Lessons from simple marine models on the bacterial regulation of eukaryotic development

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Highlights

- Cues from environmental bacteria influence the development of many marine eukaryotes
- The molecular cues produced by environmental bacteria are structurally diverse
- Eukaryotes can respond to many different environmental bacteria
- Some environmental bacteria act as “information hubs” for diverse eukaryotes
- Experimentally tractable systems, like the choanoflagellate *S. rosetta*, promise to reveal molecular mechanisms underlying these interactions

Abstract

Molecular cues from environmental bacteria influence important developmental decisions in diverse marine eukaryotes. Yet, relatively little is understood about the mechanisms underlying these interactions, in part because marine ecosystems are dynamic and complex. With the help of simple model systems, including the choanoflagellate *Salpingoeca rosetta*, we have begun to uncover the bacterial cues that shape eukaryotic development in the ocean. Here, we review how diverse bacterial cues – from lipids to macromolecules – regulate development in marine eukaryotes. It is becoming clear that there are networks of chemical information circulating in the ocean, with both eukaryotes and bacteria acting as nodes; one eukaryote can precisely respond to cues from several diverse environmental bacteria, and a single environmental bacterium can regulate the development of different eukaryotes.

Introduction

Eukaryotes evolved over two billion years ago in a world dominated by prokaryotes and have lived in close association with bacteria ever since. It has become increasingly clear that bacteria not only act as competitors and pathogens, but also promote proper health and development in eukaryotes [1,2]. Growing attention has focused on how stably associated bacteria (e.g. the microbiome) shape many aspects of eukaryotic development, from root nodule development in legumes [3] to light organ morphogenesis in the Hawaiin bobtail squid [4], and even immune system development in vertebrates [5]. Yet, bacteria in the microbiome are not the only bacteria influencing eukaryotic development. Although often overlooked, free-living environmental bacteria also provide cues that regulate essential developmental processes in diverse eukaryotes.

Many examples of interactions between environmental bacteria and eukaryotes stem from marine ecosystems, where bacterial cues elicit developmental transitions in organisms as diverse as algae and animals. Nonetheless, few of these bacterial-eukaryotic interactions are understood in molecular detail, in part because marine environments are host to dynamic and diverse bacterial communities. While it is challenging to decipher specific interactions in such complex ecosystems, the lessons learned are likely to extend to interactions between eukaryotes and other bacterial communities, such as those in the gut and soil.

Simple model systems are beginning to reveal how environmental bacteria shape eukaryotic development in the ocean. Important features of these models that facilitate the identification of molecules underlying bacterial-eukaryotic interactions include: (1)

the ability to grow and manipulate both the bacteria and the eukaryote in the lab, and (2) a clear and quantifiable response of the eukaryote to a single bacterium. Here we review mechanisms by which environmental bacteria regulate the development of choanoflagellates and other marine eukaryotes to illustrate how, and explore why, important eukaryotic developmental decisions rely on cues from specific environmental bacteria.

A choanoflagellate model for bacterial-eukaryotic interactions

One of the closest living relatives of animals, the choanoflagellate *Salpingoeca rosetta*, has emerged as an attractive model for investigating how environmental bacteria shape eukaryotic cell biology and life history. Choanoflagellates are unicellular and colony-forming microeukaryotes that live in diverse aquatic environments [6]. Every choanoflagellate cell bears an apical "collar complex" – a single flagellum surrounded by a feeding collar composed of actin-filled microvilli – that it uses to capture and phagocytose bacterial prey. Importantly, the collar complex and its role in mediating interactions with bacteria are conserved among choanoflagellates and animals [6-8]. However, choanoflagellates do not just eat bacteria, but they also undergo key life history transitions in response to molecular cues secreted by environmental bacteria.

A network of bacterial lipids flips a developmental switch in S. rosetta

In many choanoflagellates, including the emerging model choanoflagellate *S. rosetta*, a solitary cell can develop into a multicellular "rosette" colony through serial rounds of oriented cell division, with the sister cells remaining stably adherent [9,10]

(Figure 1a). Although *S. rosetta* was isolated from the ocean as a rosette, early laboratory cultures proliferated primarily in the unicellular form, producing rosettes infrequently and unpredictably. A set of unexpected observations revealed that *Algoriphagus machipongonensis,* an environmental bacterium that had been co-isolated with the choanoflagellate and persisted in laboratory cultures at very low densities*,* could induce robust and uniform rosette development in *S. rosetta* when grown at higher densities [11]*.*

Because *S. rosetta* and *Algoriphagus* could be cultured independently or together*,* and because rosette development was quantifiable (i.e. % of cells in rosettes), a straightforward rosette development bioassay could be used to investigate the molecular basis of *Algoriphagus* rosette-inducing activity. Activity-guided fractionation led to the isolation of RIF-1 (Rosette-Inducing Factor-1), a novel sulfonolipid signaling molecule that induced rosette development in *S rosetta* [11]. However, only a small fraction of *S. rosetta* cells formed rosettes in response to RIF-1, far fewer than that induced by live *Algoriphagus,* leading to the hypothesis that additional *Algoriphagus* molecules influence *S. rosetta* rosette development [12].

