Chapter 8
Sea Urchin Larvae as a Model for Postembryonic Development

Andreas Heyland, Nicholas Schuh, and Jonathan Rast

Abstract Larvae are a diverse set of postembryonic life forms distinct from juveniles or adults that have evolved in many animal phyla. Echinoids (sea urchins and sand dollars) generate rapidly developing, morphologically simple, and optically transparent larvae and are a well-established model system supported by a broad array of genomic resources, experimental approaches, and imaging techniques. As such, they provide a unique opportunity to study postembryonic processes such as endocrine signaling, immunity, host–microbe interactions, and regeneration. Here we review a broad array of literature focusing on these important processes in sea urchin larvae, providing support for the claim that they represent excellent experimental study systems. Specifically, there is strong evidence emerging that endocrine signaling, immunity, and host–microbe interactions play major roles in larval development and physiology. Future research should take advantage of sea urchin larvae as a model to study these processes in more detail.

Keywords Thyroid hormones · Nitric oxide · Histamine · Microbiota · IL-17 · Signaling · Phagocytosis · Innate immunity

A. Heyland (✉)
Integrative Biology, University of Guelph, Guelph, ON, Canada
e-mail: aheyland@uoguelph.ca

N. Schuh
Integrative Biology, University of Guelph, Guelph, ON, Canada

Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada
e-mail: nick.schuh@mail.utoronto.ca

J. Rast
Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada

Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, Georgia, USA
e-mail: jprast@emory.edu

8.1 Larval Forms as Experimental Models in Physiology and Development

Larval forms are widespread among animal phyla, and as such they represent an important part of postembryonic development (Carrier et al. 2017). While discussions about what constitutes a larva can be found elsewhere (Bishop et al. 2006a; Carrier et al. 2017; Hodin et al. 2010; McEdward 2000; McEdward and Janies 1993), they encompass several unique characteristics, which are further discussed in this chapter (Figs. 8.1 and 8.2): (1) larvae are morphologically, physiologically, and developmentally separate entities from embryos, juveniles, and reproductive adults within the life cycle of an organism; (2) larval life cycles can be contrasted with direct life cycles, where embryos transition directly into a juvenile form; (3) metamorphosis is an intricate part of the larval life cycle during which the larva is transformed into the juvenile; and (4) the transition from larva to juvenile is frequently accompanied by an ecological transition (settlement), as juveniles inhabit different ecosystems from the larvae. Here we argue that larvae present a unique opportunity to study postembryonic development, especially among marine invertebrate phyla, which feature an impressive diversification of form and function. Technically, larvae have the advantage over adult or juvenile forms in that their morphologies are relatively simple and therefore amenable to rigorous experimentation. In this review, we provide a summary of research focusing on hallmarks of postembryonic development. Research on echinoderm larvae, especially sea urchin larvae, has contributed to our understanding of the evolution of endocrine and neuroendocrine signaling systems, immunity, asexual reproduction, and regeneration.

Fig. 8.1 Larva of the sea urchin *Strongylocentrotus purpuratus* after 3 weeks of development in the lab. Specimen features eight larval arms with skeletal rods (Sk) and a developing juvenile rudiment (R). Also visible are pigment cells (PC) dispersed over the surface of the larva. The ciliated band (CB) surrounds the entire larval body and is involved in transporting food particles to the larval mouth (M). Food particles are digested in the larval stomach (S)
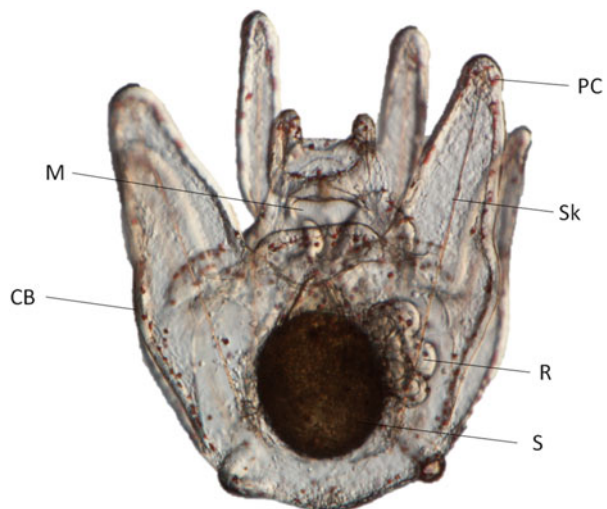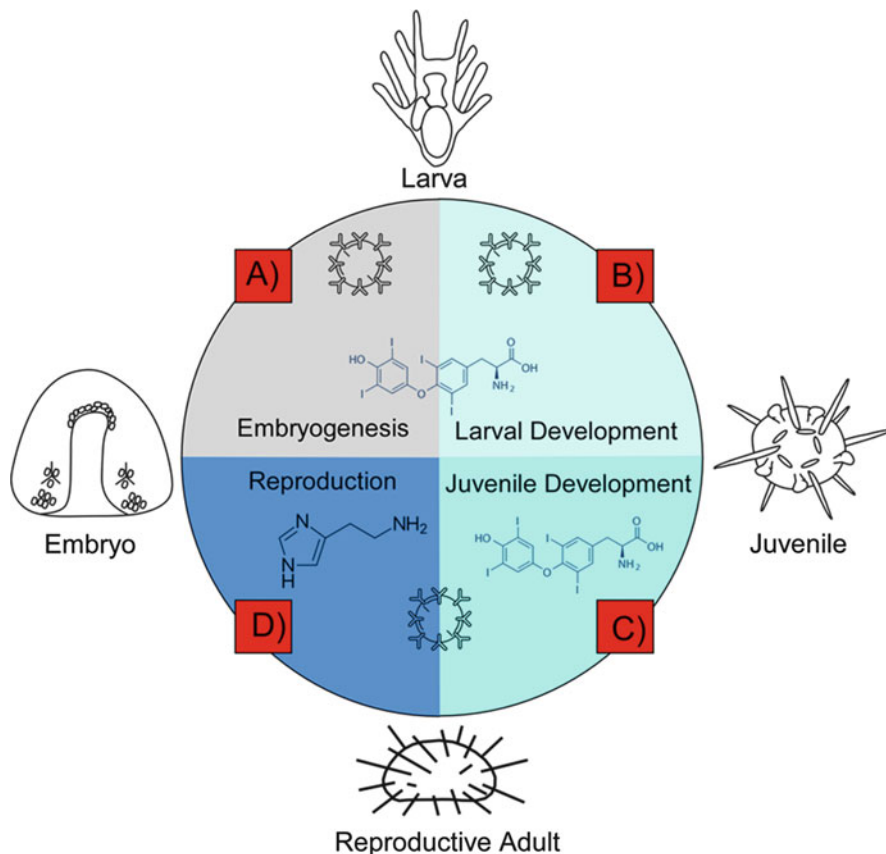
Fig. 8.2 Schematic of an indirect sea urchin life cycle with major life history stages—reproductive adult, embryo, larva, and prereproductive juvenile. The postembryonic period is indicated in blue tones and consists of larval development (the focus of this chapter), juvenile development, and reproduction. Embryogenesis (**a**) initially results in a larval form, which metamorphoses, (**b**) after a sometimes extensive period of swimming and feeding in the plankton. Settlement defines the transition from the plankton to the benthos. Juveniles require months to years, depending on the species, to reach reproductive maturity (**c**). Reproduction occurs by free-spawning gametes (sperm and eggs) into the water column (**d**). This chapter focuses on the role of endocrine signaling, immunity, and regeneration in postembryonic development. The molecules indicated in the figure are histamine (HA) and thyroxine, a thyroid hormone (TH), respectively. Circles with receptors represent immune system function

8.2 A Framework for Hormonal Action in Larval Development and Metamorphosis of the Sea Urchin

Research on the involvement of hormones in echinoderm life histories has led to the emergence of a conceptual framework of their functions and interactions in larval development and metamorphosis (Fig. 8.3). While their detailed molecular
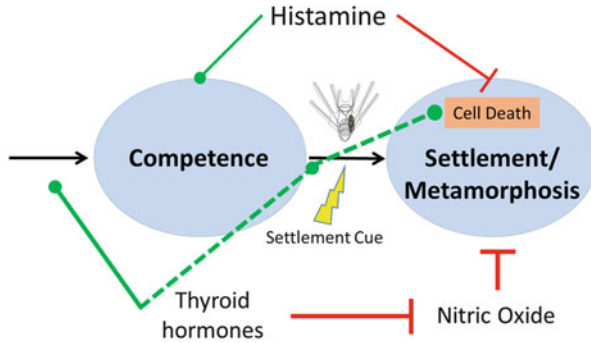
Fig. 8.3 Conceptual diagram of hypothesized endocrine regulatory network underlying larval development and metamorphosis in the purple sea urchin *S. purpuratus*. Histamine (HA), thyroxine (T4), and nitric oxide (NO) all have been shown to regulate larval development and metamorphosis. The function of these signaling molecules may converge on metamorphic competence, the stage immediately preceding settlement, via the regulation of programmed cell death (apoptosis). Round ends indicate activation while line ends indicates inhibition. For references see text. Regular lines indicate mechanisms that have been extensively tested. Broken lines indicate hypothesized mechanism for which only preliminary or no evidence exists to date

mechanisms remain elusive in many instances, their involvement in critical larval functions, such as skeletogenesis and programmed cell death (PCD), makes them attractive candidates for functional physiological studies and offers insight into the evolution of endocrine signaling. Here we will briefly discuss the current state of knowledge on the hypothesized endocrine and neuroendocrine regulatory network underlying larval development and metamorphosis (Fig. 8.3).

