h post-induction) - tail resorption.* **(F)** *In older postlarvae (8 h post-induction)* Hemps *forms a gradient in ecto- and endodermal cell layers along the anterior-posterior axis.*

metamorphosis appears to occur (i.e older larvae metamorphose quicker; Degnan and Morse, 1995; Degnan *et al.*, 1995).

In the lecithotrophic larva of *Haliotis*, the adult feeding and digestive system is non-functional and poorly formed. Nonetheless, precocious development of components of these systems (e.g. digestive enzymes, radula teeth) begins in the larva prior to obtaining competency (Barlow and Truman, 1992; Degnan *et al.*, 1995). These components continue to accrue as the competent larva ages. However, induction leads to increased rates of development and accumulation of these components. The rate of metamorphosis appears to be contingent on larval age, with older competent larvae metamorphosing more quickly. For example, chymotrypsin gene expression in a set of digestive gland cells destined to become part of the distal intestine begins around the time competence develops. Transcript prevalence slowly increases in un-induced larvae and cells expressing this gene remain in the vicinity of the digestive gland. Contact with an inductive cue results in increases in gene expression and rapid cell migration to the location of the future intestine (Degnan *et al.*, 1995). The rates of change in chymotrypsin gene expression vary greatly with age, with older competent larvae accumulating transcripts much more

rapidly. This may allow older larvae, with reduced yolk reserves, to more rapidly complete the adult morphogenetic program and commence feeding in a shorter time after settlement (Degnan and Morse, 1995).

Further molecular evidence for an anticipatory pathway comes from the tropical nudibranch *Phestilla sibogae*. When competent larvae of this species are exposed to blockers of transcription and translation, metamorphosis can still proceed to an advanced stage (Hadfield, 1998). Accumulation of transcripts and proteins prior to metamorphosis and precocious morphogenesis of juvenile structures allows molluscan larvae to metamorphose quickly (Hadfield, 2000).

Adopting the hypothesis of an anticipatory pathway, we have employed the technique of differential display RT-PCR to identify genes that are up-regulated in competent *H. asinina* larvae and to assess overall patterns of temporal gene expression during *Haliotis* development (for methodological details see Arnold *et al.*, 1997b). Analysis of differential display banding patterns reveal that a high proportion of differentially expressed genes are first activated just prior to or when competency develops in an 'anticipatory' pattern (D. Jackson and B. Degnan, unpublished). We have isolated a number of expressed sequence tags (ESTs) that display this expression profile and spatial expression patterns of some of these ESTs suggest that they are involved in either metamorphic processes or the formation of juvenile/adult structures (D. Jackson and B. Degnan, unpublished).

Sponges: Ancient Pelagobenthic Lifecycle and Origin of Larval Sensory Systems

Sponges are generally considered to have a cellular grade of organization, lacking true tissues (Simpson, 1984). While poriferans lack a true basement membrane that underlies and defines epithelial tissues in all other animals, their cells can be organized into layers which are especially evident in some larvae (Leys and Degnan, 2001, 2002), into areas with specialized functions – e.g. strands for transporting nutrients (Leys and Reiswig, 1998) or into apical or basal layers specialized for attaching to either silica- or calcium carbonate-containing substrates (Bavestrello *et al.*, 1998).

Because this grade of construction has been difficult to relate to that of bilaterians, or even to that of diploblasts, the role of developmental regulatory programs in sponge embryogenesis has been difficult to investigate. Nonetheless, as sponges are the most basal group of metazoans, analysis of sponge larval development and metamorphosis may enable the identification of developmental genes and processes that are shared among all metazoans, helping us to understand the earliest steps in animal evolution.