Further work revealed that *Algoriphagus* produces additional lipid activators, synergistic enhancers, and inhibitors that regulate rosette development [12,13] (Fig. 1a). While the RIFs (RIF-1 and a second sulfonolipid, RIF-2) were sufficient to induce low levels of rosette development, an additional class of lipid synergists, the lysophosphatidylethanolamines (LPEs), were required for robust rosette induction. Together, the RIFs and LPEs recapitulated the full rosette inducing activity of live *Algoriphagus*.

97 The importance of the LPEs had initially been obscured by the fact that they did not
98 exhibit any bioactivity on their own; only by testing bacterial lipid fractions in
99 combination with the RIFs did it become clear that these synergistic lipids helped to fully
100 potentiate the induction of rosette development. Testing bacterial fractions in
101 combination also revealed that *Algoriphagus* produces a molecule that competes with
102 and inhibits RIF-induced rosette development. The molecule, a capnine called IOR-1
103 (Inhibitor of Rosettes-1), antagonizes the RIFs, but its inhibitory activity can be
104 bypassed in the presence of LPEs, providing a possible explanation for why IOR-1 does
105 not normally prevent *Algoriphagus* rosette induction.
106 Why might *S. rosetta* rely on a network of bacterial cues before committing to
107 rosette development? We hypothesize that requiring multiple bacterial cues may ensure
108 that rosette development is not initiated in response to the wrong bacteria, or under
109 unfavorable environmental conditions. This integrated response may be especially
110 important in aquatic environments, where bacterial composition and nutrient availability
111 are constantly changing.
112
113 *A bacterial chondroitinase triggers mating in* S. rosetta
114 In addition to rosette development, *S. rosetta* can transition from asexual
115 proliferation to sexual reproduction, wherein solitary haploid cells fuse to produce a
116 diploid cell that will then undergo meiosis [14]. Despite harboring a complete meiotic
117 genetic toolkit [15,16], the *S. rosetta* sexual cycle was rarely observed in laboratory
118 cultures. Only under starvation conditions would a small fraction of the *S. rosetta*
119 population mate [17]. A serendipitous observation revealed that specific environmental

bacteria, missing from most laboratory cultures, were capable of triggering a robust, population-wide switch to sexual reproduction [18].

This discovery stemmed from the observation that *Vibrio fischeri,* an abundant marine bacterium, induced the formation of large motile aggregates or "swarms" composed of many solitary *S. rosetta* cells. Swarming had not been previously described in *S. rosetta*, and further examination revealed that during swarming, haploid *S. rosetta* cells frequently paired off and underwent cell and nuclear fusion. Genetic experiments confirmed that the diploid products of cell and nuclear fusion later generated meiotic progeny, demonstrating that *Vibrio* bacteria induce the full sexual cycle in *S. rosetta* (Figure 1b).

Because swarming was always observed prior to mating, *S. rosetta* swarming provided a robust bioassay for identifying the molecular basis of the *Vibrio* "aphrodisiac" activity. Activity-guided fractionation led to the isolation of a protein, named EroS (Extracellular Regulator of Sex) that fully recapitulated the activity of *Vibrio* bacteria. Biochemical assays revealed that EroS belongs to a class of bacterial polysaccharide-degrading enzymes called chondroitinases, and that the chondroitin-degrading activity of EroS is sufficient to induce mating in *S. rosetta*. Finally, the *S. rosetta* target of EroS was identified as the sulfated polysaccharide chondroitin sulfate, a component of the extracellular matrix previously thought to be restricted to the animal lineage. As the first example of an environmental bacterium regulating eukaryotic sexual reproduction, the interaction between *Vibrio* and *S. rosetta* raises the possibility that mating in other aquatic eukaryotes may be influenced by environmental bacteria as well.

143 *Bacteria as master regulators of* S. rosetta *life history in the marine environment*

144 Bacteria are required for rosette development and mating under laboratory
145 conditions – but can bacteria plausibly regulate *S. rosetta* development in nature?
146 Despite their underlying molecular differences, the cues that induce rosette
147 development and mating are bioactive at environmentally relevant concentrations. The
148 purified *Algoriphagus* RIFs and LPEs display activity at high nanomolar to low
149 micromolar concentrations in the laboratory; yet, the hydrophobicity of these molecules
150 makes it unlikely that *S. rosetta* encounters them as isolated lipids in the environment.
151 As constituents of the *Algoriphagus* outer membrane, it is more likely that RIFs and
152 LPEs are released into the environment within outer membrane vesicles (OMVs),
153 spherical packages of periplasmic content constitutively produced by Gram negative
154 bacteria [19,20]. Indeed, *Algoriphagus* OMVs elicit robust rosette development [12], and
155 retain their bioactivity under a wide range of conditions. Moreover, diverse bacteria
156 belonging to several marine Bacteroidetes and Actinobacteria genera induce rosette
157 development in *S. rosetta* ([11]*;* unpublished data]), raising the likelihood that *S. rosetta*
158 might encounter rosette-inducing bacteria in multiple environments