8.2.1   The Hypothesized Endocrine and Neuroendocrine Network of Sea Urchin Larval Development

Thyroid hormones (THs) are regulators of animal development and physiology. For example, these hormones are both necessary and sufficient for the metamorphic transition from a tadpole to an adult frog (for review see Tata 1996). Similarly, THs have been shown to regulate metamorphosis and other life history transitions in echinoderms, urochordates, cephalochordates, and mollusks (for review see Taylor and Heyland 2017). The function of THs (specifically T4) in echinoids (sea urchins and sand dollars) include, but is not limited to the acceleration of development, specifically juvenile skeletogenesis, and reduction of larval arm length (Flatt et al. 2006; Heyland and Hodin 2004; Heyland et al. 2005, 2009; Heyland and Moroz 2006; Heyland et al. 2001, 2004) (Fig. 8.2). THs are endogenously synthesized from incorporated iodine in the purple sea urchin *S. purpuratus* (Miller and Heyland 2010, 2013), and this endogenous synthesis can be complemented by the exogenous hormone sources such as from the consumed phytoplankton (Heyland et al. 2006,

2009; Taylor and Heyland 2017). In fact, iodine incorporation into sea urchin epithelial cells occurs via conserved mechanisms, shared between plants and animals, which are distinct from TH synthesis mechanisms in mammals and other vertebrates (Miller and Heyland 2013). Finally, it appears that THs activate programmed cell death (PCD) in the arm tips of competent sea urchin larvae, promoting the transition from larva to juvenile (Taylor et al. in review). Because of this compelling evidence for TH function in invertebrates, sea urchin larvae provide an excellent model to study underlying mechanisms.

Histamine (HA) is an essential signaling molecule across prokaryotes and eukaryotes (Kyriakidis et al. 2012), with widespread functions ranging from neuronal signaling to immunity in animals (Nassel 1999; Roeder 2003; Tabarean 2016). In sea urchins, it is functionally involved in the formation of the fertilization envelope (Leguia and Wessel 2006) and has been identified as a natural settlement cue in several Australian echinoid species (Swanson et al. 2004, 2012). Recent evidence also suggests that it functions as a regulator of metamorphic competence (Lutek et al. 2018; Sutherby et al. 2012). Specifically, these studies show that HA levels and sea urchin histamine receptor 1 (suH1R) expression increase during larval development, peaking at metamorphic competence. Furthermore, the sea urchin genome contains two putative sequences for histamine receptors, one of which we recently functionally characterized (Lutek et al. 2018). This work suggests that suH1R is expressed in neuronal clusters in the mouth region of the larva and inhibits PCD in competent larvae.

In addition to TH and HA, nitric oxide (NO) has been shown to signal in metamorphically competent sea urchin larvae (Fig. 8.2). Specifically, endogenously released NO functions as an inhibitor of settlement (Bishop and Brandhorst 2001a, b). Furthermore, this pathway appears to interact with TH signaling in TH treatment of competent sea urchin larvae results in the reduction of NOS (nitric oxide synthase—NO synthesis enzyme)-positive neurons (Bishop et al. 2006b). These results suggest that TH may function antagonistically to NO signaling in competent larvae. Still, the functional relevance of this interaction requires further studies, along with a detailed analysis of the interactions between NO, TH, and HA.

8.2.2   Putative Function of TH Signaling in Skeletogenesis

The formation of a skeleton in postembryonic development is common to many animal groups. Sea urchin larvae prominently and beautifully feature a calcareous endoskeleton (Fig. 8.1), which provides a structure for the larval arms (for review see Decker and Lennarz 1988). The formation of the larval skeleton from a subset of primary mesenchyme cells (PMCs) in the ventrolateral cluster is the result of a complex gene regulatory network (GRN) involving transcription factors and signaling systems, which have been studied in detail in *S. purpuratus* and related species (reviewed in McIntyre et al. 2014). Importantly, the PMC GRN is a target of activated (phosphorylated) mitogen-activated protein kinase (MAPK, ERK 1/2)

signaling, and the transcription factors Ets1 and Alx1 are key mediators of MAPK inputs (McIntyre et al. 2014). Intriguingly, genes involved in this mechanism are present in several other echinoderm groups lacking skeletons, suggesting that the evolution of larval skeletons involved the activation of the entire module by specific signaling factors. One such candidate is vascular endothelial growth factor (VEGF), which is essential for proper formation of larval spicules in the sea urchin embryo (Koga et al. 2016).

The function of the larval skeletons is intimately linked to swimming and feeding performance and therefore dependent on environmental input. For example, sea urchin larval arms grow longer when food availability is limited (Heyland and Hodin 2004; Miner 2005; Miner and Vonesh 2004; Strathmann et al. 1992). This response effectively increases the length of the ciliated band and allows the larva to capture more food particles, hence compensating for the lack of food in the environment (Hart and Strathmann 1994). In addition to the transcription factors and signaling molecules involved in larval skeleton formation, several genes with specific functions in skeletal elongation have been identified in the sea urchins *S. purpuratus* and *Lytechinus variegatus*, including the transmembrane protein P16, SM50, MSP130, advillin, and carbonic anhydrase. These genes are important candidates for analyzing the molecular basis of arm elongation in sea urchins under low food conditions (McAlister and Miner 2018).

Neurosecretory mechanisms have been shown to be involved in the regulation of arm length. For example, Adams et al. (2011) demonstrated that dopamine is involved in the process that induces reduced feeding structures under abundant food conditions. Furthermore, Heyland and Hodin (2004) have shown that increased levels of TH, which potentially originate from algae, can produce a phenotype similar to the high food phenotype (i.e., shorter arms in high food conditions in comparison to low food conditions). These findings suggest that receptors detecting these signals can directly or indirectly modulate the GRN underlying skeletogenesis and apoptosis (see below). However, the link between these signaling events and skeletogenesis remains largely unknown. Note that recent findings also suggest a link between this plastic response and changes in the larval microbiome (further discussed below).

8.2.3 Function of Hormonal Signaling in Programmed Cell Death (PCD)

PCD plays a central role in both normal development and disease. In animals with larval stages, PCD is linked to postembryonic development and metamorphosis (Tata 1996). Specifically, hormones such as TH in vertebrates and ecdysteroids in insects are major regulators of this process. For example, THs regulate PCD in frog tail resorption (Ishizuya-Oka et al. 2010), and ecdysteroid action is a major regulator of PCD in insect metamorphosis (Buszczak and Segraves 2000). The complement of

PCD-related proteins (i.e., caspases, CARD, Apaf, BIR, Bcl domain proteins, and others) in basal animal groups and sea urchins is more similar to vertebrates (in comparison to model systems such as fruit flies and nematodes where PCD has been historically studied), indicating that these cell death mechanisms may have evolved early in animal evolution. Hence, we can gain important insights into the mechanisms of PCD by studying it in larval development of the sea urchin (Robertson et al. 2006).

Recent research on TH and HA signaling strongly suggests a link between endocrine signals and PCD in sea urchin larvae. In addition to the work outlined above on HA signaling regulating PCD in competent larvae, our data also suggest that T4 signals upstream of the skeletogenesis GRN in PMCs (Taylor and Heyland 2017; Taylor et al. in review). Part or all of this interaction may be responsible for the observed shortening of skeletal elements in response to T4 treatment. This hypothesis requires more detailed investigations into the regulation of various proteases critical for PCD, such as caspases and matrix metalloproteinases (MPPs), by THs. Cell death is closely linked to changes in the extracellular matrix (ECM). Specifically, caspases as well as MPPs have been shown to affect cell–cell interactions in PCD (Herren et al. 1998). More importantly, MMPs have been identified as the main players in PCD during the metamorphic transition of frogs and insects, and their function is regulated by metamorphic hormones (THs and ecdysteroids, respectively) (Mathew et al. 2010; Page-McCaw et al. 2007). While the functional role of these important PCD regulators in sea urchins remains largely unknown, previous work has identified MMPs as regulators of sea urchin skeletogenesis (Ingersoll and Wilt 1998), and several forms of MMPs are expressed during embryogenesis (Ingersoll and Pendharkar 2005). Furthermore, these proteases may play a functional role in mutable collagenous tissues, a type of tissue, which has received considerable attention in echinoderms due to its ability to undergo reversible changes in mechanical properties (Ribeiro et al. 2012).

8.3   Sea Urchin Larvae as an Experimental Model for Bacterial Colonization and Host-Microbe Interactions in Postembryonic Development

Developmental biology has traditionally carried the view that multicellular organisms are discrete, individual entities. Advances in microbiology and sequencing technology have led to the emergence of a "holobiont" view: multicellular eukaryotes are hosts to vast microbial ecosystems continuous with the surrounding environment, forming indivisible biological systems (Gilbert et al. 2012; McFall-Ngai et al. 2013). These microbial residents participate in most (if not all) physiological processes, but much remains to be understood about interactions between microbes and developing hosts. The life history and habitat diversity found among echinoderms (e.g., tropical, temperate, arctic; benthic, planktonic, or brooded) could be

useful in delineating how the acquisition of distinct microbiota interacts with environment, feeding, development, and more, in the context of similar and dissimilar host genomes (Williams and Carrier 2018). This section will review the small but promising literature on bacterial microbiology in sea urchin larvae, as well as future directions for the field.

