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Bacterial Influences on Animal Origins

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Animals evolved in seas teeming with bacteria, yet the influences of bacteria on animal origins are poorly understood. Comparisons among modern animals and their closest living relatives, the choanoflagellates, suggest that the first animals used flagellated collar cells to capture bacterial prey. The cell biology of prey capture, such as cell adhesion between predator and prey, involves mechanisms that may have been co-opted to mediate intercellular interactions during the evolution of animal multicellularity. Moreover, a history of bacterivory may have influenced the evolution of animal genomes by driving the evolution of genetic pathways for immunity and facilitating lateral gene transfer. Understanding the interactions between bacteria and the progenitors of animals may help to explain the myriad ways in which bacteria shape the biology of modern animals, including ourselves.

The first bacteria evolved more than 3 billion years ago and dominated the biosphere continually thereafter, shaping the environment in which animals would eventually evolve more than 2 billion years later (Narbonne 2005; Knoll 2011). Because animals evolved in seas filled with bacteria and have lived in close association with bacteria throughout their evolutionary history, it is likely that diverse interactions with bacteria (including predation on bacteria, harboring bacterial commensals, and infection with bacterial pathogens) influenced animal origins. Nonetheless, although the potential contributions of global environmental change and genome evolution to animal origins have received a fair amount of attention (Hoffman et al. 1998; Knoll and Carroll 1999; Knoll 2003; King 2004; Canfield et al. 2007; Shen et al.

2008; Srivastava et al. 2008, 2010; Richter and King 2013), relatively little is known about how the interactions of animal progenitors with the abundant bacteria in their environment may have influenced the evolution of animals (McFall-Ngai 1999; Moran 2007; Hughes and Sperandio 2008; McFall-Ngai et al. 2013). We review here the current state of knowledge about ancient bacterial interactions and consider how these associations may have shaped the biology and evolution of the earliest animals.

BACTERIA AND THE WORLD BEFORE ANIMALS

The earliest fossil evidence for life on Earth is bacterial (Fig. 1). Layered macroscopic sedimentary structures known as “stromatolites” re-

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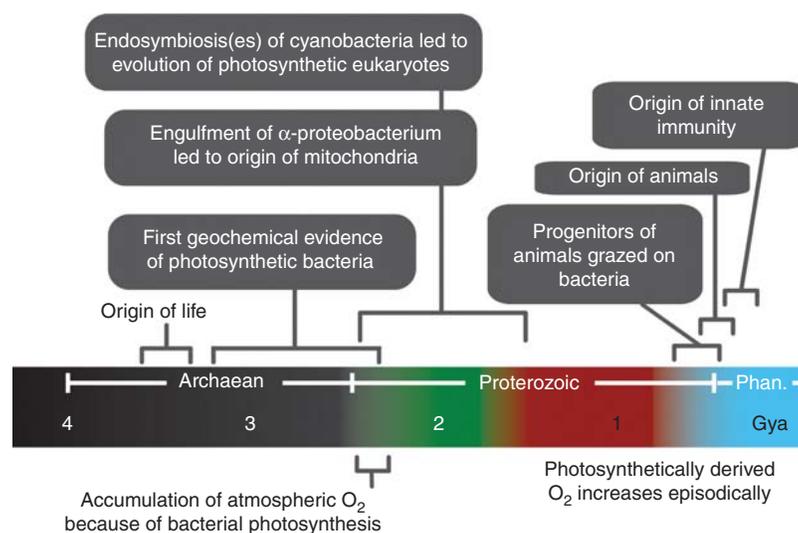


Figure 1. Major events in life's history influenced by bacteria. Bacteria have exerted critical influences on the evolution of eukaryotes and, ultimately, the origin and evolution of animals (processes indicated in gray bubbles). Bacteria and archaea contributed to the cellular and genetic building blocks for the first eukaryotic cells, and bacteria formed stable associations with early eukaryotes in the form of mitochondria and plastids. Moreover, bacteria were likely an important source of food for the progenitors of animals, as well as the first animals themselves. Finally, photosynthetic bacteria were critical for shaping the environment in which animals would evolve. The Great Oxygenation Event, estimated to have occurred 2.3 billion years ago, is likely to have been fueled by photosynthetic cyanobacteria. Moreover, photosynthetically derived oxygen, coupled with underlying geochemical processes, lead to episodic increases in oxygen levels starting in the Proterozoic. The timeline (rectangle) depicts the predominant redox state of the oceans. Anoxic surface and deep ocean waters (black) dominated the Archean Eon. During the Proterozoic Eon, surface waters became oxygenated because of mixing; however, the deep oceans remained anoxic (green). Isotopic measurements of sediments suggest that the chemistry of the ocean between ~1.8 Gya and 1 Gya was sulfidic and ferruginous (red). This period also marked the height of stromatolite abundance and diversity before their decline in the Neoproterozoic period. The Neoproterozoic is marked by the appearance of Ediacaran biota and periods of glaciation. During the Phanerozoic, more widespread oxygenation of surface and deep waters (blue) was roughly concomitant with the emergence of animals around 635 million years ago. Phan., Phanerozoic.