159 In contrast with the lipid regulators of rosette development, the mating-inducing
160 chondroitin lyase, EroS, is a soluble protein constitutively secreted by *Vibrio* bacteria.
161 Not only does EroS trigger mating at picomolar concentrations, but *S. rosetta* swarms in
162 response to as few as 400 *V. fischeri* cells/mL—a density similar to that of *V. fischeri* in
163 oligotrophic oceans [21]. In addition to *V. fischeri,* several other species of *Vibrio*
164 bacteria induce mating in *S. rosetta*, as do commercial chondroitin lyases isolated from
165 *Flavobacterium heparinum* and *Proteus vulgaris* [18], suggesting that encounters

166 between *S. rosetta* and molecules produced by mating-inducing bacteria might be
167 common occurrences in the ocean.

168 Thus, it is reasonable to infer that *S. rosetta* comes across both rosette
169 development and mating inducing bacteria in nature. In addition, because *S. rosetta* can
170 respond to cues from diverse bacteria, it seems plausible that other life history
171 transitions in choanoflagellates, such as settlement (the attachment of a planktonic cell
172 to a substrate; [6,10]), are regulated by environmental bacteria as well.

173

174 ***The widespread influences of environmental bacteria***

175 Choanoflagellates are not the only eukaryotes taking life advice from
176 environmental bacteria. Environmental bacteria also regulate developmental transitions
177 in diverse marine algae and animals, and simple model systems are beginning to
178 uncover the bacterial cues that influence eukaryotic morphogenesis.

179

180 *Algal morphogenesis*

181 It has long been known that microbial communities associated with the surfaces
182 of marine macroalgae are essential for their growth and morphogenesis [22]. Yet, the
183 bacterial species responsible for stimulating algal development remained elusive for
184 many years, due to the complex and seasonally-shifting composition of algal-associated
185 bacterial communities [23]. A key advance in studying bacterial-algal interactions was
186 the development of axenic culturing techniques (including for the seaweed *Monostroma*
187 *oxyspermum*), which provided a platform for testing individual bacterial species for
188 morphogenesis-inducing activity [24]. Although diverse bacteria that influence algal

189 growth and morphogenesis have now been identified (Table 1), only one morphogenetic
190 factor, Thallusin, has been isolated by activity-guided fractionation and characterized to
191 date [25-27]. Thallusin is an amino acid derivative produced by the *Monostroma*-
192 associated bacterium, *Zobellia uliginosa*, that is sufficient to induce thallus development
193 in *M. oxyspermum* and partially promote thallus development in *Ulva* species. With a
194 clear bioassay available, why haven't more algal morphogenetic factors been isolated?
195 One hypothesis is that the bacterial cues regulating algal growth and morphogenesis
196 are produced at very low levels. This was certainly true for Thallusin, although it was
197 ultimately possible to isolate the molecule because of its potency and stability [26].
198 Alternatively, induction may require multiple bacterial molecules. On their own, bacteria
199 belonging to *Cytophaga* and *Roseobacter* genera induce incomplete *Ulva mutabilis*
200 development, promoting either cell division or thallus differentiation, respectively [28].
201 However, the combined activities of these bacteria fully restore normal morphogenesis,
202 raising the possibility that the synergistic interactions observed at the organismal level
203 are required at the molecular level as well.
204
205 *Larval settlement*
206 Many benthic marine invertebrates have complex life histories that include stages of
207 larval settlement and metamorphosis, key developmental steps that are crucial for adult
208 success. Bacterial biofilms provide cues that trigger larval settlement and
209 metamorphosis in diverse marine invertebrates, including sponges, cnidarians,
210 molluscs, annelids, echinoderms, and urochordates (Table 1). While the majority of
211 these interactions remain poorly understood, the increased tractability of invertebrate
212 systems (e.g. the tubeworm *Hydroides elegans* [29] and the coral *Acropora millepora*

[30]) has facilitated identification of the bacterial cues that regulate larval settlement and metamorphosis. Biofilm-forming bacteria from the genus *Pseudoalteromonas* influence development in several animals, including corals and tubeworms. Interestingly, the cues produced by environmental *Pseudoalteromonas* that trigger metamorphosis in *A. millepora* and *H. elegans* are distinct; while *A. millepora* metamorphosis is induced by the small molecule tetrabromopyrrole [31], *H. elegans* metamorphosis is regulated by arrays of contractile phage-tail like structures called MACs (Metamorphosis Associated Contractile Structures) [32]. Although the structures of tetrabromopyrrole and MACs suggest that the mechanisms by which these molecules trigger metamorphosis are likely very different, both of these bacterial molecules may provide chemical evidence of a suitable surface for colonization. Because surfaces in the ocean are often limiting, cues from bacteria might indicate to animals that they have found an appropriate environment for settling down.