Previous research has isolated bacteria from adult echinoids using traditional culture techniques (Table 8.1). Unkles (1977) demonstrated that *Echinus esculentus* guts and cavity fluid were associated with *Gammaproteobacteria* (*Pseudomonas*, *Aeromonas*, and *Vibrio*) and some *Bacteroidetes* (specifically *Flavobacteria*), while the peristomial membrane had a distribution intermediate between those tissues and the surrounding environment. More recently, Hakim et al. (2015, 2016) used next-generation 16S sequencing to characterize the gut microbiome of adult *L. variegatus*, revealing not only distinct bacteria in urchin tissue and the environment, but also distinct communities associated with different parts of the gut. Pharynx and gut were dominated by *Epsilonproteobacteria* relative to the digesta, which were primarily colonized by *Gammaproteobacteria*, other *Proteobacteria*, and *Bacteroidetes* (including *Flavobacteria*). The surrounding seagrass and ocean water were relatively enriched for *Cyanobacteria* and *Alphaproteobacteria*, respectively. *Strongylocentrotus intermedius* and *Strongylocentrotus nudus* gonads were reported to be exclusively colonized by *Bacteroidetes*, although this may be a result of the *Bacteroidetes* (specifically *Tenacibaculum*)-focused 16S PCR primers used (Balakirev et al. 2008). Regardless, distinct relative abundances of *Flavobacteria* and *Sphingobacteria* were associated with the "usual" and "gray" *S. intermedius* phenotypes, but not with their depth below sea level, which may indicate a functional role associated with these phenotypes (Balakirev et al. 2008). Kiselev et al. (2013) isolated 22 strains (predominantly *Gammaproteobacteria* but also *Bacteroidetes* and *Firmicutes*) of 9 genera (*Aliivibrio*, *Bizionia*, *Colwellia*, *Olleya*, *Paenibacillus*, *Photobacterium*, *Pseudoalteromonas*, *Shewanella*, *Vibrio*) from adult *Strongylocentrotus pallidus* cavity fluid and exposed them to congeneric *S. intermedius* embryos. *Aliivibrio*, *Vibrio*, *Colwellia*, *Shewanella*, and *Photobacterium* spp. slowed or arrested growth by 3 days postfertilization; the remaining strains had no noticeable effect (Kiselev et al. 2013). Conversely, some of these strains induced apparent increases in pigment cell numbers (also see below) and viability, which may imply a degree of bacteria-immune synergy (Kiselev et al. 2013). These studies illustrate several recurring themes: adult echinoids in diverse habitats (Clyde Sea for *E. esculentus*, Gulf of Mexico for *L. variegatus*, Sea of Japan for *S. intermedius* and *S. nudus*, Sea of Okhotsk for *S. pallidus*) are similarly colonized by culturable *Proteobacteria* and *Bacteroidetes* relative to their diets and surrounding environments. Guts and coelomic fluid are associated with *Gammaproteobacteria* (particularly the genera *Colwellia*, *Pseudoalteromonas*, *Pseudomonas*, *Shewanella*, and *Vibrio*) and *Bacteroidetes* (particularly class *Flavobacteria*). These taxa are similarly overrepresented using culture-independent 16S sequencing approaches (Hakim et al. 2015, 2016) and may be associated with an array of functions (De Ridder and Foret 2006; Williams and Carrier 2018).

Table 8.1 Bacterial phyla associated with adult and larval sea urchins

Stage	Tissue(s)	Sea urchin species	Method	Bacterial phyla	Reference
Adult	Gut, cavity fluid, peristomial membrane	*Echinus esculentus*	Culture, morphology, staining	*Bacteroidetes Proteobacteria* (γ) Other	Unkles (1977)
Adult	Gonad	*Strongylocentrotus intermedius*	Culture, 16S	*Bacteroidetes*	Balakirev et al. (2008)
Adult	Gonad	*Strongylocentrotus nudus*	Culture, 16S	*Bacteroidetes*	
Adult	Cavity fluid	*Strongylocentrotus pallidus*	Culture, 16S	*Bacteroidetes Firmicutes Proteobacteria* (γ)	Kiselev et al. (2013)
Adult	Pharynx, gut tissue, gut digesta, feces	*Lytechinus variegatus*	16S	*Bacteroidetes Proteobacteria* (α, β, γ, δ, ε) Other	Hakim et al. (2015) and Hakim et al. (2016)
4-arm larva	–	*Strongylocentrotus purpuratus*	Culture, 16S	*Actinobacteria Bacteroidetes Cyanobacteria Proteobacteria* (α, β, γ) Other	Ho et al. (2017)
4-, 6-, 8-arm larvae	–	*Strongylocentrotus purpuratus*	16S	*Bacteroidetes Cyanobacteria Planctomycetes Proteobacteria* (α, β, γ) *Verrucomicrobia* Other	Carrier and Reitzel (2018)
4-, 6-, 8-arm larvae	–	*Mesocentrotus franciscanus*	16S	*Bacteroidetes Cyanobacteria Planctomycetes Proteobacteria* (α, β, γ) *Verrucomicrobia* Other	
4-, 6-, 8-arm larvae	–	*Strongylocentrotus droebachiensis*	16S	*Bacteroidetes Cyanobacteria Planctomycetes Proteobacteria* (α, β, γ) *Verrucomicrobia* Other	

Among echinoderms, fewer studies have focused on endogenous microbiota of sea urchin larvae (Table 8.1). Ho et al. (2017) characterized 89 bacterial strains associated with 4-arm feeding *S. purpuratus* larvae; the vast majority were *Gammaproteobacteria*, notably genera *Alteromonas*, *Colwellia*,

Pseudoalteromonas, *Pseudomonas*, and *Vibrio*. Representatives of the *Actinobacteria*, *Bacteroidetes*, and *Cyanobacteria* were also present. Repeated isolation experiments and comprehensive 16S sequencing corroborate these results: larvae are associated primarily with *Proteobacteria*, *Bacteroidetes*, and *Cyanobacteria*, distinct from the communities present in their surrounding environment (Ho et al. 2017). Culturable diversity turned out to be lower than that revealed with 16S sequencing, and associated diversity was lower when larvae were raised in the laboratory than in the field (unpublished data). *Cyanobacteria* and some types of *Proteobacteria* may be especially difficult to culture, at least using traditional methods. However, much of the microbiota appears to be relatively stable, even when larvae are raised in artificial seawater, suggesting that the host–microbe interactions underlying these associations could be studied in the lab.

Microbial transmission can occur in several dimensions, (vertical vs. horizontal, parental vs. exogenous, larva to adult, etc.), and many larvae-associated taxa overlap with taxa previously identified in adults, raising questions about the dynamics of the host–microbiota relationship in echinoid development and life history. Likely compartments for bacterial colonization are the digestive tract and the ectoderm, either its external surface or the subcuticular space (Cameron and Holland 1983, 1985). Subcuticular bacteria have been observed in adult echinoids (De Ridder and Foret 2006; Holland and Nealson 1978) and some echinoderm larvae (Cameron and Holland 1983, 1985; Holland and Nealson 1978; Cerra et al. 1997). Still, to our knowledge, this phenomenon has not been observed in sea urchin larvae. 16S fluorescent in situ hybridization reveals that larval bacterial load is miniscule until about 3–4 days of development, coinciding loosely with the onset of feeding, after which the vast majority of associated bacteria are present in the mouth and gut lumen (unpublished data) (Smith et al. 2008). Small numbers of bacteria are sometimes associated with the external ectoderm, but are rarely or never seen in the cuticle. Blastocoelar bacteria are generally only seen in injured or dead larvae, or under experimental conditions, where they are rapidly cleared by phagocytes (Ho et al. 2017).

In the face of environmental variation, sea urchin larvae acclimate physiologically by differentially expressing genes as well as adjusting phenotypic traits (see also discussion of phenotypic plasticity above). For many other animal taxa, acclimation is a combined physiological as well as microbial response, whereas in the latter, the community composition and their relative proportions of the host-associated microbiome are restructured. In a recent study, Carrier and Reitzel (2018) tested the hypothesis that feeding-induced plasticity in urchin larvae (a host response to environmental variation) correlates with a phenotype-specific microbiome, a proxy for hologenomic acclimation. In their study, they find support that across a phenotypic continuum, urchin larvae associate with phenotype-specific microbiome, of which was independent of dietary state (i.e., food quantity), developmental stage, and ecological drift. This pattern was convergent between *S. purpuratus*, *S. droebachiensis*, and *Mesocentrotus franciscanus* larvae, even though they associated with species-specific microbiomes. Furthermore, the authors find support that the magnitude that phenotypic plasticity is expressed strongly

correlates with the degree to which the larval host differentially associates with the microbiome. Collectively, these data could represent an additional layer of adaptation to changing environmental feeding conditions, reinforcing the view that echinoid larval microbiota are associated with feeding.