cord the existence of bacteria (and possibly archaea) dating back 3.45 billion years (Walter et al. 1980; Mojzsis et al. 1996; Grotzinger and Knoll 1999; Rosing 1999; Kaiser 2001; Allwood et al. 2007). Over the succeeding 2 billion years, bacteria and archaea infiltrated almost every available ecological niche and played key roles in one of life's major evolutionary transitions (Szathmáry and Smith 1995): the origin of eukaryotes. There is debate as to whether the primordial nucleus-bearing (but amitochondriate) eukaryote arose autogenously or through the fusion of a eubacterium and an archeon because genes thought to be derived from both lineages have been observed in the nuclear

genomes of modern eukaryotes (Margulis 1996; Rivera and Lake 2004; Cox et al. 2008; Embley and Martin 2006; Keeling 2014; Koonin and Yutin 2014). In either event, an ancestor of all modern eukaryotes engulfed an α -proteobacterium (Sagan 1967; Cavalier-Smith 2009), leading to the establishment of a stable endosymbiotic relationship that gave rise to the mitochondrion. Thus, the cell biology and genomes of all modern eukaryotes were built on a bacterial foundation.

Although the exact timing of eukaryotic origins is unknown, macroscopic structures found in Gabon, West Africa have been interpreted as eukaryotic fossils that are at least 2.1 billion



years old (El Albani et al. 2010). Undisputed multicellular eukaryotes did not appear in the fossil record until ~ 1.2 billion years ago (Butterfield 2000). Animals apparently lagged even further behind and were not preserved in the fossil record until ~ 635 million years ago (Narbonne 2005; Love et al. 2009; Maloof et al. 2010; Knoll 2011), although animals may have evolved as early as 800 million years ago (Knoll and Carroll 1999; Douzery et al. 2004; Hedges et al. 2004; Peterson 2004; Erwin et al. 2011; Parfrey et al. 2011).

What caused the delay? One hypothesis posits that high levels of oxygen were a prerequisite for the evolution of multicellularity, in part, because of the limits of diffusion. Before mechanisms for active transport of oxygen evolved, an organism's size was limited by the efficiency of oxygen diffusion to inner cell layers (Erwin 1993). In addition, high levels of oxygen may have been required for the structure and function of certain classes of animal proteins that are required for multicellularity, including collagen, a key component of animal extracellular matrix (Towe 1970). Although the reasons underlying the protracted interval between the origin of eukaryotes and evolution of multicellular eukaryotes (including animals) may never be fully understood, the low concentration of oxygen in oceans and atmosphere throughout much of the Proterozoic may have suppressed the proliferation of large multicellular life (Nursall 1959; Knoll and Carroll 1999).

As a result of the photosynthetic activity of marine cyanobacteria, atmospheric oxygen levels reached what is thought to be the minimum concentration supportive of animal life, $\sim 1\%$ of present atmospheric levels, at least 2.3 billion years ago (Budd and Jensen 2000; Kasting and Siefert 2002; Newman and Banfield 2002; Canfield et al. 2013). The emergence of the Ediacara biota ~ 580 million years ago, including stem animals and other multicellular eukaryotes, coincided with the oxygenation of the deep oceans, providing circumstantial evidence linking pO_2 levels to the evolution of animal multicellularity (Knoll and Carroll 1999; Knoll 2003; Canfield et al. 2008; 2007). Thus, by contributing to the establishment of a prolonged

stable oxic environment, bacteria inadvertently helped set the stage for animal origins.

RECONSTRUCTING THE BIOLOGY OF THE FIRST ANIMALS

The fossil record is silent when it comes to the cell and organismal biology of the last common ancestor of animals, also known as the Urmetazoan (Fig. 2) (Haeckel 1874). Nevertheless, aspects of stem animal biology can be inferred by identifying those features that are conserved among nearly all extant animals. For example, because all animals are multicellular and produce differentiated cell types, including eggs and sperm, the Urmetazoan likely also had these character traits (Nielsen 2008; Richter and King 2013). Proteins and domains required for immune defense against pathogens, including the Toll/Interleukin 1 receptor/resistance domain (TIR), the interleukin-1 receptor-like domain, and interferon regulatory factor-like proteins (required for antiviral defense), are also universal among animals and likely evolved before animal origins (Srivastava et al. 2010). Moreover, several animal developmental signaling pathways (Wnt, TGF- β , RTK, Notch, Hedgehog, and Jak-STAT) were present in the last common ancestor of animals, suggesting that the potential for elaborate developmental programs may have evolved early (Pires-daSilva and Sommer 2003; Nichols et al. 2006; Adamska et al. 2007; Manning et al. 2008; Pincus et al. 2008; Srivastava et al. 2010).