Larval Development: Gastrulation and Polarity

Most sponges produce short-lived lecithotrophic larvae (Fell, 1983). While four kinds of larvae are found in the Porifera (coeloblastula, amphiblastula, parenchymella and trichimella), the majority of sponge larvae are parenchymellae. Here we focus on this larval type. Parenchymella larvae have a solid core of 5-8 cell types among a collagenous extracellular matrix, and a ciliated columnar epithelium that is the result of gastrulation by cellular migration or delamination (Fig. 5; Efremova, 1997; Boury-Esnault *et al.*, 1999; Leys and Degnan, 2001, 2002). Analysis of developmental genes expressed in the larva and during embryogenesis and metamorphosis strongly suggests that members of many conserved transcription factor gene families play a central role in sponge development (D. Liubicich, V. Hinman, S. Leys, C. Larroux and B. Degnan, unpublished).

Polarity of the parenchymella larva is determined in practice by larval swimming direction, which, in turn, is governed by the development of a region of lightly or heavily pigmented cells that surround and give rise to long cilia that control the speed and direction of swimming (Fig. 5; Woollacott and Hadfield, 1989; Woollacott, 1993; Maldonado and Young, 1996). Micromeres that

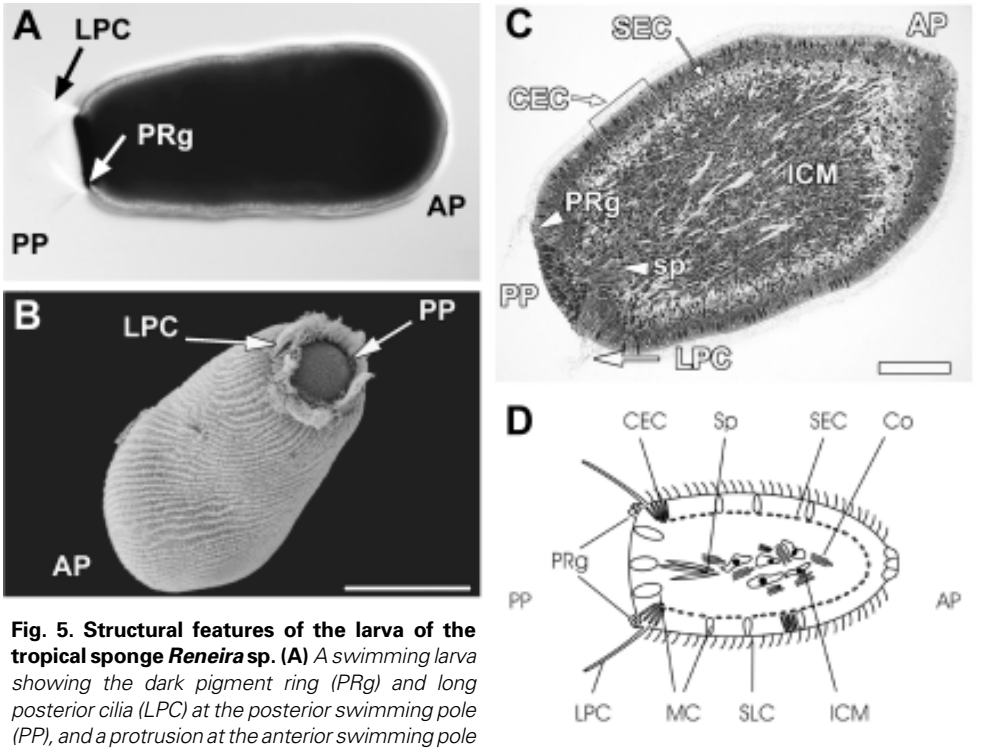

Fig. 5. Structural features of the larva of the tropical sponge *Reneira* sp. (A) *A swimming larva showing the dark pigment ring (PRg) and long posterior cilia (LPC) at the posterior swimming pole (PP), and a protrusion at the anterior swimming pole (AP).* **(B)** *A larva viewed by scanning electron microscopy showing the long posterior cilia and the unciliated posterior pole. Scale bar, 100 μm.* **(C)** *Transmission electron micrograph of a longitudinal section through a 2-hour-old larva. Short (20 μm) cilia arise from columnar epithelial cells (CEC) except at the anterior and posterior pole. Long posterior cilia arise from pigment filled columnar epithelial cells primarily in the anterior portion of the pigment ring. Inside the CECs is a layer of sub-epithelial cells (SEC) that run circumferentially around the larva. Cells in the central region (inner cell mass, ICM) are aligned along the anterior-posterior axis of the larva, with spicules (sp) at the posterior pole. Scale bar, 250 μm.* **(D)** *Schematic of the organization of cell types in the larva. Co, collagen; MC, mucous cell; SLC, short lateral cilia.*