Several bacterial taxa found in sea urchin larvae are consistently associated with settlement and metamorphosis and have been hypothesized to be functionally involved in the induction of settlement (Table 8.2). For example, Huggett et al. (2006) isolated culturable bacteria from coralline algae and demonstrated settlement of the sea urchin *Heliocidaris erythrogramma* in response to biofilms produced by *Pseudoalteromonas*, *Vibrio*, *Shewanella*, *Photobacterium*, and *Pseudomonas* strains. Settlement is significantly reduced or blocked in *H. erythrogramma* and *Tripneustes gratilla* sea urchin larvae, as well as *Acanthaster planci* sea star larvae, when inducing macroalgae or rocks are cleared of their associated bacteria (Dworjanyn and Pirozzi 2008; Huggett et al. 2006; Johnson et al. 1991). *A. planci* larvae were observed by electron microscopy to settle and metamorphose only on regions colonized by bacteria, which included *Alteromonas* or *Pseudomonas*, and *Vibrio* (Johnson et al. 1991). Mos et al. (2011) tested a broad set of potential settlement cues and concluded that seaweeds with their associated bacteria were the most effective settlement cue for *T. gratilla*. Furthermore, *H. erythrogramma* settlement was found to be strongly correlated with the diversity of *Amphiroa anceps* and *Corallina officinalis* macroalgae-associated bacterial communities, but no significant relationship was observed for *Holopneustes purpurascens*, which settled consistently in response to algae-derived HA (Nielsen et al. 2015). Together, these results suggest that competent larvae may respond to specific bacterial strains or at least algal–bacterial consortia, depending on the echinoid and bacterial species involved. Further study is required to elucidate the molecular cues associated with bacteria and algae that regulate this process and the pathways of bacterial transfer and colonization between food sources, adults, larvae, and juveniles.

8.4 Sea Urchin Immunity in Larval Development

Echinoderms have had an important place in the history of immunology ever since Ilya Metchnikoff first described phagocytosis in asteroid and echinoid larvae (Metchnikoff 1893; Tauber 2003). Though the field of immunology has been primarily focused on vertebrate systems, with strong contributions from insects, there is every reason to believe that sea urchins have need of a complex immune system, and much research has confirmed that this is the case. Sea urchins are relatively long-lived invertebrates, and some species are among the animals with the longest lifespans (Ebert and Southon 2013). Marine environments are dense microbial ecosystems full of potential pathogens. Prolonged life expectancy in such environments implies broad and robust antimicrobial immunity (Bodnar and Coffman 2016; Buckley and Rast 2012). Embryos and larvae must survive to metamorphic competence in similar environments as their adults, suggesting a

Table 8.2 Bacterial genera associated with induction of larval settlement

Echinoderm species	Bacterial phyla	Bacterial genera	Induction cue	Method	Reference
Acanthaster planci	*Proteobacteria* (γ)	*Alteromonas/Pseudomonas* *Vibrio*	Live bacteria associated with the macroalgae *Lithothamnium pseudosorum*	Culture, morphology, biochemistry	Johnson et al. (1991)
Holopneustes purpurascens	*Proteobacteria* (γ)	*Photobacterium* *Thalassomonas* *Vibrio*	Monoculture biofilms	Culture, 16S	Swanson et al. (2006)
Heliocidaris erythrogramma	*Actinobacteria*, *Bacteroidetes*, *Proteobacteria* (α,γ)	*Alteromonas* *Aestuariibacter* *Cytophaga* *Micrococcus* *Thalassomonas* *Photobacterium* *Pseudoalteromonas* *Pseudomonas* *Shewanella* *Vibrio*	Monoculture biofilms	Culture, 16S	Huggett et al. (2006)
Tripneustes gratilla	*Proteobacteria* (γ)	*Pseudoalteromonas* *Thalassomonas* *Vibrio*	Live bacteria, bacterial supernatants, mixed biofilms	Culture, 16S	Mos et al. (2011)

need for broad immune competence in these stages as well. Today, there is much work describing the immune systems of adult echinoids, and immunity in other life history stages are beginning to receive renewed attention (reviewed in Smith et al. 2018). This work has been greatly aided by access to genome sequences from the purple sea urchin and several other echinoderms, which reveal the presence of a broad suite of genes encoding innate immune receptors, signals, and effectors (Buckley and Rast 2017; Hibino et al. 2006; Sea Urchin Genome Sequencing et al. 2006). Although sea urchins do not have the same adaptive immune system that characterizes vertebrate immunity, they share developmental and regulatory systems with vertebrates that are also important in the larva. This section will review literature and emerging perspectives on sea urchin larval immunity and address potential future directions for the field.

Several mesenchymal cell types in sea urchin embryos and larvae are capable of rapid recognition and phagocytosis of foreign particles in the blastocoel as demonstrated in both *L. variegatus* (Silva 2000) and in *S. purpuratus* (Ho et al. 2017). Embryos and larvae also express humoral immune effectors such as complement factors (Shah et al. 2003) and antibacterial peptides (Li et al. 2014). The development and morphology of several larval mesenchymal cell lineages have been well described (Gibson and Burke 1987; Ruffins and Ettensohn 1993; Tamboline and Burke 1992), and a subset of these was recently shown to have specific immune functions (Buckley et al. 2017; Buckley and Rast 2017; Ho et al. 2017). In the purple sea urchin embryo, two populations of secondary mesenchymal cells are specified at the blastula stage, which give rise to several types of immune cells in the larva. These include five cell categories (Fig. 8.4): (1) *pigment cells* contain red granules and are motile with several dendritic cell-like pseudopodia (Gibson and Burke 1987; Ho et al. 2017). In unchallenged larvae, these cells are closely associated with the ectoderm and form clusters near the apex and arm tips. Upon bacterial challenge, they become rounded and hypermotile and often enter the blastocoelar space (Ho et al. 2017). (2) *Globular cells* are large cells filled with round vesicles that impart an unusual, popcorn-like appearance. Expression of a lineage-specific membrane attack complex/perforin family (MACPF)-like factor suggests possible effector function for these cells (MACPFA2 Ho et al. 2017). Several motile globular cells typically patrol the blastocoel, consistent with an immune surveillance function. (3) *Filopodial cells* include the primary phagocytes of echinoid larvae. This heterogeneous cell category possesses small, rounded cell bodies and extended long (10–50 μm) cytoplasmic projections, forming a dynamic intercellular network connecting the basal surfaces of the gut and ectodermal epithelia throughout the blastocoelar space (Ho et al. 2017). Upon bacterial challenge, a subpopulation of filopodial cells activates the echinoid-specific antimicrobial effector SpTransformer (185/333). (4) *Ovoid cells* are elliptical phagocytes that appear when bacteria are introduced into the blastocoel. These cells are not observed in unchallenged larvae. The developmental origin of these cells is not known but they may be an activated form of filopodial cell (Ho et al. 2017). (5) *Amoeboid cells* are small, highly motile comma-shaped cells (Gibson and Burke 1987; Ho et al. 2017). Their role in
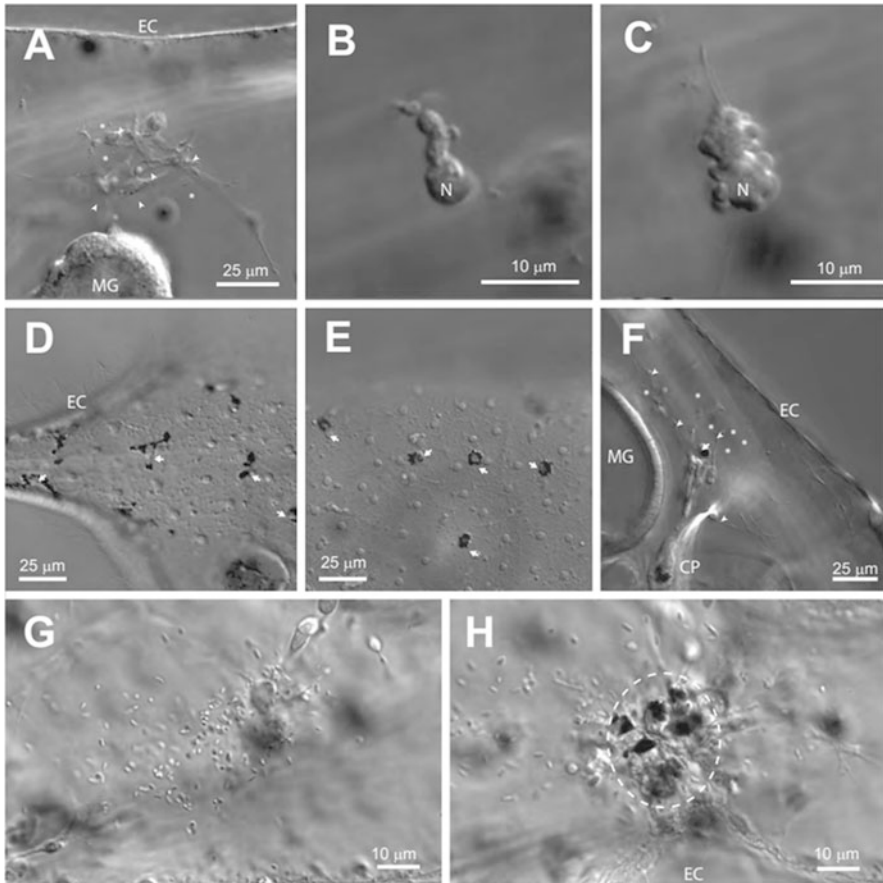
Fig. 8.4 Cellular immune response in the sea urchin larva. (**a**) A complex of several filopodial cells phagocytosing injected *Vibrio diazotrophicus* (*V.d.*). (**b**) An amoeboid cell in the blastocoel. (**c**) A globular cell with several filopodia in the blastocoel. (**d**) Pigment cells apposed to the ectoderm just after injection of *V.d.* showing inactivated extended morphology. (**e**) Pigment cells several hours after injection showing rounded activated morphology. (**f**) A group of interacting filopodial cells and pigment cells at site of injected *V.d.* (**g**) Site of injection 5 min after introduction of *Vibrio lentus*. Some cells have already begun to accumulate in area of bacteria. (**h**) Same region after 30 min. A large complex of several cell types has accumulated including filopodial cells, globular cells, pigment cells, and other uncharacterized cell types (surrounded by dotted line). *CP* coelomic pouch, *EC* ectoderm, *MG* midgut, *N* nucleus; arrow, pigment cell nucleus; arrowhead, filopodial cells; asterisk, selected bacterial cells

immunity is unclear, but they migrate rapidly to bacterial infection sites and carry out complex interactions with epithelia and other immunocytes (Ho et al. 2017).