In eumetazoans, early embryogenesis is marked by a process of invagination called "gastrulation" in which an infolding of the blastoderm leads to the development of new tissue layers, including mesoderm. Like eumetazoans, some sponges produce hollow larvae that invaginate (Byrum and Martindale 2004; Leys and Eerkes-Medrano 2005; Leys and Ereskovsky 2006; Leys et al. 2009), raising the possibility that development in the Urmetazoan involved a gastrulation-like infolding of cell layers. Sponges also form epithelia that display several hallmarks of eumetazoan epithelia, including an underlying basement membrane and, in some species, proteinaceous intercellular junc-

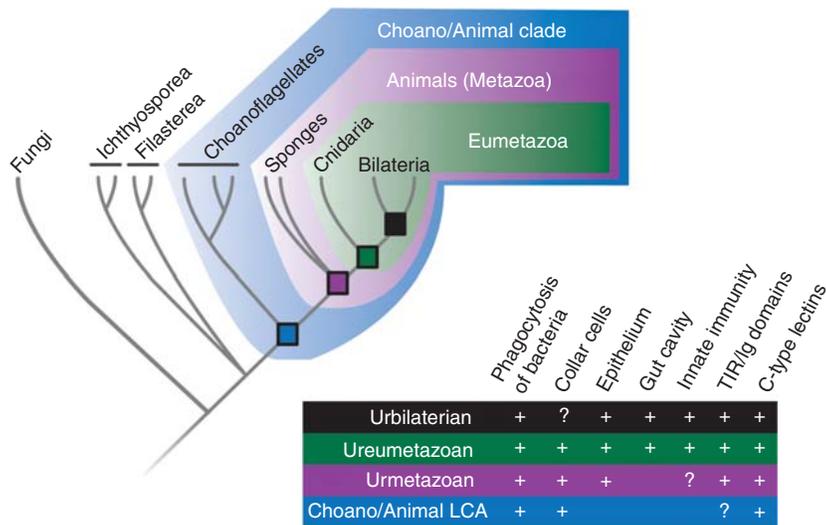


Figure 2. Ancestry and evolution of animal–bacterial interactions. Bacterial influences on the origin and evolution of animals can be inferred by comparing the organismal biology and genome content of extant choanoflagellates and diverse animals within a robust phylogenetic framework (*upper left*). Features shared among choanoflagellates and diverse animals were likely present in their last common ancestor (Choano/Animal LCA, blue square; *bottom right*). Likewise, the biology of the Urmetazoan (purple square), Ureumetazoan (green square), and Urbilaterian (black square) can be reconstructed from features that are shared among diverse animals, eumetazoans (i.e., tissue-grade animals), and bilaterians, respectively. The conservation of collar cells in choanoflagellates, sponges, and diverse eumetazoa suggests that the progenitors of choanoflagellates and animals likely used collar cells to capture bacteria. Epithelia, an animal cell type that may be derived from an ancestral collar cell, are found in diverse animals and were likely present in the Urmetazoan. Pattern recognition protein domains, including TIR/Ig domains and C-type lectins, are expressed by diverse animals and likely evolved in stem animals, if not earlier. Interactions with bacteria were mediated solely at the cellular level in the Choano/Animal LCA and the Urmetazoan, whereas eumetazoans have evolved specialized tissue and organ systems, including the gut cavity, to harbor bacteria and regulate interactions with bacteria. Question marks indicate uncertainty about the presence or absence of a character for a given ancestor.

tions (Haeckel 1872; Falk et al. 1998; Leys and Degnan 2002; Maldonado and Bergquist 2002; Degnan et al. 2005; Nichols et al. 2009). Importantly, epithelia are essential for mediating interactions between modern animals and bacteria (e.g., by lining the gut and thereby forming a barrier to gut bacteria) (Fraune and Bosch 2010; McFall-Ngai et al. 2013). The phylogenetic distribution of epithelia and their conserved roles in regulating interactions between animals and bacteria suggests that epithelia were present and may have served similar roles in the Urmetazoan (Tyler 2003; Nielsen 2008; Nichols et al. 2009; Gilbert et al. 2012; Richter and King 2013).

Comparisons among animals and their closest relatives, the choanoflagellates, reveal additional features of Urmetazoan organismal

biology (Medina et al. 2003; Philippe et al. 2004; Steenkamp et al. 2006; Carr et al. 2008; King et al. 2008; Ruiz-Trillo et al. 2008). Choanoflagellates are unicellular and colony-forming flagellates that resemble the feeding cells of sponges, called “collar cells” or choanocytes (Figs. 3A,B) (James-Clark 1868; Fjordingstad 1961; Leys and Degnan 2002; Gonobobleva and Maldonado 2009). Each sponge collar cell and choanoflagellate cell has an ovoid cell body and single apical flagellum that is surrounded by a feeding collar of rigid, actin-filled microvilli (Karpov and Leadbeater 1997, 1998). The sinusoidal beat of the flagellum generates water currents that trap bacterial prey and organic detritus against the surface of the collar before engulfment (Lapage 1925; Pettitt et al. 2002).