Bacterial cues are proxies for environmental conditions

As more bacterial cues are isolated, it is becoming clear that interactions between eukaryotes and their environmental bacteria exhibit remarkable molecular specificity. Even slight modifications to the structures of bacterial cues can completely eliminate inducing activity, as is the case with the choanoflagellate rosette-inducing molecules and the algal morphogenetic factor Thallusin [13,27,33]. Nonetheless, multiple environmental bacteria can elicit the same eukaryotic developmental responses, and in each case the molecular cues seem to be distinct (this has been demonstrated for *S. rosetta* rosette development, *Monostroma* morphogenesis, and *H. elegans* larval settlement; Table 1). Because marine microbial communities are highly

dynamic, it may be beneficial for eukaryotes to interpret developmental cues from diverse bacteria. The molecular stringency we observe likely allows eukaryotes to be responsive to many different environmental bacteria, whilst maintaining tight regulation over important developmental decisions.

Interestingly, we find that select bacterial genera (including *Flavobacteriia, Pseudoalteromonas,* and *Vibrio*) have a high level of influence on the development of diverse marine eukaryotes. It is possible that many eukaryotes may rely on cues from Bacteroidetes and Gammaproteobacteria because these bacteria flourish in nutrient and carbon rich environments, and can thus serve as proxies for favorable environmental conditions. Indeed, many of the bacteria that regulate eukaryotic development are well equipped for rapidly responding to increasing nutrient availability, boasting an assortment of extracellular enzymes that break down polysaccharides, lipids, and proteins [34]. For example, the polysaccharide degrading abilities of Bacteroidetes and Gammaproteobacteria allow these bacteria to utilize algal-derived polysaccharides and promptly proliferate when phytoplankton bloom (often as a result of increased mineral levels) [35]. Even more impressive is the ability of some inducing-bacteria (notably *Vibrio* spp.) to pursue nutrient dense microenvironments through chemotaxis [36]. Thus, diverse eukaryotes may have converged on certain bacteria as indicators of nutrient-rich environments.

Finally, it is enticing to consider how environmental bacteria might benefit from these interactions. Many of the bacteria that induce eukaryotic developmental transitions also frequently associate with eukaryotes, for example by accumulating on surfaces of macroalgae and invertebrates. Because these bacteria produce

exoenzymes that help them utilize plant and animal-derived molecules for nutrition [34], it is possible that inducing eukaryotic development allows specific bacteria to rapidly colonize valuable “real estate.”

Conclusion

Although the influences of environmental bacteria on the development of marine eukaryotes has been observed for decades, we are just beginning to gain a molecular understanding of these interactions. With the help of simple model systems and straightforward bioassays, it is becoming clear that environmental bacteria produce structurally diverse cues that govern eukaryotic development with a high degree of molecular specificity. Nonetheless, even simple bacterial-eukaryotic interactions can be challenging to characterize when we rely solely on activity-guided fractionation. Furthermore, most interactions with bacteria are not simple, and may rely upon multiple molecular cues produced by one or more bacterial species. To understand these complex interactions, future studies should likely combine genetic and activity-guided approaches in the study of environmentally-relevant pairs or communities of eukaryotes and bacteria.

Importantly, many of the environmental bacteria that regulate eukaryotic development in marine ecosystems interact with eukaryotes in other pathogenic and commensal contexts. For example, the bacterium *Vibrio fischeri* forms a symbiosis with and triggers morphogenesis in the Hawaiian bobtail squid (*Euprymna scolopes*), while other members of the Vibrionaceae are common animal enteric commensals, pathogens, and mutualists [37]. In addition, Bacteroidetes bacteria closely related to

283 *Algoriphagus* are abundant mammalian gut commensal bacteria that are important for
284 proper intestinal development and homeostasis [38]. Therefore, understanding how
285 environmental bacteria shape the development of marine eukaryotes may also provide
286 insight into broadly applicable mechanisms of bacterial-eukaryotic interactions.
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288
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1. Mcfall-Ngai M, Hadfield MG, Bosch TCG, Carey HV, Domazet-Lošo T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF, et al.: **Animals in a bacterial world, a new imperative for the life sciences.** *Proc Natl Acad Sci USA* 2013, **110**:3229–3236.

2. Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A: **The importance of the microbiome of the plant holobiont.** *New Phytol.* 2015, **206**:1196–1206.

3. Badri DV, Weir TL, van der Lelie D, Vivanco JM: **Rhizosphere chemical dialogues: plant–microbe interactions**. *Current Opinion in Biotechnology* 2009, **20**:642–650.

4. McFall-Ngai MJ, Ruby EG: **Symbiont recognition and subsequent morphogenesis as early events in an animal-bacterial mutualism**. *Science* 1991, **254**:1491–1494.

5. Sommer F, Bäckhed F: **The gut microbiota--masters of host development and physiology.** *Nat Rev Microbiol* 2013, **11**:227–238.

6. Leadbeater BSC: *The Choanoflagellates: Evolution, Ecology, and Biology*. Cambridge University Press; 2015.

7. James-Clark H: **On the Spongiae ciliatae as Infusoria flagellata; or, observations on the structure, animality and relationship of *Leucosolenia botryoides* Bowerbank.** *Ann. Mag. Nat. His.* 1868, **1**:133–142.

8. Brunet T, King N: **The origin of animal multicellularity and cell differentiation.** *Dev Cell* 2017, **43**:124–140.