In four-arm purple sea urchin larvae, exposure to laboratory *Escherichia coli*, either through blastocoelar microinjection or from the surrounding seawater, does not induce a strong response (Ho et al. 2017). However, *Vibrio diazotrophicus*, a marine bacterium isolated from adult *Strongylocentrotus droebachiensis* (Guerinot

et al. 1982), in culture seawater induces reproducible and synchronous changes in larval immunocyte behavior. Specifically, the midgut epithelium becomes dramatically thickened, and pigment cells retract their projections and migrate to the blastocoelar space and basal surfaces of the mouth, esophagus, and gut. Increased interactions between pigment cells and the various classes of blastocoelar cells are also observed (Ho et al. 2017). Pigment cell migration by 24 h is reduced considerably when larvae are exposed to killed vs. live *Vibrio* (Ho et al. 2017), so the response likely depends on activity of the living bacteria. In addition, a rapid and vigorous phagocytic response is induced when *Vibrio* are injected into the larval blastocoel (Ho et al. 2017).

8.4.1   The Larval Immune Gene Response and IL-17

As mentioned above, the purple sea urchin genome encodes a broad panel of immune factors, including orthologs of transcription factors involved in vertebrate immunity (e.g., SCL/TAL2, E2A/HEB/ITF2, GATA1/2/3, PU.1), cytokines and receptors (e.g., IL-1, IL-17, TNF), complement factors (e.g., C3/4/5, Bf), antimicrobial peptides (e.g., Strongylocins), and an expansive repertoire of innate immune receptors (e.g., TLRs, NLRs, SRCRs) (for a comprehensive list, see Buckley and Rast 2017; Hibino et al. 2006). However, the degree to which this immune toolkit is utilized in the embryo and larval stages was initially unclear. This question was addressed by sequencing whole transcriptomes from four-arm *Strongylocentrotus purpuratus* larvae exposed to *Vibrio diazotrophicus* in a time series over a 24-hour period (Buckley et al. 2017). Coincident with the changes in immune cell behavior described above, expression of homologs of genes involved in every layer of vertebrate immune response also is evident, including transcription factors (Atf2, CEBPα, and γ, NF-κB), signaling adaptors (NFkBIz, TNFAIP3, Traf6), cytokines (IL-17, TNF), and effectors (SpSoul1 and the echinoid-specific SpTransformer (185/333) family) (Buckley et al. 2017). Genomic analysis finds 35 *Strongylocentrotus purpuratus* IL-17 homologs separated into 10 subfamilies; a small group of IL-17 ligands belonging to two subfamilies (SpIL17-1 and SpIL17-4) are strongly upregulated early in response to bacterial exposure. Whole-mount in situ hybridization (WMISH) localizes expression of both families to the midgut and hindgut epithelia. Since these events happen very early in response to exposure and the gut lumen is a primary site of bacterial exposure and recognition, these results implicate the gut epithelium as a primary regulator of immune response. Another group, SpIL17-9, is expressed in a similarly rapid fashion in adult coelomocytes following *Vibrio diazotrophicus* injection.

Two IL-17 receptors (IL17-R1 and IL17-R2) have been identified in the purple sea urchin genome via the presence of SEF/interleukin-1 receptor (SEFIR) domains (Buckley et al. 2017; Buckley and Rast 2017; Hibino et al. 2006). WMISH experiments localize expression of both receptors at low levels to multiple tissues and cells throughout the larva (Buckley and Rast 2017). Larvae raised from zygotes

microinjected with antisense morpholinos targeting the IL17-R1 exhibit reduced
expression of an array of downstream genes, including IL17-4, after *Vibrio
diazotrophicus* exposure, suggesting a feedback mechanism (Buckley et al. 2017).
Further study is required to elaborate other potential immune pathways, but these
studies demonstrate that the IL17/IL17-R system is a major regulator of immune
response in sea urchin larvae.

8.4.2 Partitioning the Immune System Between Embryo, Larva, and Adult

Although immune genes are expressed from the beginning of development, analysis
of embryonic RNAseq profiles (Tu et al. 2014) indicates a sharp increase in the
complexity of immune gene expression at the onset of feeding. This coincides with
the differentiation of several immune cell types (Ho et al. 2017; Solek et al. 2013),
the establishment of robust immune signaling systems (Buckley et al. 2017), and
increasing complexity of microbial associations (see above). The immune system is
further elaborated in terms of immune cell number and efficiency of response as the
larva grows. The initial wave of immune cells is derived from embryonic mesoderm
that is subdivided by a GRN involving transcription factors that have important
homologs in vertebrate hematopoietic systems (Schrankel et al. 2016; Solek et al.
2013). The source of later larval immune cells remains unknown. In larvae, expres-
sion of the transcription factors necessary for early immune cell specification is
maintained in the coelomic pouches and in a small group of mesenchymal cells at the
aboral larval apex (Solek et al. 2013). These areas are candidate sources for the
expansion of larval immune cells as the larva grows.

   The immune cells of larvae and adults have similarities as well as differences
(Ho et al. 2017; Solek et al. 2013). The red spherule cells of adults share expression
of transcriptional regulators and enzymes with larval pigment cells, as do adult and
larval phagocytic cells. Larval amoeboid cells share morphology and behavior with
the amoeboid forms of adult colorless spherule cells. In contrast, there are no
obvious morphological counterparts of vibratile cells and several other adult immune
cell types in the larva. In terms of recognition and effector gene expression, there
appear to be distinct differences between larvae and adults. Although some of these
may fall away as different immune activation mechanisms are explored, it seems that
the larva expresses a set of immune genes that overlap, but also differ, from those of
the adult and that to some extent the genome encodes two immune systems that
operate in these distinct life stages (Ho et al. 2017). How the immune system is
elaborated during the course of larval development, how it may function at meta-
morphosis, and how the larval immune system contributes to that of the adult are
questions that remain to be explored.

8.5 Asexual Reproduction and Regeneration

8.5.1 Regeneration in Echinoderm Larvae

Regeneration is an essential component of postembryonic development in animals, as it provides the option to rebuild vital organs and tissues. This process is widespread among invertebrates and has been extensively studied in adult echinoderms for over a century (Candia and Paolo 2010). Still, it has been known for some time that the capability of regeneration is not restricted to adults and that larvae can regenerate body parts as well. Generally, regeneration proceeds through a series of processes, which include wound healing, cell fate changes, cell migration and proliferation, as well as cell–cell signaling. In some animals, such as cnidarians, regeneration originates from highly proliferative stem cells (Holstein et al. 2003). The generality of this mechanism among animals remains unclear however.

Bipinnaria larvae of sea stars can regenerate most larval structures after bisection (i.e., cutting the animal into an anterior and posterior half), including the stomach, mouth, intestine, and anal opening (Vickery et al. 1999a, b, 2001a, b; Vickery and McClintock 1998). A recent study on cell proliferation during regeneration in the bat star *Patiria miniata* shows a marked reduction immediately following bisection, prior to the onset of wound-proximal proliferation (Hinman and Cary 2017). Transcriptome analysis of bisected larvae also suggests conservation of gene expression changes associated with wound healing and proliferation with those found in other animals with regenerative capabilities. Still, localization of specific target genes (e.g., SRAP—sea start regeneration-associated protease, vasa, dysferlin, and vitellogenin) and a detailed analysis of cellular changes occurring after bisection during the regeneration process (Oulhen et al. 2016) suggest that larval regeneration in *P. miniata* also involves broad cellular changes instead of just proliferation by pre-specified stem cell populations. These findings suggest that regeneration follows a distinct differentiation pattern. Future research will have to clarify these regenerative mechanisms in more detail, and sea urchin larvae provide an ideal model organism for such investigations.

8.5.2 Asexual Reproduction Via Budding in Sea Urchins

In contrast to vertebrates, where sexual reproduction is the predominant reproductive strategy, other animal taxa have evolved asexual life cycles involving the generation of larval or adult forms without a preceding embryogenesis. Larval budding has been described in four of the five echinoderm classes, i.e., ophiuroids (Balser 1998), asteroids (Rao et al. 1993), and more recently, echinoids (Eaves and Palmer 2003; Vaughn and Strathmann 2008) and holothuroids (Eaves and Palmer 2003). While the mechanisms underlying this process remain largely unknown, its widespread distribution warrants a more detailed analysis. A variety of abiotic factors such as

pH, salinity, temperature, and turbulence have been shown to induce budding (Allen et al. 2018). Additionally, it has been shown that conspecific larvae in the environment as well as predators may elicit a budding response in sea urchin larvae (Allen et al. 2018). Still, the mechanisms underlying these events, as well as their adaptive significance within the life cycle of an organism, remain elusive. Presumably, some mechanisms involved in regeneration are also important for budding as the larval body needs to be rebuilt. Other processes however may be quite different as regeneration likely involves a stronger response of the immune system during wound healing. Moreover, bisected larvae can draw from a pool of differentiated cells, while buds, which typically consist of only one or two cell types, need to transdifferentiate these cells into a variety of other cell types. It will be interesting to investigate the similarities and differences of molecular and cellular mechanisms between budding and regeneration among sea urchin larvae.

