Symbiosis as a General Principle in Eukaryotic Evolution

Angela E. Douglas

Department of Entomology, Cornell University, Ithaca, New York 14853

Correspondence: aes326@cornell.edu

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Eukaryotes have evolved and diversified in the context of persistent colonization by nonpathogenic microorganisms. Various resident microorganisms provide a metabolic capability absent from the host, resulting in increased ecological amplitude and often evolutionary diversification of the host. Some microorganisms confer primary metabolic pathways, such as photosynthesis and cellulose degradation, and others expand the repertoire of secondary metabolism, including the synthesis of toxins that confer protection against natural enemies. A further route by which microorganisms affect host fitness arises from their modulation of the eukaryotic-signaling networks that regulate growth, development, behavior, and other functions. These effects are not necessarily based on interactions beneficial to the host, but can be a consequence of either eukaryotic utilization of microbial products as cues or host–microbial conflict. By these routes, eukaryote–microbial interactions play an integral role in the function and evolutionary diversification of eukaryotes.

Eukaryotes do not live alone. They bear living cells of bacteria (Eubacteria and Archaea), and often eukaryotic microorganisms, on their surfaces and internally without any apparent ill effect. Furthermore, there is now persuasive evidence that all extant eukaryotes are derived from an association with intracellular bacteria within the *Rickettsiales* that evolved into mitochondria (Williams et al. 2007), with the implication that this propensity to form persistent associations has very ancient evolutionary roots. In this respect, the eukaryotes are different from the bacteria, among which only a subset associate with eukaryotes, specifically members of about 11 of an estimated 52 phyla of Eubacteria (Sachs et al. 2011) and a tiny minority of Archaea (Gill and Brinkman 2011).

The current interest in the microbiota associated with eukaryotes stems from key technological advances for culture-independent analysis of microbial communities, especially high-throughput sequencing methods to identify and quantify microorganisms (Caporaso et al. 2011; Zaneveld et al. 2011). The Human Microbiome Project (commonfund.nih.gov), MetaHIT (metahit.eu), and other initiatives are yielding unprecedented information on the taxonomic diversity and functional capabilities of microorganisms associated with humans, other animals, and also plants, fungi, and unicellular eukaryotes (the protists), as well as abiotic habitats (Qin et al. 2010; Muegge et al. 2011; Human Microbiome Project 2012a; Lundberg et al. 2012; Bourne et al. 2013). Much of this

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research has focused on the Eubacteria, but eukaryotic members of the microbiota, especially the fungi, are increasingly being investigated (Iliev et al. 2012; Findley et al. 2013).

Although driven by technological change, these culture-independent studies of the microbiota of humans and other eukaryotes are having profound consequences for our conceptual understanding. In particular, there is a growing appreciation that the germ theory of disease, which has played a crucial role in improving public health and food production through the 20th century, has also led to the widespread but erroneous belief that all microorganisms associated with animals and plants are pathogens. This outmoded perception is increasingly being replaced by the recognition that eukaryotes are chronically infected with benign and beneficial microorganisms, and that disease can result from disturbance to the composition or activities of the microbiota (McFall-Ngai et al. 2013; Stecher et al. 2013).

This article reviews the pervasive impact of symbiosis with microorganisms on the traits of their eukaryotic hosts and the resultant consequences for the evolutionary history of eukaryotes. For the great majority of associations, the effects of symbiosis can be attributed to two types of interaction. The first interaction—"symbiosis as a source of novel capabilities"—is founded on metabolic or other traits possessed by the microbial partner but not the eukaryotic host. By gaining access to these capabilities, eukaryotes have repeatedly derived enhanced nutrition, defense against natural enemies, or other selectively important characteristics. The second interaction—"the symbiotic basis of health"—comprises the improved vigor and fitness that eukaryote hosts gain through microbial modulation of multiple traits, including growth rates, immune function, nutrient allocation, and behavior, even though the effects cannot be ascribed to specific microbial capabilities absent from the host. There is increasing evidence that the health benefits of symbiosis are commonly a consequence of microbial modulation of the signaling networks by which the growth and physiological function of eukaryote hosts are coordinated.

This article comprises three sections: the two types of interaction are considered in turn, with the key patterns and processes illustrated by specific examples from a range of symbioses in animals, plants, and other eukaryotes; and the concluding comments address some key unanswered questions about symbiosis in eukaryotes. This article does not review the full diversity of associations made in this article on the general principles of symbiosis in eukaryotic evolution; interested readers are referred to Douglas (2010).