9. Fairclough SR, Dayel MJ, King N: **Multicellular development in a choanoflagellate**. *Curr Biol* 2010, **20**:R875–6.

10. Dayel MJ, Alegado RA, Fairclough SR, Levin TC, Nichols SA, McDonald K, King N: **Cell differentiation and morphogenesis in the colony-forming choanoflagellate *Salpingoeca rosetta*.** *Developmental biology* 2011, **357**:73–82.

11. Alegado RA, Brown LW, Cao S, Dermenjian RK, Zuzow R, Fairclough SR, Clardy J, King N: **A bacterial sulfonolipid triggers multicellular development in the closest living relatives of animals.** *Elife* 2012, **1**:e00013.

12. Woznica A, Cantley AM, Beemelmanns C, Freinkman E, Clardy J, King N: **Bacterial lipids activate, synergize, and inhibit a developmental switch in choanoflagellates.** *Proc Natl Acad Sci USA* 2016, **113**:7894–7899.

13. Cantley AM, Woznica A, Beemelmanns C, King N, Clardy J: **Isolation and synthesis of a bacterially produced inhibitor of rosette development in choanoflagellates**. *J. Am. Chem. Soc.* 2016, **138**:4326–4329.

14. Levin TC, King N: **Evidence for sex and recombination in the choanoflagellate *Salpingoeca rosetta***. *Current Biology* 2013, **23**:2176–2180.

15. Carr M, Leadbeater BSC, Baldauf SL: **Conserved meiotic genes point to sex in the choanoflagellates.** *J. Eukaryot. Microbiol.* 2010, **57**:56–62.

16. Fairclough SR, Chen Z, Kramer E, Zeng Q, Young S, Robertson HM, Begovic E, Richter DJ, Russ C, Westbrook MJ, et al.: **Premetazoan genome evolution and the regulation of cell differentiation in the choanoflagellate *Salpingoeca rosetta***. *Genome Biol.* 2013, **14**:R15.

17. Levin TC, Greaney AJ, Wetzel L, King N: **The Rosetteless gene controls development in the choanoflagellate *S. rosetta***. *Elife* 2014, **3**.

18. Woznica A, Gerdt JP, Hulett RE, Clardy J, King N: **Mating in the closest living relatives of animals is induced by a bacterial chondroitinase**. *Cell* 2017, doi:10.1016/j.cell.2017.08.005.

19. Kulp A, Kuehn MJ: **Biological functions and biogenesis of secreted bacterial outer membrane vesicles**. *Annu Rev Microbiol* 2010, **64**:163–184.

20. Lynch JB, Alegado RA: **Spheres of hope, packets of doom: the good and bad of outer membrane vesicles in interspecies and ecological dynamics.** *Journal of Bacteriology* 2017, **199**:e00012–17.

21. Jones BW, Maruyama A, Ouverney CC, Nishiguchi MK: **Spatial and temporal distribution of the Vibrionaceae in coastal waters of Hawaii, Australia, and France**. *Microb Ecol* 2007, **54**:314–323.

22. Wichard T: **Exploring bacteria-induced growth and morphogenesis in the green macroalga order Ulvales (Chlorophyta).** *Front Plant Sci* 2015, **6**:86.

23. Burke C, Thomas T, Lewis M, Steinberg P, Kjelleberg S: **Composition, uniqueness and variability of the epiphytic bacterial community of the green alga *Ulva australis*.** *ISME J* 2011, **5**:590–600.

24. Singh RP, Reddy CRK: **Seaweed–microbial interactions: key functions of seaweed-associated bacteria**. *FEMS Microbiol Ecol* 2014, **88**:213–230.

25. Matsuo Y, Suzuki M, Kasai H, Shizuri Y, Harayama S: **Isolation and phylogenetic characterization of bacteria capable of inducing**

differentiation in the green alga *Monostroma oxyspermum*. *Environ Microbiol* 2003, **5**:25–35.

26. Matsuo Y, Imagawa H, Nishizawa M, Shizuri Y: **Isolation of an algal morphogenesis inducer from a marine bacterium.** *Science* 2005, **307**:1598–1598.

27. Nishizawa M, Iyenaga T, Kurisaki T, Yamamoto H, Sharfuddin M, Namba K, Imagawa H, Shizuri Y, Matsuo Y: **Total synthesis and morphogenesis-inducing activity of (±)-thallusin and its analogues**. *Tetrahedron Letters* 2007, **48**:4229–4233.

28. Spoerner M, Wichard T, Bachhuber T, Stratmann J, Oertel W: **Growth and thallus morphogenesis of *Ulva mutabilis* (Chlorophyta) depends on a combination of two bacterial species excreting regulatory factors**. *Journal of Phycology* 2012, **48**:1433–1447.

29. Huang S, Hadfield MG: **Composition and density of bacterial biofilms determine larval settlement of the polychaete *Hydroides elegans***. *Marine Ecology Progress …* 2003, **260**:161–172.