8.6 Synthesis

- Larval forms are morphologically distinct from the juvenile. Functional evidence on endocrine and immunity functions during larval development indicates the existence of similarities and differences between the larva and the adult.
- While hormones fulfill critical functions in the internal regulation of metamorphosis and settlement, the ecological transition from the planktonic to the benthic environment appears to involve interactions with bacteria, through biofilms or secreted factors.
- While cell death plays a key role in postembryonic development, its function is strongly linked to endocrine signaling and potentially the immune response.
- The complexity of immune gene expression and of the cellular immune system increases over the course of larval development.
- The acquisition of complex microbiota and the elaboration of cellular immunity and immune gene expression are temporally and functionally associated with the onset of feeding in planktotrophic larvae.

Box 1

Adaptive immunity: Also known as anticipatory immunity, adaptive immunity is found in all vertebrates where immune receptors are somatically assembled from incomplete genomic elements and selected for binding to nonself in the individual. Immunoglobulins and T-cell receptor systems in jawed vertebrates and variable lymphocyte receptor (VLR) systems in jawless vertebrates are thus far the only well-described examples of this type of receptor system.

Budding: Form of asexual reproduction, which has been observed in a diversity of marine invertebrate larvae. It results in the formation of a new

Box 1 (continued)

individual by cleaving off ectoderm and endodermal cells from the larva, which can then form a completely new larval body.

Holobiont: A multicellular eukaryote host and its associated microbial symbionts (or microbiota). The combined genomes of the host and its microbiota (host genome + microbiome) are the hologenome.

Innate immunity: Protective systems based on nonself-recognition receptors that are directly encoded in the genome like any other gene. Receptors that mediate this type of immunity generally target highly conserved microbial molecules and associated infection signals. These systems are found throughout animals.

Larva: Developmental stage in animals with indirect life histories, preceding juvenile and/or adult stages and following embryogenesis. Larvae are dispersed through the water column, transition into a juvenile via a sometimes drastic metamorphosis, and frequently occupy a different habitat than the juvenile and/or adult. Bipinnaria and pluteus larvae are example of sea star and sea urchin larval stages, respectively.

Larval skeletogenesis: The formation of an endoskeleton in sea urchin and other larval forms. Sea urchin larvae require the skeleton to swim and feed. Note that in sea urchin larvae, skeletogenesis occurs in both, the larval and juvenile compartment.

Metamorphosis: Developmental transition from a larva to a juvenile, which typically involves the development of juvenile structures inside the larva as either a rudiment or imaginal disks. Once juvenile structures matured, larval structures will be destroyed as part of the metamorphic transition.

Programmed cell death (apoptosis): A form of cell death, which is mediated by specific intracellular programs.

Settlement: Transition from the planktonic to the benthic habitat upon induction by specific environmental cues. In marine invertebrate larvae, settlement links the planktonic with the benthic part of the live cycle.

16S: A gene encoding the RNA component of the prokaryotic 30S small ribosomal subunit. Useful in both universal and specific identification of microbes due to the presence of both highly conserved regions, present in all prokaryotes, and hypervariable regions, which evolve more rapidly, and can distinguish closely related taxa to genus- or species-level resolution.

References

Adams DK, Sewell MA, Angerer RC, Angerer LM (2011) Rapid adaptation to food availability by a dopamine-mediated morphogenetic response. Nat Commun 2:592

Allen JD, Reitzel AM, Jaeckle W (2018) Asexual reproduction of marine invertebrate embryos and larvae. In: Carrier T, Reitzel A, Heyland A (eds) Evolutionary ecology of marine invertebrate larvae. Oxford University Press, Oxford, pp 67–82

Balakirev ES, Pavlyuchkov VA, Ayala FJ (2008) DNA variation and symbiotic associations in phenotypically diverse sea urchin *Strongylocentrotus intermedius*. Proc Natl Acad Sci USA 105:16218–16223

Balser EJ (1998) Cloning by ophiuroid echinoderm larvae. Biol Bull 194:187–193

Bishop CD, Brandhorst BP (2001a) NO/cGMP signaling and HSP90 activity represses metamorphosis in the sea urchin *Lytechinus pictus*. Biol Bull 201:394–404

Bishop CD, Brandhorst BP (2001b) The role of NO/cGMP and HSP90 in regulating metamorphosis of the sea urchin *Lytechinus pictus*. Dev Biol 235:251

Bishop CD, Erezyilmaz DF, Flatt T, Georgiou CD, Hadfield MG, Heyland A, Hodin J, Jacobs MW, Maslakova SA, Pires A, Reitzel AM, Santagata S, Tanaka K, Youson JH (2006a) What is metamorphosis? Integr Comp Biol 46:655–661

Bishop CD, Huggett MJ, Heyland A, Hodin J, Brandhorst BP (2006b) Interspecific variation in metamorphic competence in marine invertebrates: the significance for comparative investigations into the timing of metamorphosis. Integr Comp Biol 46:662–682

Bodnar AG, Coffman JA (2016) Maintenance of somatic tissue regeneration with age in short- and long-lived species of sea urchins. Aging Cell 15:778–787

Buckley KM, Rast JP (2012) Dynamic evolution of toll-like receptor multigene families in echinoderms. Front Immunol 3:136

Buckley KM, Rast JP (2017) An organismal model for gene regulatory networks in the gut-associated immune response. Front Immunol 8:1297

Buckley KM, Ho ECH, Hibino T, Schrankel CS, Schuh NW, Wang G, Rast JP (2017) IL17 factors are early regulators in the gut epithelium during inflammatory response to *Vibrio* in the sea urchin larva. Elife 6:e23481

Buszczak M, Segraves WA (2000) Insect metamorphosis: out with the old, in with the new. Curr Biol 10(22):R830–R833

Cameron RA, Holland ND (1983) Electron-microscopy of extracellular materials during the development of a sea star, *Patiria-miniata* (echinodermata, asteroidea). Cell Tissue Res 234:193–200

Cameron RA, Holland ND (1985) Demonstration of the granular layer and the fate of the hyaline layer during the development of a sea-urchin (*Lytechinus-variegatus*). Cell Tissue Res 239:455–458

Candia CMD, Paolo B (2010) Regeneration in echinoderms and ascidians, encyclopedia of life sciences (ELS). Wiley, Chichester

Carrier TJ, Reitzel AM (2018) Convergent shifts in host-associated microbial communities across environmentally elicited phenotypes. Nature Communications 9:952

Carrier TJ, Reitzel A, Heyland A (2017) Evolutionary ecology of marine invertebrate larvae. Oxford University Press, Oxford

Cerra A, Byrne M, Hoegh-Guldberg O (1997) Development of the hyaline layer around the planktonic embryos and larvae of the asteroid and the presence of associated bacteria. Invertebrate Reproduction & Development 31(1–3):337–343

De Ridder C, Foret TW (2006) Non-parasitc symbioses between echinoderms and bacteria. In: Jangoux M, Lawrence JM (eds) Echinoderm studies. Balkema Publishers, Leiden, pp 111–169

Decker GL, Lennarz WJ (1988) Skeletogenesis in the sea-urchin embryo. Development 103:231–247

Dworjanyn SA, Pirozzi I (2008) Induction of settlement in the sea urchin *Tripneustes gratilla* by macroalgae, biofilms and conspecifics: a role for bacteria? Aquaculture 274:268–274

Eaves AA, Palmer AR (2003) Reproduction: widespread cloning in echinoderm larvae. Nature 425:146

Ebert TA, Southon JR (2013) Red sea urchins (*Strongylocentrotus franciscanus*) can live over 100 years: confirmation with A-bomb 14carbon. Fish Bull 101:915–922

Flatt T, Moroz LL, Tatar M, Heyland A (2006) Comparing thyroid and insect hormone signaling. Integr Comp Biol 46:777–794

Gibson AW, Burke RD (1987) Migratory and invasive behavior of pigment cells in normal and animalized sea urchin embryos. Exp Cell Res 173:546–557

Gilbert SF, Sapp J, Tauber AI (2012) A symbiotic view of life: we have never been individuals. Q Rev Biol 87:325–341

Guerinot ML, West PA, Lee JV, Colwell RR (1982) *Vibrio-diazotrophicus* sp-nov, a marine nitrogen-fixing bacterium. Int J Syst Bacteriol 32:350–357

Hakim JA, Koo H, Dennis LN, Kumar R, Ptacek T, Morrow CD, Lefkowitz EJ, Powell ML, Bej AK, Watts SA (2015) An abundance of Epsilonproteobacteria revealed in the gut microbiome of the laboratory cultured sea urchin, *Lytechinus variegatus*. Front Microbiol 6:1047

Hakim JA, Koo H, Kumar R, Lefkowitz EJ, Morrow CD, Powell ML, Watts SA, Bej AK (2016) The gut microbiome of the sea urchin, *Lytechinus variegatus*, from its natural habitat demonstrates selective attributes of microbial taxa and predictive metabolic profiles. Fems Microbiol Ecol 92(9):fiw146