SYMBIOSIS AS A SOURCE OF NOVEL CAPABILITIES

The Ancient Evolutionary Roots of Symbioses Founded on Metabolism

Eukaryotes have repeatedly gained access to complex metabolic capabilities by forming symbioses with other organisms that possess these functions. By acquiring the organism (not just the genes), eukaryotes have gained not only the genes coding specific enzymes, but also the genetic and cellular machinery for their regulated expression.

The capacity to acquire metabolic capabilities by symbiosis is evident in the common ancestor of all extant eukaryotes and can be linked to the metabolic limitations in the lineage giving rise to the eukaryotes. In particular, this lineage lacked the capacity for autotrophic carbon fixation and aerobic respiration; these traits had evolved in the Eubacteria, presumably more recently than the common ancestor of the two groups. The association with the α-proteobacterial ancestor of mitochondria probably evolved 1–2 billion years ago. All extant eukaryotes have mitochondria or are descended from mitochondriate ancestors, raising the possibility that the acquisition of mitochondria defined the evolutionary origin of eukaryotes (Embley and Martin 2006). (We cannot, however, exclude the alternative scenario that the ancestor of mitochondria was acquired by bona fide eukaryotic cells, with the subsequent extinction of all amitochondriate eukaryotic lineages.) Furthermore, the mitochondria have undergone

substantial evolutionary diversification, including the transformation into hydrogenosomes, functionally distinct organelles that generate ATP with the production of hydrogen and lack oxidative phosphorylation (Hjort et al. 2010). Hydrogenosomes have evolved multiple times in secondarily anaerobic eukaryotes, including various ciliate protists and the lorificeran animals in anoxic marine sediments (van der Giezen et al. 2005; Danovaro et al. 2010). The evolutionary history of eukaryotic acquisition of oxygenic photosynthesis by symbiosis with the cyanobacterial ancestor of chloroplasts is also complex. Unlike mitochondria, which evolved just once, chloroplasts have evolved from two different cyanobacterial groups (Keeling 2013). One cyanobacterial-derived chloroplast is allied to *Synechoccus* and has been reported in the rhizarian amoeba *Paulinella chromatophora* (Nakayama and Ishida 2009; Nowack and Grossman 2012). The other lineage is in the very successful Archaeplastids, including both algae (e.g., Rhodophytes, Chlorophytes) and terrestrial plants, with the subsequent evolution of some algae into secondary, and even tertiary, chloroplasts in further groups of eukaryotes (Keeling 2013).

Without doubt, the evolutionary and ecological success of the eukaryotes is founded on their acquisition of aerobic respiration and photosynthesis by symbiosis, with the transition of bacterial symbionts to organelles. Without these capabilities, the eukaryotes as a group would have been excluded from oxic habitats (occupied by the photosynthetic cyanobacteria and aerobic bacteria) and restricted to the low-energy lifestyle dictated by hypoxia and anoxia. The localization of electron transport chain in aerobic respiration and photosynthesis to the intracellular compartments of mitochondria and plastids, respectively, provided additional evolutionary opportunities for eukaryotes. The restriction of energy production to the organellar membranes, separate from cell membrane function, permitted large cell size and associated reduction in the ratio of (cell membrane surface area)/(cell volume) without prejudicing energy production (Lane and Martin 2010). (Bacterial cells with the respiratory chain localized to the cell membrane are generally restricted to small size with a high surface area:volume ratio, although some bacteria can attain relatively large cell sizes through various adaptations (Schulz and Jorgensen 2001). Furthermore, the bacterial-derived organelles represent additional subcellular compartments with key roles in metabolism (e.g., β-oxidation of fatty acids, steroid hormone synthesis) and signaling (e.g., apoptosis, calcium signaling), thereby enhancing cellular efficiency and capacity to mediate complex interactions.

Symbioses Founded on Primary Metabolism of Microbial Symbionts

Symbiosis with microorganisms is not restricted to associations involving eubacterial endosymbionts that evolved in the distant ancestors of modern eukaryotes. Rather, the capacity to acquire bacterial or eukaryotic microorganisms is a persistent theme in the biology of the eukaryotes and a major factor shaping adaptation of eukaryotes to different habitats and lifestyles, as well as the evolutionary diversification of many groups. Particularly striking examples are provided by the acquisition of photosynthetic algae (eukaryotic microorganisms that are, themselves, the product of symbiosis) by fungi, generating the lichens that account for nearly half of all Ascomycete fungal species, and by various animals, including the corals, whose capacity to form reefs in the photic zone is symbiosis dependent.