have moved to the periphery of the larva at gastrulation become pigmented and migrate to the posterior pole (Leys and Degnan, 2002).

Sponge cells are highly labile and are capable of regenerating the entire organism from a few cells (Wilson and Penney, 1930; Buscema *et al*., 1980). The capacity of archaeocytes from adult sponges to transdifferentiate into all the other cell types in a sponge (Van de Vyver and Buscema, 1981) is a defining characteristic of this group of animals. We have recently confirmed work by Amano and Hori (1996) that revealed a similar pluripotency of the larval ciliated epithelial cells. During metamorphosis, the columnar epithelial cells lose their cilia and migrate inwards, eventually transdifferentiating into the flagellated choanocytes of the juvenile sponge (Leys and Degnan, 2002).

Settlement Cues

Photosensitivity has been demonstrated in many sponge larvae (reviewed in Wapstra and van Soest, 1987). Maldonado and Young (1996) demonstrated that four species of parenchymella larvae settle preferentially in shaded sites even in the presence of current faster than their speed of swimming. Although they determined that positive rheotaxis was directly correlated with the length of a 'tuft' or ring of long cilia at the posterior swimming end of the larvae, sponges are not known to possess statocysts or sensory receptors that would provide positional information to the larva. Indeed, the absence of neurons and electrical coupling between cells in either adult sponges or larvae (Mackie, 1979; Leys and Mackie, 1997), makes it especially challenging to determine how they are able to sense environmental cues that might influence settlement and metamorphosis.

Recently we were able to demonstrate that the long posterior cilia (LPC) in parenchymellae larvae are highly sensitive to abrupt changes in light intensity (Leys and Degnan, 2001). Rapid increases in light intensity causes the LPC to abruptly straighten, and rapidly reduced light levels cause the LPC to abruptly bend. Experiments with the posterior portions of larvae that were bisected medially support the hypothesis that the sequential response to changes in light intensity of individual ciliated cells in the ring of LPC causes the larva to swim away from sources of bright light (Leys and Degnan, 2001).

Although earlier work suggested that sponge larvae are non-specific in substrate preference for settlement, larvae from the demosponge *Aplysilla* sp. can be induced to settle and undergo metamorphosis by elevated KCl and CsCl (Woollacott and Hadfield, 1996). Experiments to test the effectiveness on sponge larvae of chemical inducers of metamorphosis in other invertebrate groups, demonstrated that exposure to 30 mM KCl promoted metamorphosis of the parenchymella larvae, but only in the presence of a biofilm on the artificial substratum. However, 30 mM CsCl was effective at inducing metamorphosis even in the absence of a biofilmed substratum, but settlement was augmented when CsCl was applied in the presence of a biofilmed substratum. Our own attempts to induce metamorphosis in parenchymella larvae of *Reneira* sp. show that poorly-defined coral rubble and non-geniculate coralline algal substrata induce settlement (Fig. 6), suggesting that these larvae can respond to specific chemical and/or structural features.

Although parenchymella larvae have no identifiable apical sensory organ (or, again, the neurons to co-ordinate a response in the cilia), the anterior end of many parenchymella larvae is formed of large cuboidal cells that contain clear inclusions and single cilium arising from a deep invagination in the cell's surface (Fig. 5; Leys and Degnan, 2001). These cells protrude considerably at the larval anterior end, and in most sponge larvae, are the initial site of attachment during settlement and metamorphosis. These cells have cytological hallmarks that suggest that they may have the capacity to recognise specific environmental cues and coordinate early metamorphosis.