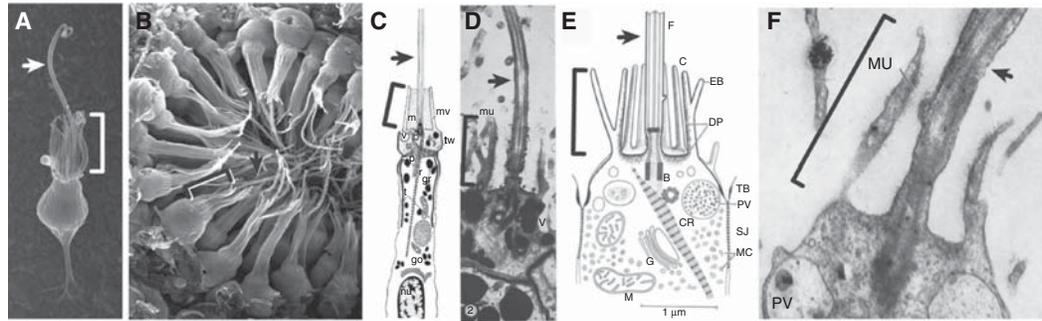


Figure 3. Variations on a collar cell theme in choanoflagellates and animals. Collar cells are typified by the presence of a single apical flagellum (indicated with an arrow) surrounded by a collar of actin-filled microvilli (indicated with a bracket). (A) Choanoflagellates are heterotrophic microeukaryotes that undulate their apical flagellum to generate water currents that draw bacteria against their actin-filled collar before phagocytosis (image of *Salpingoeca rosetta* courtesy of Mark Dayel). (B) Like choanoflagellates, sponge collar cells also have a single apical flagellum and collar of microvilli that they use to capture bacterial prey (image of choanocyte chamber from *Oscarella carmela* courtesy of Scott Nichols). (C,D) Collar cells have also been observed in cnidarians (adapted from data in Lyons 1973) and (E,F) echinoderms (adapted from data in Nerrevang and Wingstrand 1970). Original labels for C: go, Golgi body; gr, granules; l, lipid; m, mucus; mv, collar microvilli; nu, nucleus; p, plaque; r, ciliary rootlet; t, cylindrical thickening; tw, terminal web; v, vacuole. Original labels for D: mu, mucus coat; v, vacuole. Original labels for E: B, basal bodies; C, collar; DP, dense plasma; EB, external branch of microvillus; F, flagellum; G, Golgi apparatus; MC, medium-contrast clumps without membrane; M, mitochondria; PV, phagocytic vacuole; TB, terminal bar; SJ, septate junction. Original labels for F: MU, mucus; PV, phagocytic vacuole.

The conserved structure and function of collar cells in choanoflagellates and sponges suggest that collar cells were present in the Urmetazoan and last common ancestor of choanoflagellates and animals (Brusca and Brusca 2002; Knoll 2011; McFall-Ngai et al. 2013; Richter and King 2013). Moreover, it suggests that at least some modern animal cell types evolved from an ancestral collar cell that phagocytosed bacteria.

The notion that the first animals used a filter-feeding collar cell to capture bacteria provides a useful starting point for reconstructing bacterial influences on animal origins. Although collar cells were once thought to be restricted to choanoflagellates and sponges, collar cells have now been documented in cnidarians (Lyons 1973; Goldberg and Taylor 1989b; Peterson 2004), echinoderms (Nerrevang and Wingstrand 1970; Walker 1979; Martinez et al. 1991), ascidians (Milanesi 1971), and hemichordates (Nerrevang 1964). In cnidarians, some presumptive collar cells act as phagocytic feeding cells in the gastrodermis (Lyons 1973). Other cnidarian collar cells function as sensory cells

in the tentacles of adults and serve unknown functions on the outer surfaces of motile larvae (Goldberg and Taylor 1989a). Epithelial cells, which are conserved in all animal lineages, may also be related to the ancient bacteria-eating collar cells of the Urmetazoan. The apical-basal polarity of epithelial cells, their possession of an apical primary cilium surrounded by microvilli, and phagocytic activity provide parallels to some of the most diagnostic features of collar cells (Nerrevang 1964; Nerrevang and Wingstrand 1970; Walker 1979; Martinez et al. 1991; Singla and Reiter 2006).

Previous analyses of the paleontological record provide independent support for the idea that bacterivory was important during the origin and early evolution of animals (Stanley 1973). For example, Cambrian trace fossils hint that early bilaterians grazed on dense microbial assemblages growing on hard substrates (Dornbos et al. 2004; Bottjer 2005; Gaidos et al. 2007). The emergence and diversification of animals coincided with a rapid decline in the abundance of stromatolites leading some to hy-

pothesize that bacteria involved in stromatolite formation were also prey targets of early animals (Garrett 1970; Awramik 1971; Walter and Heys 1985). Thus, the phylogenetic distribution of phagocytic collar cells coupled with analyses of the fossil record suggest that bacteria may have served as important food sources for early animals.