30. Tebben J, Tapiolas DM, Motti CA, Abrego D, Negri AP, Blackall LL, Steinberg PD, Harder T: **Induction of larval metamorphosis of the coral *Acropora millepora* by tetrabromopyrrole isolated from a pseudoalteromonas bacterium**. *PloS one* 2011, **6**:e19082–8.

31. Tebben J, Tapiolas DM, Motti CA, Abrego D, Negri AP, Blackall LL, Steinberg PD, Harder T: **Induction of larval metamorphosis of the coral *Acropora millepora* by tetrabromopyrrole isolated from a *Pseudoalteromonas* bacterium**. *PloS one* 2011, **6**:e19082.

32. Shikuma NJ, Pilhofer M, Weiss GL, Hadfield MG, Jensen GJ, Newman DK: **Marine tubeworm metamorphosis induced by arrays of bacterial phage tail-like structures.** *Science* 2014, **343**:529–533.

33. Beemelmanns C, Woznica A, Alegado RA, Cantley AM, King N, Clardy J: **Synthesis of the rosette-inducing factor RIF-1 and analogs.** *J. Am. Chem. Soc.* 2014, **136**:10210–10213.

34. Stal LJ, Cretoiu MS (Eds): *The Marine Microbiome*. Springer; 2016.

35. Teeling H, Fuchs BM, Bennke CM, Krüger K, Chafee M, Kappelmann L, Reintjes G, Waldmann J, Quast C, Glöckner FO, et al.: **Recurring patterns in bacterioplankton dynamics during coastal spring algae blooms.** *Elife* 2016, **5**:e11888.

36. Stocker R: **Marine microbes see a sea of gradients.** *Science* 2012, **338**:628–633.

403 37. Thompson FL, Austin B, Swings J: *The biology of Vibrios.* ASM Press; 2006.

404 38. Thomas F, Hehemann J-H, Rebuffet E, Czjzek M, Michel G: **Environmental**
405 **and gut bacteroidetes: the food connection.** *Front Microbiol* 2011, **2**:93.

406 39. Grueneberg J, Engelen AH, Costa R, Wichard T: **Macroalgal**
407 **morphogenesis induced by waterborne compounds and bacteria in**
408 **coastal seawater.** *PloS one* 2016, **11**:e0146307.

409 40. Marshall K, Joint I, Callow ME, Callow JA: **Effect of marine bacterial**
410 **isolates on the growth and morphology of axenic llantlets of the green**
411 **alga *Ulva linza***. *Microb Ecol* 2006, **52**:302–310.

412 41. Singh RP, Mantri VA, Reddy CRK, Jha B: **Isolation of seaweed-associated**
413 **bacteria and their morphogenesis-inducing capability in axenic**
414 **cultures of the green alga *Ulva fasciata***. *Aquatic Biology* 2011, **12**:13–21.

415 42. Joint I, Tait K, Callow ME, Callow JA, Milton D, Williams P, Cámara M: **Cell-**
416 **to-cell communication across the prokaryote-eukaryote boundary**.
417 *Science* 2002, **298**:1207–1207.

418 43. Whalan S, Webster NS: **Sponge larval settlement cues: the role of**
419 **microbial biofilms in a warming ocean**. *Sci Rep* 2014, **4**:srep04072.

420 44. Neumann R: **Bacterial induction of settlement and metamorphosis in**
421 **the planula larvae of *Cassiopea andromeda***. *Marine Ecology Progress*
422 *Series* 1979, **1**:21–28.

423 45. Schmahl G: **Induction of stolon settlement in the scyphopolyps of**
424 ***Aurelia aurita* by glycolipids of marine bacteria**. *Helgolthder wiss.*
425 *Meeresuntersuchungen* 1985, **39**:117–127.

426 46. Fitt WK, Coon SL, Walch M, Weiner RM, Colwell RR, Bonar DB: **Settlement**
427 **behavior and metamorphosis of oyster larvae (*Crassostrea gigas*) in**
428 **response to bacterial supernatants**. *Marine Biology* 1990, **106**:389–394.

429 47. Freckelton ML, Nedved BT, Hadfield MG: **Induction of invertebrate larval**
430 **settlement; different bacteria, different mechanisms?** *Sci Rep* 2017,
431 **7**:42557.

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

436 49. Szewzyk U, Holmström C, Wrangstadh M: **Relevance of the**
437 **exopolysaccharide of marine *Pseudomonas* sp. strain S9 for the**
438 **attachment of *Ciona intestinalis* larvae**. *Ecology Progress Series* 1991,

439 doi:10.2307/24825861.