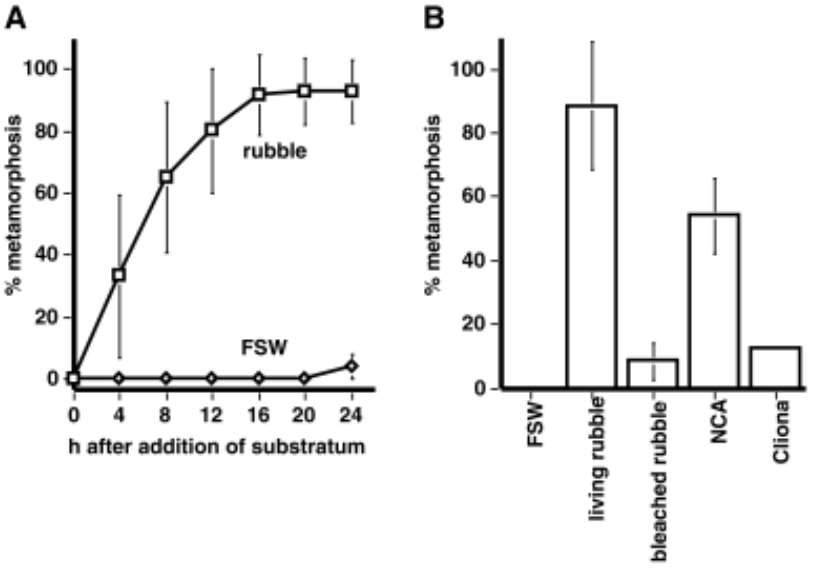

Fig. 6. Substrata-induced settlement and metamorphosis of *Reneira* sp. larvae. *Substrata were introduced to 10 competent larvae in 5 ml of 0.2 µm filtered sea water (FSW); 6 replicates were performed for each treatment (see Degnan* et al.*,1997 for detailed methodologies). These cultures were maintained in the dark until being scored. Rubble were chips of decaying coral skeletons collected from the normal habitat of* Reneira *sp. and consisted of nongeniculate coralline algae (NCA), the boring sponge* Cliona *sp. and undescribed biofilm biota.* **(A)** *Percentage of larvae initiating metamorphosis at different times after being exposed to rubble or FSW.* **(B)** *Percentage of larvae initiating metamorphosis 16 h after being expose to different substrata. Bleached rubble - rubble collected from the natural habitat was placed in 10% bleach overnight and rinsed in FSW for at least 24 h. NCA and Cliona were separated from the rubble before being combined with the larvae.*

Conclusions

Marine invertebrates with a pelagobenthic life cycle provide excellent models for understanding interplay between the environment and endogenous developmental programs. Specific signals associated with inductive substrata activate a cascade of intra- and inter-cellular events that results in a marked change in the developmental state of the animal. This change results in the formation of a new body plan adapted to exist within a different trophic level and habitat. Currently we do not have a detailed understanding of the molecular mechanisms underlying the induction of metamorphosis in this conserved and ancient lifecycle although inroads are being made in the study of

ascidians and gastropod molluscs. Sponges being the most basal metazoan phylum have the potential to shed light on the origin of the pelagobenthic life cycle and the basal sensory systems required to induce settlement and metamorphosis in a favourable location. Analysis of the pleiotropic role of developmental genes during embryogenesis and metamorphosis, in view of the distinct selective forces that sculpt larval and adult body plans, provides an opportunity to gain insight into the role of the environment in the evolution of metazoan body plans. Also, analysis of the environmental control of regulatory genes that may play a crucial role in phase transitions in metazoan lifecycles (e.g. *let-7*; Pasquinelli *et al.*, 2000) may contribute to our understanding of the molecular basis of metamorphosis.

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