CHOANOFLAGELLATE COLONY DEVELOPMENT AS A MODEL FOR ANIMAL ORIGINS

The evolution of animals from their single-celled ancestors is hypothesized to have involved a transition through a hollow, spherical colony of undifferentiated cells (Fig. 4A) (Haeckel 1874; Buss 1987; Grosberg and Strathmann 2007; Mikhailov et al. 2009; Knoll 2011). Many choanoflagellate species can transition between unicellular and simple multicellular (i.e., “colonial”) morphologies, recapitulating the evolutionary transition that likely occurred in the animal stem lineage. Indeed, colonies formed by some choanoflagellate species resemble morula stage embryos (Fig. 4B). Moreover, just as animal embryos develop from a zygote through repeated rounds of cell division, choanoflagellate colonies develop from a single founder cell when daughter cells from each round of cell division remain attached (Fairclough et al. 2010). In diverse species of choanoflagellates, neighboring cells within colonies remain physically linked by intercellular bridges (Hibberd 1975; Karpov and Coupe 1998; Fairclough et al. 2010; Dayel et al. 2011) that leave the colony resembling the coenocytic syncytia formed by diverse invertebrates during embryogenesis (Foe and Alberts 1983; Carlson and Handel 1988; Kojima 1992; Leys 2006; Greenbaum et al. 2007; Ong and Tan 2010). The morphological parallels with animal development and the prevalence of colony formation among diverse choanoflagellates have raised the possibility that the last common ancestor of choanoflagellates and animals may have been capable of forming simple colonies (Carr et al. 2008). Thus, the last common ancestor of animals and choanoflagellates may have displayed many features of mod-

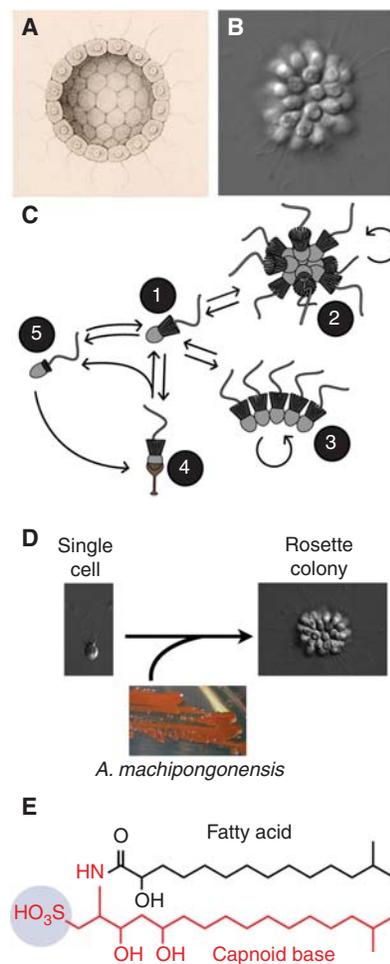


Figure 4. Choanoflagellate colony development as a model for animal origins. (A) The evolution of animals from their single-celled ancestors is hypothesized to have involved a transition through a simple, flagellated colonial form termed the Blastaea by Ernst Haeckel (1874). (B) *S. rosetta* produces rosette-shaped colonies that resemble Haeckel’s hypothesized Blastaea (Dayel et al. 2011). (C) *S. rosetta* undergoes transient differentiation into slow swimming cells (1), attached “thecate” cells (4), fast swimmer cells (5), rosette colonies (2), and chain colonies (3) (adapted from data in Dayel et al. 2011). Arrows indicate differentiation events that have been observed under laboratory conditions. (D) A prey bacterium, *Algoriphagus machipongonensis*, produces a bioactive sulfonolipid, rosette-inducing factor-1 (RIF-1), that is sufficient to stimulate rosette colony development in *S. rosetta* (Alegado et al. 2012). (E) RIF-1 is composed of a fatty acid chain (black), capnoid base (red), and sulfonic acid head group (gray circle).



ern choanoflagellate biology including collar cells, nutrient acquisition through filter feeding on bacteria, and the ability to form stable cell–cell or cell–surface contacts (Steenkamp et al. 2006; Carr et al. 2008; Nielsen 2008; Richter and King 2013).

The regulation of cell differentiation during animal development may have evolved from preexisting mechanisms for regulating transient cell differentiation during the life cycles of the single-celled ancestors of animals (Bonner 1998; King 2004; Nielsen 2008). A modern example of transient cell differentiation can be found in the choanoflagellate *S. rosetta*, which produces diverse unicellular and colonial morphotypes in response to a variety of environmental cues (Fig. 4C) (Dayel et al. 2011; Alegado et al. 2012; Levin and King 2013). One of the colonial morphotypes, the rosette colony, has been shown to undergo development involving multiple layers of regulation. The first committed step toward a multicellular state occurs when a subset of solitary swimming cells differentiates, becoming competent to form a rosette colony. Rosette-competent solitary swimmers can be distinguished from morphologically identical (but rosette-incompetent) cells by the fact that they can be specifically stained by wheat germ agglutinin (WGA) lectin (Dayel et al. 2011), a commonly used marker of cell differentiation in animals (Falk et al. 1994). Although the molecular basis of the change in WGA staining is not clear, competence to form rosette colonies may be influenced by bacteria; the abundance of WGA⁺ solitary cells increases in rapidly growing cultures that have ample bacterial prey (Dayel et al. 2011).