Hart MW, Strathmann RR (1994) Functional consequences of phenotypic plasticity in echinoid larvae. Biol Bull 186:291–299

Herren B, Levkau B, Raines EW, Ross R (1998) Cleavage of beta catenin and plakoglobin and shedding of VE-cadherin during endothelial apoptosis: evidence for a role for caspases and metalloproteinases. Mol Biol Cell 9:1589–1601

Heyland A, Hodin J (2004) Heterochronic developmental shift caused by thyroid hormone in larval sand dollars and its implications for phenotypic plasticity and the evolution of nonfeeding development. Evolution 58:524–538

Heyland A, Moroz LL (2006) Signaling mechanisms underlying metamorphic transitions in animals. Integr Comp Biol 46:743–759

Heyland A, Reitzel AM, Hodin J (2001) Facultative feeding in an obligatorily feeding sand dollar larva: the role of thyroid hormones in echinoderm life history evolution. Am Zool 41:1470–1471

Heyland A, Reitzel AM, Hodin J (2004) Thyroid hormones determine developmental mode in sand dollars (Echinodermata: Echinoidea). Evol Dev 6:382–392

Heyland A, Hodin J, Reitzel AM (2005) Hormone signaling in evolution and development: a non-model system approach. Bioessays 27:64–75

Heyland A, Price DA, Bodnarova-Buganova M, Moroz LL (2006) Thyroid hormone metabolism and peroxidase function in two non-chordate animals. J Exp Zool B Mol Dev Evol 306:551–566

Heyland A, Reitzel A, Hodin J (2009) Thyroid hormone signaling in echinoderms: comparative genomics, cross-kingdom signaling and life history evolution. Integr Comp Biol 49:E242

Hibino T, Loza-Coll M, Messier C, Majeske AJ, Cohen AH, Terwilliger DP, Buckley KM, Brockton V, Nair SV, Berney K, Fugmann SD, Anderson MK, Pancer Z, Cameron RA, Smith LC, Rast JP (2006) The immune gene repertoire encoded in the purple sea urchin genome. Dev Biol 300:349–365

Hinman V, Cary G (2017) Conserved processes of metazoan whole-body regeneration identified in sea star larvae. bioRxiv. https://doi.org/10.1101/118232

Ho EC, Buckley KM, Schrankel CS, Schuh NW, Hibino T, Solek CM, Bae K, Wang G, Rast JP (2017) Perturbation of gut bacteria induces a coordinated cellular immune response in the purple sea urchin larva. Immunol Cell Biol 95:647

Hodin J, Bishop CD, Heyland A (2010) Towards a metamorphic and settlement signaling network in echinoids. Integr Comp Biol 50:E76

Holland ND, Nealson KH (1978) The fine structure of the echinoderm cuticle and the subcuticular space of echinoderms. Acta Zool 59:169–185

Holstein TW, Hobmayer E, Technau U (2003) Cnidarians: an evolutionarily conserved model system for regeneration? Dev Dyn 226:257–267

Huggett MJ, Williamson JE, de Nys R, Kjelleberg S, Steinberg PD (2006) Larval settlement of the common Australian sea urchin *Heliocidaris erythrogramma* in response to bacteria from the surface of coralline algae. Oecologia 149:604–619

Ingersoll EP, Pendharkar NC (2005) Characterization and expression of two matrix metalloproteinase genes during sea urchin development. Gene Expr Patterns 5:727–732

Ingersoll EP, Wilt FH (1998) Matrix metalloproteinase inhibitors disrupt spicule formation by primary mesenchyme cells in the sea urchin embryo. Dev Biol 196:95–106

Ishizuya-Oka A, Hasebe T, Shi YB (2010) Apoptosis in amphibian organs during metamorphosis. Apoptosis 15:350–364

Johnson CR, Sutton DC, Olson RR, Giddins R (1991) Settlement of crown-of-thorns starfish: role of bacteria on surfaces of coralline algae and a hypothesis for deep-water recruitment. Mar Ecol Prog Ser 71:143–162

Kiselev KV, Ageenko NV, Kurilenko VV (2013) Involvement of the cell-specific pigment genes pks and sult in bacterial defense response of sea urchins *Strongylocentrotus intermedius*. Dis Aquat Organ 103:121–132

Koga H, Fujitani H, Morino Y, Miyamoto N, Tsuchimoto J, Shibata TF, Nozawa M, Shigenobu S, Ogura A, Tachibana K, Kiyomoto M, Amemiya S, Wada H (2016) Experimental approach reveals the role of alx1 in the evolution of the echinoderm larval skeleton. PLoS One 11(2): e0149067

Kyriakidis DA, Theodorou MC, Tiligada E (2012) Histamine in two component system-mediated bacterial signaling. Front Biosci Landmark 17:1108–1119

Leguia M, Wessel GM (2006) The histamine H1 receptor activates the nitric oxide pathway at fertilization. Mol Reprod Dev 73:1550–1563

Li C, Blencke HM, Haug T, Jorgensen O, Stensvag K (2014) Expression of antimicrobial peptides in coelomocytes and embryos of the green sea urchin (*Strongylocentrotus droebachiensis*). Dev Comp Immunol 43:106–113

Lutek K, Dhaliwal RS, Van Raay TJ, Heyland A (2018) Sea urchin histamine receptor 1 regulates programmed cell death in larval *Strongylocentrotus purpuratus*. Scientific Reports 8:4002

Mathew S, Fu LZ, Hasebe T, Ishizuya-Oka A, Shi YB (2010) Tissue-dependent induction of apoptosis by matrix metalloproteinase stromelysin-3 during amphibian metamorphosis. Birth Defects Res C 90:55–66

McAlister JS, Miner BG (2018) Phenotypic plasticity of feeding structures in marine invertebrate larvae. In: Carrier T, Reitzel A, Heyland A (eds) Evolutionary ecology of marine invertebrate larvae. Oxford University Press, Oxford, pp 103–123

McEdward LR (2000) Adaptive evolution of larvae and life cycles. Semin Cell Dev Biol 11:403–409

McEdward LR, Janies DA (1993) Life-cycle evolution in asteroids: what is a larva. Biol Bull 184:255–268

McFall-Ngai M, Hadfield MG, Bosch TCG, Carey HV, Domazet-Loso T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF, Hentschel U, King N, Kjelleberg S, Knoll AH, Kremer N, Mazmanian SK, Metcalf JL, Nealson K, Pierce NE, Rawls JF, Reid A, Ruby EG, Rumpho M, Sanders JG, Tautz D, Wernegreen JJ (2013) Animals in a bacterial world, a new imperative for the life sciences. Proc Natl Acad Sci USA 110:3229–3236

McIntyre DC, Lyons DC, Martik M, McClay DR (2014) Branching out: origins of the sea urchin larval skeleton in development and evolution. Genesis 52:173–185

Metchnikoff E (1893) Lectures on the comparative pathology of inflammation: delivered at the Pasteur Institute in 1891. In: Starling FA, Starling EH (eds). Kegan Paul, Trench, Trubner & Co. Ltd., London

Miller AE, Heyland A (2010) Endocrine interactions between plants and animals: implications of exogenous hormone sources for the evolution of hormone signaling. Gen Comp Endocrinol 166:455–461

Miller AE, Heyland A (2013) Iodine accumulation in sea urchin larvae is dependent on peroxide. J Exp Biol 216:915–926

Miner BG (2005) Evolution of feeding structure plasticity in marine invertebrate larvae: a possible trade-off between arm length and stomach size. J Exp Mar Biol Ecol 315:117–125

Miner BG, Vonesh JR (2004) Effects of fine grain environmental variability on morphological
    plasticity. Ecol Lett 7:794–801

Mos B, Cowden KL, Nielsen SJ, Dworjanyn SA (2011) Do cues matter? Highly inductive
    settlement cues don't ensure high post-settlement survival in sea urchin aquaculture. PLoS
    One 6(12):e28054

Nassel DR (1999) Histamine in the brain of insects: a review. Microsc Res Tech 44:121–136

Nielsen SJ, Harder T, Steinberg PD (2015) Sea urchin larvae decipher the epiphytic bacterial
    community composition when selecting sites for attachment and metamorphosis. Fems
    Microbiol Ecol 91:1–9

Oulhen N, Heyland A, Carrier TJ, Zazueta-Novoa V, Fresques T, Laird J, Onorato TM, Janies D,
    Wessel G (2016) Regeneration in bipinnaria larvae of the bat star Patina miniata induces rapid
    and broad new gene expression. Mech Dev 142:10–21

Page-McCaw A, Ewald AJ, Werb Z (2007) Matrix metalloproteinases and the regulation of tissue
    remodelling. Nat Rev Mol Cell Bio 8:221–233

Rao PS, Rao KH, Shyamasundari K (1993) A rare condition of budding in bipinaria larva
    (Asteroidea). Curr Sci 65:792–793

Ribeiro AR, Barbaglio A, Oliveira MJ, Ribeiro CC, Wilkie IC, Carnevali MDC, Barbosa MA
    (2012) Matrix metalloproteinases in a sea urchin ligament with adaptable mechanical properties.
    PLoS One 7(11):e49016

Robertson AJ, Croce J, Carbonneau S, Voronina E, Miranda E, McClay DR, Coffman JA (2006)
    The genomic underpinnings of apoptosis in *Strongylocentrotus purpuratus*. Dev Biol
    300:321–334