Furthermore, aerobic respiration and photosynthesis are not the only complex metabolic traits acquired by eukaryotes through symbiosis, although none of the microbial partners contributing these other traits are known to have evolved into organelles. Nitrogen fixation and chemosynthesis are two metabolic capabilities that are apparently absent from the ancestral eukaryote and have been acquired by multiple eukaryotic groups by symbiosis. Angiosperm plants of the eurosid clade are particularly predisposed to associate with nitrogen-fixing bacteria, notably the rhizobia (including *Rhizobium* and *Bradyrhizobium* in the α-proteobacteria) and the actinobacteria *Frankia*. The access to atmospheric nitrogen in these

plants facilitates their utilization of nitrogen-poor soils, for example, in early succession communities, and is recognized as a contributory factor to the evolutionary diversification of legumes, with their large, nitrogen-rich seeds protected by nitrogen-containing toxins, including alkaloids (Houlton et al. 2008; Corby et al. 2011). Nitrogen-fixing symbioses have also been reported in protists, for example, in marine diatoms (Foster et al. 2011), and some animals, notably the wood-feeding shipworms and some termites (Lechene et al. 2007; Desai and Brune 2012).

Chemosynthesis (the capacity to fix carbon dioxide using the energy derived from the oxidation of reduced substrates, such as hydrogen, hydrogen sulfide, and methane) is widely exploited by animals. The habitats where these associations flourish are zones of oxic/anoxic mixing, including aquatic sediments, hydrothermal vents, and hydrocarbon seeps. Many animals in these habitats bear chemosynthetic symbionts on their body surface (e.g., annelid worms), in gills (e.g., bivalve mollusks), or in a central tissue called the trophosome (in pogonophoran worms), and many display reduced capacity for feeding and a digestive tract that is much reduced or absent (Petersen et al. 2011; Roeselers and Newton 2012).

Various eukaryotes have compounded the metabolic limitations of their eukaryotic inheritance by the loss of further metabolic capabilities. Reduction of metabolic scope is particularly evident in the animals, which, as a group, lack the capacity to synthesize at least nine of the 20 protein amino acids (the essential amino acids) and various cofactors required for the function of enzymes central to metabolism (e.g., various vitamins). The arthropods have, additionally, lost the capacity for sterol synthesis. Generally, these lost metabolic capabilities comprise many reactions and tend to be energetically demanding (Wagner 2005). Organisms with an ample dietary supply of amino acids or cofactors would gain no advantage from retaining these capabilities (relaxed selection) and arguably the selective advantage of energetic efficiency from losing them. As predicted from these considerations, the sponges and choanoflagellates (the most basal animals, and closest relative of animals, respectively) feed on living organisms, especially bacteria, which provide a nutritionally balanced diet.

Repeatedly in the evolution of animals, symbiosis with microorganisms has enabled animals to escape from the nutritional constraint of feeding on a nutritionally balanced diet. Many microbial symbionts of animals have probably evolved by the progressive delay in animal digestion of food-associated microorganisms, raising the evolutionary scenario of a shift in the animal to a poor-nutrient diet coupled to the transition of nutrient acquisition by digestion to biotrophic release from intact microbial cells. Two diets for which animals require nutritional support from microbial symbionts are vertebrate blood and plant sap, as indicated by the possession of microbial symbionts by all animals feeding on these diets through the life cycle (Buchner 1965). For example, symbiotic microorganisms are borne in the gut or specialized cells of the obligately blood-feeding leeches, ticks, lice, and bedbugs, and they are believed to provide their animal hosts with B vitamins. Plant sap feeding has also evolved multiple times, but only among insects of the order Hemiptera (including whiteflies, aphids, cicadas, and planthoppers). These insects derive essential amino acids, nutrients in short supply in the plant phloem and xylem sap, and possibly vitamins from their microbial partners (Douglas 2009).