The second step in rosette colony development involves a developmental switch in which WGA⁺ solitary cells undergo incomplete cytokinesis along the apical-basal axis and subsequently remain attached in a radial orientation that leads to the formation of a compact rosette colony. Transcriptome sequencing has revealed that solitary cells and colonies have distinct expression profiles and the initiation of colony development preferentially involves the up-regulation of genes that are exclusively shared by choanoflagellates and animals (Fairclough et al.

2013). Thus, rosette colonies show a number of hallmarks of animal development, including clonal multicellularity, the expression of conserved sets of regulatory genes, and morphological similarity to early animal embryos. Given the parallels between choanoflagellate rosette colony formation and animal development, elucidating the molecular mechanisms of *S. rosetta* rosette colony development may provide a window into the biology of the earliest animals.

BACTERIAL CUES REGULATE A DEVELOPMENTAL SWITCH IN THE CLOSEST RELATIVES OF ANIMALS

Despite being isolated from the environment as a rosette colony more than a decade ago (King et al. 2003), the triggers of rosette colony development in *S. rosetta* were, until recently, unknown. Early efforts to investigate the phenomenon of rosette formation were stymied by severe overgrowth of coisolated environmental bacteria and seemingly stochastic shifts in the relative abundances of different choanoflagellate cell types in laboratory cultures. This all changed serendipitously when it was discovered that serial rounds of antibiotic treatment, intended to decrease the diversity of cocultured prey bacteria, also resulted in an irreversible loss of rosette colony development in choanoflagellate cultures. Subsequent investigation revealed that rosette colony development in *S. rosetta* is regulated extrinsically by coisolated environmental bacteria (Dayel et al. 2011; Alegado et al. 2012). Thus, environmental bacteria are not just a food source for *S. rosetta*, but also regulators of its life cycle.

Inclusion of microbial cues into the regulation of development is hardly a new theme (McFall-Ngai et al. 2013). Larvae produced by diverse basal animals, including sponges (Woollacott and Hadfield 1996), cnidarians (Webster et al. 2004), bryozoans in the *Bugula* genus (Bertrand and Woollacott 2003; Dobretsov and Qian 2006), the ascidian *Ciona intestinalis* (Wieczorek and Todd 1997; Webster et al. 2004), and the polychaete *Hydroides elegans* (Hadfield 2011), all settle in response to uncharacterized compounds released by environ-



mental bacteria. In addition, commensal bacteria can regulate animal development, as in the case of the light organ in the squid *Euprymna scolopes* (Montgomery and McFall-Ngai 1994; McFall-Ngai and Ruby 2000), and gut morphogenesis in mammals (Mazmanian et al. 2005; Bates et al. 2006). In fact, nearly all animals have stable associations with bacteria, but investigating how these interactions have shaped animal evolution has been difficult, in part, because of a dearth of tractable and phylogenetically relevant model systems.

Rosette colony development in *S. rosetta* is triggered by a chemical cue that originates from the bacterium *A. machipongonensis*, a member of the Bacteroidetes phylum (Alegado et al. 2012, 2013). Notably, Bacteroidetes bacteria regulate development and cell differentiation in at least three independent multicellular lineages: red algae, green algae, and mammals (Table 1). Thus, identifying and characterizing signaling molecules produced by *A. machipongonensis* may help to reveal mechanisms by which Bacteroidetes influence the development and evolution of multicellular organisms.

A bioassay based on rosette colony development revealed that a signaling molecule produced by *A. machipongonensis*, the previously undescribed sulfonolipid RIF-1, was sufficient to trigger rosette colony development by *S. rosetta* (Alegado et al. 2012). Sulfonolipids are structurally similar to sphingolipids, a group of bioactive molecules that regulate cell growth,

apoptosis, adhesion, cell migration, and intercellular trafficking in diverse eukaryotes (Hannun and Obeid 2008). Sphingolipids, although best understood as eukaryotic signaling molecules, are also synthesized by bacteria in the Bacteroidetes phylum (Kunsman and Caldwell 1974; Lev 1979; Miyagawa et al. 1979; Yano et al. 1982; Nichols 1998; Batrakov et al. 1999), members of genus *Sphingomonas* (Batrakov et al. 1998; Naka et al. 2000), and *Bdellovibrio* genera (Steiner et al. 1973), but their presence in other bacterial groups is unknown. Sphingolipids make up a significant portion of total lipids (40%–70%) in several *Bacteroidetes* species (Olsen and Jantzen 2001) and can even replace lipopolysaccharide in the membrane of *Sphingomonas paucimobilis* (Kawasaki et al. 1994), leading to the hypothesis that bacterial sphingolipids play integral roles in the cell envelope. Bacterial sphingolipids have also been documented to affect eukaryotic signaling pathways, for example, by inducing apoptosis (Minamino et al. 2003) or activating Toll-like receptor 4 (Fujiwara et al. 2013). Sulfonolipids are distinguished from sphingolipids by their sulfonic acid head group and have thus far only been isolated from bacteria (Godchaux and Leadbetter 1984; Drijber and McGill 1994; Baronio et al. 2010). The roles of sulfonolipids in bacterial physiology are unknown, but the discovery that a sulfonolipid can signal to *S. rosetta* raises the possibility that these molecules may mediate other signaling interactions between bacteria and eukaryotes.