440

Table 1. Environmental bacteria regulate development in marine eukaryotes

Eukaryote	Bacteria (Phylum)	Developmental outcome	Molecular cue	Reference
Chlorophyta (green algae)				
Ulva mutabilis	*Cytophaga* (B)	Thallus differentiation	Unknown	[28]
	Roseobacter (B)	Cell division	Unknown	[28]
	Algoriphagus (B); *Polaribacter* (B)	Cell division and thallus differentiation	Unknown	[39,40]
Monostroma oxyspermum	*Zobellia uliginosa* (B)	Morphogenesis	Thallusin	[26]
Ulva pertussa	*Zobellia uliginosa* (B)	Morphogenesis	Unknown	[25]
Ulva conglobata	*Zobellia uliginosa* (B)	Morphogenesis	Unknown	[25]
Ulva fasciata	*Marinomonas* (F); *Bacillus* (F)	Morphogenesis/ growth of zoospores	Unknown	[41]
Enteromorpha	*Vibrio anguillarum* (G)	Zoospore settlement	AHLs	[42]
Choanoflagellate				
Salpingoeca rosetta	*Algoriphagus machipongonensis* (B)	Rosette development	Lipid cofactors (Sulfonolipids, LPEs, capnine)	[11-13]
	Zobellia uliginosa (B); *Demequina* (A)	Rosette development	Uncharacterized lipids	[11]; unpublished
	Vibrio fischeri (G); *Flavobacterium heparinum* (B)	Sexual reproduction	Chondroitin lyase	[18]
Porifera (sponges)				
Rhopaloeides odorabile	Bacterial biofilm	Larval settlement	Unknown	[43]
Cnidaria				
Acropora millepora	*Pseudoalteromonas* (G)	Larval metamorphosis	Tetrabromopyrrole	[30,31]
Acropora willisae	*Pseudoalteromonas* (G)	Larval metamorphosis	Tetrabromopyrrole	[44]
Porites astreoides (coral)	*Pseudoalteromonas* (G)	Larval metamorphosis	Tetrrabromopyrrole	[44]
Cassiopea andromeda (jellyfish)	*Vibrio alginolyticus* (G)	Larval metamorphosis	Unknown	[44]
Aurelia aurita (jellyfish)	*Micrococcacae* (A)	Larval settlement	Glycolipids	[45]
Mollusca				
Crassostrea gigas (oyster)	*Altermonas colwelliana* (G); *Vibrio cholerae* (G)	Larval settlement / metamorphosis	Unknown	[46]
Annelida				
Hydroides elegans (marine tubeworm)	*Pseudoalteromonas luteoviolacea* (G);	Larval settlement	Tailocin MACs	[32]
	Cellulophaga lytica (B);	Larval settlement	Unknown	[47]
	Bacillus aquimaris (F);	Larval settlement	Unknown	[47]
	Staphylococcus warneri (F)	Larval settlement	Unknown	[47]
Echinodermata				

Heliocidaris erythrogramma (sea urchin)	*Pseudoalteromonas luteoviolacea* (G); *Vibrio* (G); *Shewanella* (G)	Larval settlement	Unknown	[48]
Chordata				
Ciona intestinalis (sea squirt)	*Pseudomonas* (G)	Larval attachment	Exopolysaccharide	[49]

Bacterial phylogeny key: (B) Bacteroidetes; (G) Gammaproteobacteria; (F) Firmicutes; (A) Actinobacteria

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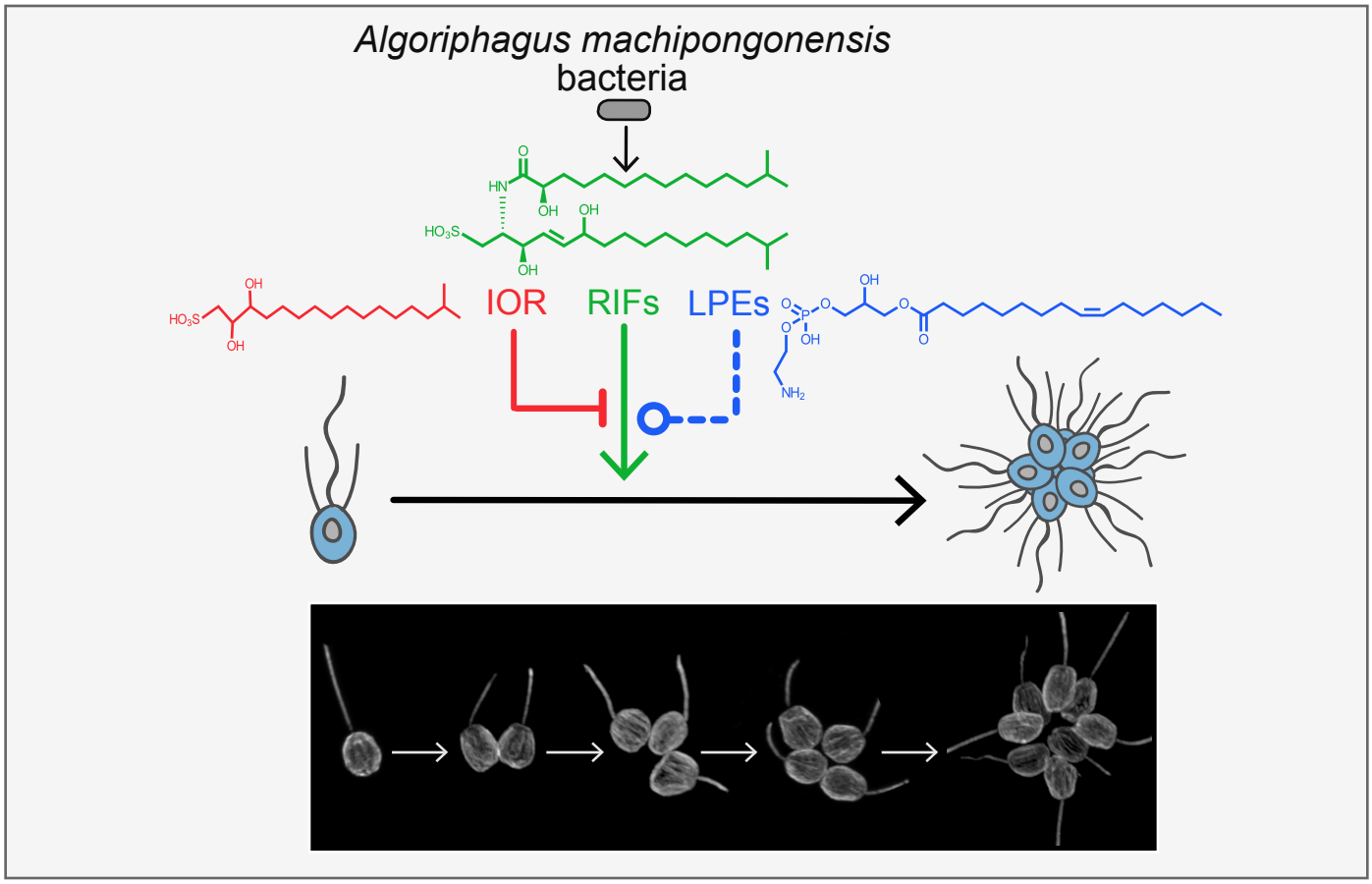
Figure Legends