Microbial symbioses have also played an important role in animal utilization of structural plant material, including lignocellulose. Herbivory has evolved multiple times in the vertebrates and, where investigated, the extraction of energy from the ingested cellulose is dependent on cellulolysis by symbiotic bacteria in an anaerobic portion of the gut, known as the fermentation chamber. The waste products of the bacterial fermentation are short-chain fatty acids, such as acetic acid and butyric acid. These products are transported into the epithelial cells of the animal and via the blood to other organs, where they are used as a substrate for aerobic respiration (Karasov and Douglas 2013). Microbial degradation of ingested lignocellulose and

fermentation of cellulose are generally less important for invertebrate, especially insect, herbivores, but can make an important contribution to the carbon economy of insects, such as termites, wood roaches, wood wasps, and some beetles, that feed on wood products of very low nutritional content (Geib et al. 2008; Suen et al. 2010; Adams et al. 2011). Multiple factors may contribute to the greater contribution of microbial symbionts to vertebrates than insects feeding on structural plant material. They include the greater anatomical barriers to a fermentation chamber in a small, highly aerobic insect than in a larger vertebrate with a predominantly anoxic gut lumen, and the widespread occurrence of intrinsic cellulases (i.e., of animal origin) in invertebrates, including many insects, but apparently in no vertebrates (Davison and Blaxter 2005; Calderon-Cortes et al. 2012). Consequently, microbial symbiosis is a general principle for the evolution of herbivory in vertebrates, but its significance for invertebrate animals has to be assessed on a case-by-case basis.

Symbioses and Secondary Metabolism

Symbiotic microorganisms can also contribute to the secondary metabolism of their eukaryotic hosts. "Secondary metabolism" refers to metabolic reactions and pathways that do not contribute directly to organismal growth and reproduction but that can be crucial to the fitness of the organism in the natural environment. Secondary metabolism includes the synthesis of toxins, antibiotics, pheromones and allomones, and pigments, as well as the degradation of toxins and other secondary metabolites. The capacity of eukaryotes for secondary metabolism varies widely, even among closely related species, and the technical difficulties in working with secondary metabolism has led to various failures to recognize the role of microorganisms in the secondary chemistry of some eukaryotes, as well as some unsubstantiated claims of symbiotic secondary metabolism.

Two complementary approaches provide an excellent test for the role of microbial symbionts in secondary metabolism: identification of the relevant genes in the sequenced genome of the microbial partner, and perfect correlation between expression of the secondary metabolic capability in the host and presence of the microbial partner with these genes. This can be illustrated by the contribution of bacterial symbionts to the production of a biologically active class of secondary compounds, the polyketides. For example, the beetle *Paederus* bears bacteria of the genus *Pseudomonas*, which have the genes for polyketide synthase and linked reactions required for the production of the polyketide profile of these animals (Piel et al. 2004a), and elimination of the *Pseudomonas* in *Paederus* results in loss of polyketide production (Kellner 2001). Similarly, many grasses are protected from herbivory by alkaloids that are synthesized by clavicipitaceous fungal endophytes that ramify through the plant tissues (Fletcher and Harvey 1981), and the genetic basis of the fungal synthesis of one key alkaloid loline by the fungal endophyte in the grass *Lolium* has been established (Spiering et al. 2005). Secondary metabolites synthesized by microbial symbionts have also been shown to contribute to the protection against predators and pathogens in multiple benthic marine animals, including sponges (Piel et al. 2004b), bryozoans (Sharp et al. 2007), and tunicates (Kwan et al. 2012).

Just as secondary chemistry is very diverse, so are the routes by which eukaryotes have gained access to these often-complex biosynthetic pathways for secondary metabolite synthesis. This is illustrated by recent research on the source of carotenoid pigments in animals. Carotenoids have antioxidant properties and are also used as pigments. Animals, generally, are unable to synthesize carotenoids, and many derive carotenoids from their diet. However, the carotenoid profile of some insects is independent of a dietary supply. In one group of insects, the whiteflies, the carotenoids are of symbiotic origin, with the carotenoid biosynthesis genes coded by its bacterial symbiont *Portiera* (Sloan and Moran 2013). In two other groups, the aphids and spider mites, the carotenoid biosynthesis genes are encoded in the animal genome, following independent lateral transfers from fungi (Moran and Jarvik 2010; Altincicek et al. 2012; Novakova and Moran 2012).