Table 1. Signaling by Bacteroidetes bacteria influences the biology of diverse eukaryotes

Eukaryote	Lineage	Bacteria/ μ m	Signaling outcome	Molecule	References
Human	Mammal	Bacteroides	Obesity	Unknown	Turnbaugh et al. 2009
Mouse	Mammal	Bacteroides	Gut maturation	PSA	Mazmanian et al. 2005
Zebrafish	Bony fish	Consortia	Gut maturation	Unknown	Bates et al. 2006
<i>Ciona intestinalis</i> (sea squirt)	Ascidian	Bacteroidetes consortia	Larval settlement/ metamorphosis	Unknown	Wieczorek and Todd 1997
<i>Acropora micro- pthalma</i> (coral)	Cnidarian	Bacteroidetes consortia	Larval settlement/ metamorphosis	Unknown	Webster et al. 2004
<i>Salpingoeca rosetta</i>	Choanoflagellate	<i>Algoriphagus</i> spp.	Colony formation	RIF-1	Alegado et al. 2012
<i>Ulva pertusa</i> (sea lettuce)	Green alga	<i>Zobellia uglinosa</i>	Thallus differentiation	Thallusin	Matsuo et al. 2005

PSA, polysaccharide A; RIF-1, rosette-inducing factor-1.

It is unknown why *S. rosetta* colony development is regulated extrinsically by environmental bacteria rather than intrinsically. In the coastal estuarine environment in which *S. rosetta* was isolated, the distribution of prey bacteria within the water column fluctuates dramatically. It is possible that *S. rosetta* uses the presence or absence of distinct bacterial cues to govern cell differentiation pathways (e.g., thecate cell vs. fast swimmer vs. rosette colony) (Fig. 4C). The case of RIF-1 provides an example of how this might work in a dynamic environment with variable concentrations of a changing cast of bacteria. The initiation of rosette development in *S. rosetta* does not require direct cell contact with *A. machipongonensis*. Instead, RIF-1 is released into the aquatic environment where it can trigger rosette colony development over a broad range of concentrations, from 10^{-2} to 10^7 fM. At these concentrations, RIF-1 is seemingly as potent as or more potent than other marine-signaling molecules (Schaefer et al. 1996; Ziegler and Forward 2007). Importantly, RIF-1 is constitutively synthesized whether or not the choanoflagellate predator is present, suggesting that its production is likely intrinsic to the biology of *A. machipongonensis* rather than a response to predation. But why would a bacterial signal induce the formation of rosette colonies? A hint comes from the fact that fluid flow around rosette colonies is higher than that around solitary cells, leading to increased contact rates with bacterial prey (Orme et al. 2001; Roper et al. 2013). Thus, the connection between bacterial signals and rosette colony development may stem from enhanced prey capture by rosette colonies under certain environmental conditions. Investigating whether bacterial signals also regulate colony development in other choanoflagellate species may provide further insight into the biology of the last common ancestor of choanoflagellates and animals.

IMPLICATIONS OF A HISTORY OF BACTERIVORY ON THE GENETIC TOOLKIT FOR ANIMAL MULTICELLULARITY

The composition of the first animal genomes was the product of both gene co-option and

innovation (Rokas et al. 2003; Putnam et al. 2007; King et al. 2008; Rokas 2008; Srivastava et al. 2008; 2010; Richter and King 2013). Comparing features of animal genomes with those of choanoflagellates has to reveal the identity of those genes that arose on the stem lineage leading to animals. For example, comparisons of genomes from animals, choanoflagellates, and other relatives of animals and fungi has revealed that much of the molecular toolkit for animal development originated in the premetazoan era (Lang et al. 2002; King et al. 2003, 2008; Ruiz-Trillo et al. 2004; Ruiz-Trillo et al. 2006; Steenkamp et al. 2006; Abedin and King 2008; Li et al. 2008; Shalchian-Tabrizi et al. 2008; Liu et al. 2009; de Mendoza et al. 2010; Sebé-Pedrós et al. 2010; Young et al. 2011; Nichols et al. 2012; Fairclough et al. 2013; Paps et al. 2013; Sebé-Pedrós et al. 2013; Suga et al. 2013).

Because the progenitors of animals were likely bacterivores, bacterivory may have impacted genome evolution on the cusp of animal origins. For example, lateral gene transfer (LGT) from ingested prey may have provided one source of new genetic material in the progenitors of animals. As put forth by W. Ford Doolittle, the “you are what you eat” hypothesis proposes that because phagotrophy is an ancient means of obtaining nutrients and common among free-living heterotrophic protists, gene acquisition from food sources might have been a frequent basis for LGT (Doolittle 1998). Acquiring genes from the environment in this manner would require that intact DNA from donor species not only be integrated into the nucleus of a host eukaryote, but also appropriately expressed.