Roeder T (2003) Metabotropic histamine receptors: nothing for invertebrates? Eur J Pharmacol
    466:85–90

Ruffins SW, Ettensohn CA (1993) A clonal analysis of secondary mesenchyme cell fates in the sea
    urchin embryo. Dev Biol 160:285–288

Schrankel CS, Solek CM, Buckley KM, Anderson MK, Rast JP (2016) A conserved alternative
    form of the purple sea urchin HEB/E2-2/E2A transcription factor mediates a switch in E-protein
    regulatory state in differentiating immune cells. Dev Biol 416:149–161

Sea Urchin Genome Sequencing Consortium, Sodergren E, Weinstock GM, Davidson EH, Cam-
    eron RA, Gibbs RA, Angerer RC, Angerer LM, Arnone MI, Burgess DR, Burke RD, Coffman
    JA, Dean M, Elphick MR, Ettensohn CA, Foltz KR, Hamdoun A, Hynes RO, Klein WH,
    Marzluff W, McClay DR, Morris RL, Mushegian A, Rast JP, Smith LC, Thorndyke MC,
    Vacquier VD, Wessel GM, Wray G, Zhang L, Elsik CG, Ermolaeva O, Hlavina W,
    Hofmann G, Kitts P, Landrum MJ, Mackey AJ, Maglott D, Panopoulou G, Poustka AJ,
    Pruitt K, Sapojnikov V, Song X, Souvorov A, Solovyev V, Wei Z, Whittaker CA, Worley K,
    Durbin KJ, Shen Y, Fedrigo O, Garfield D, Haygood R, Primus A, Satija R, Severson T,
    Gonzalez-Garay ML, Jackson AR, Milosavljevic A, Tong M, Killian CE, Livingston BT, Wilt
    FH, Adams N, Belle R, Carbonneau S, Cheung R, Cormier P, Cosson B, Croce J, Fernandez-
    Guerra A, Geneviere AM, Goel M, Kelkar H, Morales J, Mulner-Lorillon O, Robertson AJ,
    Goldstone JV, Cole B, Epel D, Gold B, Hahn ME, Howard-Ashby M, Scally M, Stegeman JJ,
    Allgood EL, Cool J, Judkins KM, McCafferty SS, Musante AM, Obar RA, Rawson AP,
    Rossetti BJ, Gibbons IR, Hoffman MP, Leone A, Istrail S, Materna SC, Samanta MP,
    Stolc V, Tongprasit W, Tu Q, Bergeron KF, Brandhorst BP, Whittle J, Berney K, Bottjer DJ,
    Calestani C, Peterson K, Chow E, Yuan QA, Elhaik E, Graur D, Reese JT, Bosdet I, Heesun S,
    Marra MA, Schein J, Anderson MK, Brockton V, Buckley KM, Cohen AH, Fugmann SD,
    Hibino T, Loza-Coll M, Majeske AJ, Messier C, Nair SV, Pancer Z, Terwilliger DP, Agca C,
    Arboleda E, Chen N, Churcher AM, Hallbook F, Humphrey GW, Idris MM, Kiyama T, Liang S,
    Mellott D, Mu X, Murray G, Olinski RP, Raible F, Rowe M, Taylor JS, Tessmar-Raible K,
    Wang D, Wilson KH, Yaguchi S, Gaasterland T, Galindo BE, Gunaratne HJ, Juliano C,
    Kinukawa M, Moy GW, Neill AT, Nomura M, Raisch M, Reade A, Roux MM, Song JL, Su
    YH, Townley IK, Voronina E, Wong JL, Amore G, Branno M, Brown ER, Cavalieri V,
    Duboc V, Duloquin L, Flytzanis C, Gache C, Lapraz F, Lepage T, Locascio A, Martinez P,

Matassi G, Matranga V, Range R, Rizzo F, Rottinger E, Beane W, Bradham C, Byrum C, Glenn T, Hussain S, Manning G, Miranda E, Thomason R, Walton K, Wikramanayke A, Wu SY, Xu R, Brown CT, Chen L, Gray RF, Lee PY, Nam J, Oliveri P, Smith J, Muzny D, Bell S, Chacko J, Cree A, Curry S, Davis C, Dinh H, Dugan-Rocha S, Fowler J, Gill R, Hamilton C, Hernandez J, Hines S, Hume J, Jackson L, Jolivet A, Kovar C, Lee S, Lewis L, Miner G, Morgan M, Nazareth LV, Okwuonu G, Parker D, Pu LL, Thorn R, Wright R (2006) The genome of the sea urchin *Strongylocentrotus purpuratus*. Science 314:941–952

Shah M, Brown KM, Smith LC (2003) The gene encoding the sea urchin complement protein, SpC3, is expressed in embryos and can be upregulated by bacteria. Dev Comp Immunol 27:529–538

Silva JR (2000) The onset of phagocytosis and identity in the embryo of *Lytechinus variegatus*. Dev Comp Immunol 24:733–739

Smith LC, Arizza V, Barela Hudgell MA, Barone G, Bodnar AG, Buckley KM, Cunsolo V, Dheilly NM, Franchi N, Fugmann SD, Furukawa R, Garcia-Arraras J, Henson JH, Hibino T, Irons ZH, Li C, Lun CM, Majeske AJ, Oren M, Pagliara P, Pinsino A, Raftos, DA, Rast JP, Samasa B, Schillaci D, Schrankel CS, Stabili L, Stensväg K, Sutton E (2018) The complex immune system in echinoderms. Springer (in press)

Smith MM, Smith LC, Cameron RA, Urry LA (2008) The larval stages of the sea urchin, *Strongylocentrotus purpuratus*. J Morphol 269:713–733

Solek CM, Oliveri P, Loza-Coll M, Schrankel CS, Ho ECH, Wang GZ, Rast JR (2013) An ancient role for Gata-1/2/3 and Scl transcription factor homologs in the development of immunocytes. Dev Biol 382:280–292

Strathmann RR, Fenaux L, Strathmann MF (1992) Heterochronic developmental plasticity in larval sea-urchins and its implications for evolution of nonfeeding larvae. Evolution 46:972–986

Sutherby J, Giardini JL, Nguyen J, Wessel G, Leguia M, Heyland A (2012) Histamine is a modulator of metamorphic competence in *Strongylocentrotus purpuratus* (Echinodermata: Echinoidea). BMC Dev Biol 12:14

Swanson RL, Williamson JE, De Nys R, Kumar N, Bucknall MP, Steinberg PD (2004) Induction of settlement of larvae of the sea urchin *Holopneustes purpurascens* by histamine from a host alga. Biol Bull 206:161–172

Swanson RL, de Nys R, Huggett MJ, Green JK, Steinberg PD (2006) In situ quantification of a natural settlement cue and recruitment of the Australian sea urchin *Holopneustes purpurascens*. Marine Ecology Progress Series 314:1–14

Swanson RL, Byrne M, Prowse TAA, Mos B, Dworjanyn SA, Steinberg PD (2012) Dissolved histamine: a potential habitat marker promoting settlement and metamorphosis in sea urchin larvae. Mar Biol 159:915–925

Tabarean IV (2016) Histamine receptor signaling in energy homeostasis. Neuropharmacology 106:13–19

Tamboline CR, Burke RD (1992) Secondary mesenchyme of the sea urchin embryo: ontogeny of blastocoelar cells. J Exp Zool 262:51–60

Tata JR (1996) Metamorphosis: an exquisite model for hormonal regulation of post-embryonic development. Biochem Soc Symp:123–136

Tauber AI (2003) Metchnikoff and the phagocytosis theory. Nat Rev Mol Cell Bio 4:897–901

Taylor E, Heyland A (2017) Evolution of thyroid hormone signaling in animals: non-genomic and genomic modes of action. Mol Cell Endocrinol 459:14–20

Taylor E, Wynen H, Heyland A (in review) Thyroid hormone exposure of sea urchin embryos and larvae leads to acceleration of skeletogenesis. Front Endo

Tu Q, Cameron RA, Davidson EH (2014) Quantitative developmental transcriptomes of the sea urchin *Strongylocentrotus purpuratus*. Dev Biol 385:160–167

Unkles SE (1977) Bacterial flora of the sea urchin *Echinus esculentus*. Appl Env Microbiol 34:347–350

Vaughn D, Strathmann RR (2008) Predators induce cloning in echinoderm larvae. Science 319:1503

Vickery MS, McClintock JB (1998) Regeneration in metazoan larvae. Nature 394:140–140

Vickery MCL, Vickery MS, Amsler CD, McClintock JB (1999a) Characterization of novel genes expressed during regeneration in larval sea stars. Am Zool 39:51a

Vickery MS, Vickery MCL, McClintock JB (1999b) Regeneration in echinoid larvae. Am Zool 39:50a–51a

Vickery MCL, Vickery MS, Amsler CD, McClintock JB (2001a) Regeneration in echinoderm larvae. Microsc Res Tech 55:464–473

Vickery MCL, Vickery MS, McClintock JB, Amsler CD (2001b) Utilization of a novel deuterostome model for the study of regeneration genetics: molecular cloning of genes that are differentially expressed during early stages of larval sea star regeneration. Gene 262:73–80

Williams EA, Carrier TJ (2018) An -omics perspective on marine invertebrate larvae. In: Carrier T, Reitzel A, Heyland A (eds) Evolutionary ecology of marine invertebrate larvae. Oxford University Press, Oxford, pp 284–300