There is one further aspect to secondary metabolism that deserves careful consideration: the contribution of resident microorganisms to the detoxification of secondary metabolites. This is currently a "hot topic" in biomedical research, in the light of evidence that the activities of the gut microbiota can determine the half-life and metabolic fate of orally administered drugs, with clinically important implications for drug efficacy and toxicity (Haiser and Turnbaugh 2012; Holmes et al. 2012; Haiser et al. 2013). An analogous issue has been of persistent concern in the discipline of herbivory for decades. Although herbivorous animals generally possess a diverse array of detoxifying enzymes, including cytochrome P450 monooxygenases, glutathione *S*-transferases, and esterases (Despres et al. 2007), symbiotic microorganisms have long been invoked to mediate the detoxification of plant allelochemicals (Jones 1984; Berenbaum 1988; Dillon and Dillon 2004). Supportive evidence comes from a few systems. The microbiota in the foregut fermentation chamber (the rumen) of ruminant mammals has the potential to detoxify ingested allelochemicals in the food, as illustrated by the reported capacity of the rumen microbiota of indigenous Indonesian cattle to degrade the toxic amino acid mimosine in the tropical legume *Leucaena leucocephala* (Cheeke 1994). Similarly, the capacity of reindeer to use reindeer moss (a lichen, *Cladonia rangiferina*) has been attributed to a bacterium *Eubacterium rangiferina* in the reindeer rumen, which can metabolize usnic acid, an abundant metabolite in the lichen that is toxic to most animals (Sundset et al. 2008, 2010). Plant allelochemical detoxification has also been implicated in certain insect symbioses, notably bark-feeding beetles and leaf-cutting ants. The gut microbiota of the mountain pine beetle *Dendroctonus ponderosae* include bacteria of the genera *Pseudomonas*, *Serratia*, *Rahnella*, and *Burkholderia* that bear genes involved in the degradation of terpenes, the principal defensive compounds of the trees infested by these beetles (Adams et al. 2013). The leaf-cutting ant *Acromyrmex echinatior* maintains, and feeds on, the symbiotic fungus *Leucocoprinus gongylophorus* in its nest. The fungus genome has multiple genes for laccases (a type of phenoloxidase). Enzymatically active laccase enzyme is passed through the gut of ants feeding on the fungus and released onto plant material, where it can degrade plant compounds, such as tannins and flavonoids (De Fine Licht et al. 2013).

THE SYMBIOTIC BASIS OF HEALTH

The function of all living organisms is orchestrated by signaling networks that coordinate processes within individual cells and, for multicellular forms, also among cells, tissues, organs, and so on. It has long been known that these regulatory networks can be manipulated by pathogens and parasites, with various consequences ranging from the reorganization of the cytoskeleton and intracellular trafficking to the restructuring of host growth patterns, reproductive schedules, and behavior (Gandon et al. 2002; Bhavsar et al. 2007; Hughes et al. 2012). It is now becoming increasingly clear that the nonpathogenic resident microorganisms can also modulate the signaling networks of their eukaryotic hosts and that these manipulations are generally advantageous for the host. Although the data are still fragmentary, various lines of evidence are consistent with the hypothesis that eukaryotes derive health benefits from symbiosis because their regulatory networks are structured to function in the context of interactions with the resident microbiota.

The health benefits of symbiosis can be illustrated by plant-growth-promoting rhizobacteria (PGPRs), including strains of *Azospirillum*, *Bacillus subtilis*, *Pseudomonas putida*, and *Enterobacter cloacae*. As the term suggests, PGPRs are associated with plant roots and promote plant growth. The underlying processes are complex and variable, but very commonly involve the capacity to synthesize signaling molecules, including plant hormones, for example, the auxin indole-3-acetic acid (IAA), and volatiles, for example, 2,3-butanediol (Spaepen et al. 2007; Lugtenberg and Kamilova 2009; Roca et al. 2013). The best-studied interaction relates to PGPR-derived IAA, which triggers increased root branching and a higher densi-

ty of root hairs. This altered root morphology enhances nutrient uptake from the soil, thereby promoting plant growth (Dobbelaere et al. 1999; Steenhoudt and Vanderleyden 2000). The important implication of these results is that the plant regulatory networks controlling the pattern of plant growth are not structured to generate optimal growth of microbe-free plants, which is achieved only in the context of signal exchange with associated rhizosphere bacteria.

The IAA-producing PGPRs are an example of resident microorganisms that influence host growth by the release of molecules that are also an integral part of the host-signaling network (in this case, a plant hormone). Other compounds produced by microbial symbionts are not known elements of the host-signaling network but, nevertheless, act to modulate (amplify or dampen) host signaling. One example comes from research on the association between the *Drosophila* fruit fly and its gut microbiota. When the microorganisms are eliminated, the *Drosophila* display depressed development rates and elevated levels of lipid and circulating glucose, similar to the phenotypic traits of flies with impaired insulin signaling (Shin et al. 2011). Flies infected with a mutant of a dominant bacterial symbiont, *Acetobacter pomorum*, that cannot produce the enzyme pyrroloquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH) also display these traits, together with reduced expression of the genes for insulin-like peptides (*dilp-3* and *dilp-5*). PQQ-ADH mediates the oxidation of ethanol to acetic acid. When acetic acid was supplied in the food, development rates, lipid and glucose contents, and *dilp* gene expression in flies bearing the mutant bacteria shifted to values found in conventional flies. These data suggest that acetic acid produced by wild-type *A. pomorum* stimulates insulin signaling in the fly. Although the processes by which acetic acid interacts with insulin signaling are unknown, the resultant speeding of larval development is advantageous for the insect, which develops in rotting fruit and must complete larval development before the fruit resources are exhausted. As with the IAA-producing PGPRs associated with plant roots, the acetic-acid-producing bacteria in fruit fly guts promote host health and vigor by increasing the amplitude of host signaling.