Choanoflagellate genomes show evidence of rampant gene acquisition through LGT. Yue et al. (2013) have calculated that ~4.4% of the *Monosiga brevicollis* genome derives from LGT, one of the highest rates reported for unicellular eukaryotes to date. The phosphofructokinase gene from *Monosiga ovata* may have originated in spirochetes (Baptiste et al. 2003) and pathways for amino acid biosynthesis in *M. brevicollis* also seem to have been acquired from bacteria (Torruella et al. 2009; Sun and Huang 2011). Intriguingly, *M. brevicollis* also appears to carry



a significant number of genes of algal origin (Nedelcu et al. 2008; Sun et al. 2010), and stramenopiles may have contributed biosilicification genes found in the genome of a loricate choanoflagellate (Marron et al. 2013) suggesting that an ancestor of extant choanoflagellates may have used both bacteria and other protists as food sources. The frequency of LGT in choanoflagellates suggests that LGT may also have been common in the phagocytic progenitors of animals.

The evolutionary history of teneurin, a neuronal membrane protein that regulates gene transcription during development, provides one example of a gene potentially acquired through bacterivory that persists in the animal lineage (Tucker et al. 2012). The extracellular domains of teneurins mediate homotypic cell–cell adhesion and are most similar to YD-repeat proteins, adhesins found in the cell envelopes of several bacterial groups. Acquisition of such a bacterial protein may have facilitated heterotypic interactions between ancient prey bacteria and their animal predators.

The constant exposure of the first animals to environmental bacteria likely provided opportunities for new interactions, both beneficial and antagonistic. Mediating these different kinds of interactions may have required animal progenitors to evolve mechanisms for distinguishing between different types of bacteria (e.g., prey vs. pathogen). Moreover, the process of phagotrophy involves processes—including cell adhesion, incorporation, and interpretation of cues and signals from the extracellular environment, and intercellular communication—that are also fundamental requirements for multicellularity (King 2004; Grosberg and Strathmann 2007). For example, phagocytosis is initiated by contact between prey and predator cells. Animal receptors that recognize bacterial molecules include C-type lectin receptors and Toll-like receptors (Beutler 2004), some of which contain immunoglobulin domains. Choanoflagellate genomes encode C-type lectins and immunoglobulin domains (King et al. 2003, 2008; Fairclough et al. 2013) suggesting that the last common ancestor of choanoflagellates and animals also may have used these do-

main for recognition of nonself/prey ligands. C-type lectins mediate cell adhesion in animals (Weis et al. 1998), therefore, it is also conceivable that mechanisms for predator–bacteria adhesion were co-opted for intercellular adhesion during the evolution of multicellularity.

Associations between animals and bacteria also may have provided adaptive advantages during mass extinctions, such as those occurring during the Phanerozoic (McFall-Ngai et al. 2013). By extending the nutritional capacity of their hosts, commensal bacteria may have allowed animals to adapt to changing environments and expand into new ecological niches. Bacteria enhance the biology of their animal hosts in a number of ways. In sap-sucking insects, gut bacteria synthesize essential amino acids (Baumann 2005). Gut bacteria also increase the bioavailability of nutrients, such as complex carbohydrates, in some mammals (Ley et al. 2008), and microbial consortia are essential for the proper maturation of the vertebrate immune system (Mazmanian et al. 2005; Bates et al. 2006). Bacteria also exert heritable effects on host gene function by affecting epigenetic factors such as histone modification, DNA methylation, noncoding RNA, and RNA splicing (Bierne et al. 2012). Thus, bacteria likely contributed to the evolution of the Urmetazoan genome at many levels.

CONCLUDING REMARKS

As the earliest organisms that evolved on Earth, bacteria and archaea laid the biological and ecological foundations for eukaryotic origins. Evidence of our bacterial past persists today in modern eukaryotes in the form of mitochondria, plastids, membrane composition, and certain classes of nuclear genes. Moreover, the earliest animals evolved in an environment already occupied by abundant and diverse bacteria. Bacteria likely had diverse ecological interactions with the first animals, including acting as pathogens, food sources, and purveyors of chemical signals that potentially influenced the physiology and morphology of eukaryotes, including the Urmetazoan. Animals are the only obligate multicellular organisms capable



of phagotrophy, and the bacterivorous lifestyle of the first animals potentially influenced animal genome evolution both directly through LGT and indirectly through the co-option of proteins required for prey capture into roles (e.g., intercellular adhesion and signaling) that were required for multicellularity.

The study of extant early-branching animals and their unicellular relatives promises to help illuminate the contributions of bacteria to animal origins. The conservation of collar cells in choanoflagellates and sponges suggests that bacterivory was important to the organismal biology of the first animals. Future studies of the mechanisms by which filter-feeding choanoflagellates and animals capture prey bacteria will help to explain the potential connections between bacterivory and evolution of animal multicellularity. Moreover, identifying the genes in choanoflagellates, sponges, and other early-branching animals that mediate interactions with bacteria may help to reveal the ancestry of development and innate immunity in animals. Finally, studying mechanisms by which environmental bacteria regulate the life cycles of choanoflagellates and diverse animals may begin to explain why stable associations with bacteria, once considered anathema to human health, are actually essential for the biology of most animals.

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R.A. Alegado and N. King

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R.A. Alegado and N. King

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