Figure 1. Bacteria regulate rosette development and sexual reproduction in the choanoflagellate, *S. rosetta*. (A) *Algoriphagus machipongonensis* bacteria regulate the development of *S. rosetta* from a solitary cell into a multicellular "rosette" colony through serial rounds of cell division. *Algoriphagus* produces three classes of lipids – sulfonolipids (RIFs), lysophosphatidylethanolamines (LPEs), and a capnine (IOR-1)– that interact to alternately induce, enhance, or inhibit rosette development. While the sulfonolipid RIFs are sufficient to initiate rosette development in *S. rosetta*, they require the synergistic enhancing activity of the LPEs for robust rosette development. *Algoriphagus* also produces the inhibitory IOR-1 that inhibits the RIFs, but cannot overcome the synergistic inducing activity of the RIFs + LPEs. Immunofluorescence images illustrate stages of *S. rosetta* rosette development; tubulin staining (grey) highlights the cell body and apical flagellum. **(B)** *Vibrio fischeri* bacteria induce sexual reproduction in *S. rosetta*. EroS, a chondroitin lyase secreted by *V. fischeri*, triggers solitary *S. rosetta* cells (arrows) to form large swarms (brackets) through cell aggregation. During swarming, *S. rosetta* cells pair off and mate, a process that involves the cell and nuclear fusion of two haploid cells into one diploid cell, followed by meiosis to generate haploid progeny. Immunofluorescence images depict mating stages in *S. rosetta*; tubulin staining (grey) highlights the cell body and apical flagellum, and Hoechst staining (magenta) highlights the nucleus.

Figure 2. Distinct molecular cues from environmental Bacteroidetes and Gammaproteobacteria regulate developmental transitions in diverse marine eukaryotes. The Bacteroidetes bacteria *Algoriphagus* and *Zobellia uliginosa* regulate morphogenesis in organisms as diverse as algae and choanoflagellates. **(1)** Uncharacterized factors produced by *Algoriphagus* induce morphogenesis in the macroalgae *Ulva mutabilis.* **(2)** *Algoriphagus machipongonensis* lipids [sulfonolipids, lysophosphatidylethanolamines, and a capnine] regulate rosette development in the choanoflagellate *Salpingoeca rosetta*. **(3)** Thallusin, and amino acid derivative produced by *Zobellia uliginosa*, induces morphogenesis in the macroalgae *Monostroma oxyspermum*. **(4)** Uncharacterized molecules from *Zobellia uliginosa* induce rosette development in the choanoflagellate *Salpinogeca rosetta*. Gammaproteobacteria can likewise elicit developmental responses in diverse animals and choanoflagellates. **(5)** Tetrabromopyrrole produced by *Pseudoalteromonas* spp. induces larval metamorphosis in corals *Acropora millepora* and *Acropora willisae*, and larval settlement (attachment and metamorphosis) in the coral *Porites astreoides*. **(6)** Uncharacterized cues from *Pseudoalteromonas* bacteria induce larval settlement in the sea urchin *Heliocidaris erythrogramma*. **(7)** *Pseudoalteromonas luteoviolacea* produces arrays of contractile phage tail-like structures (MACs) that trigger metamorphosis of the tubeworm *Hydroides elegans*. **(8)** A chondroitinase (EroS) secreted by *Vibrio fischeri* induces mating in the choanoflagellate *S. rosetta.* **(9)** Unknown cues secreted by *Vibrio alginolyticus* induce larval metamorphosis in the jellyfish *Cassiopea andromeda*

A



B