The hyperlipidemia and hyperglycemia displayed by *Drosophila* containing mutant *A. pomorum* are reminiscent of the elevated lipid and glucose levels in the laboratory mouse treated with antibiotics that alter the composition of the gut microbiota (Cho et al. 2012). The gut microbiota of the mouse can also be altered by diet or mutation, especially of the mouse immune system and nutrient signaling, with correlated phenotypic lesions, especially in nutrient allocation and immune function (Maslowski and Mackay 2011; Claesson et al. 2012; Maynard et al. 2012). Transplant studies suggest that the altered microbial community likely contributes to the altered host phenotype. When introduced to germ-free control mice (reared on the standard diet/of wild-type genotype), the recipients display similar deleterious phenotypic traits (Vijay-Kumar et al. 2010; Smith et al. 2013). These various data sets reinforce the growing appreciation that the composition and activities of the resident microbiota are important for host health, such that perturbation of the microbiota can contribute to chronic ill health, a condition known as "dysbiosis." The concept of dysbiosis, first coined a century ago (Metchnikoff 1910), has gained relevance in the context of hypotheses put forward to account for the increase in immunological and metabolic disease in humans, with reduced exposure of children to environmental microorganisms (hygiene hypothesis) or to specific members of the human resident microbiota (disappearing microbiota hypothesis) as possible drivers (Strachan 1989; Blaser and Falkow 2009).

Given the fitness costs of dysbiosis, the susceptibility of the regulatory circuits of animals and plants to modulation by their resident microbiota appears as a very real vulnerability. How is it that eukaryote-signaling networks are not insulated from the influence of resident microorganisms, but appear to be under joint host–microbial control?

Two sets of processes may contribute to the symbiotic basis of health. The first is that microorganisms provide a reliable cue for current or

future environmental conditions. Eukaryotes that respond appropriately to the microbial products, that is, display regulated changes in growth or developmental patterns, behavior, and the like, would be at a selective advantage. For example, the profile of metabolic end-products from bacteria associated with rotting fruit may provide a reliable cue for the longevity of the fruit resource, enabling *Drosophila* larvae to titrate their developmental time to environmental circumstance. Specifically, the amplified insulin production in response to *Acetobacter*-derived acetic acid increases developmental rates, and this may be highly adaptive in the natural environment. Microbial products are used as cues by various eukaryotes. For example, a sulfonolipid released from specific bacteria, *Algoriphagus machipongonensis*, induces colony formation in choanoflagellates (Alegado et al. 2012); *N*-acyl homoserine-lactones (quorum-sensing molecules) released from *Vibrio* bacteria promote settling of motile zoospores of the green alga *Enteromorpha* (Joint et al. 2002); and compounds released from the bacterium *Pseudoalteromonas* associated with the substrate in marine environments promote the settling and metamorphosis of barnacle larvae (Hadfield 2011).

I describe these various compounds as cues because their production by microorganisms is independent of the response of the eukaryotic host, that is, whether and how a eukaryote derives information from microbial compounds has no effect on their production by the microorganisms. If a microbe-derived compound is a reliable cue, the signaling network of the eukaryote may evolve to increasing dependence on that cue through reduced responsiveness to other environmental factors, and this may result in dependence on specific microbial products for sustained function of the signaling network and, ultimately, host health.

The alternative basis for microbial impacts on host-signaling networks derives from conflict between the microorganisms and host. Conflict is inevitable because of incomplete selective overlap between a eukaryotic host and its resident microbiota. A eukaryote is a nutrient-rich patch in which microorganisms tolerant of the immune system and other defenses can proliferate, and it is also frequently a route for dispersal. Consequently, many microorganisms have no selective interest in the reproductive output of their host. (Exceptionally, the various maternally inherited forms are in conflict with the host over host sex ratio.) Thus, microorganisms in the animal gut may favor hyperphagia despite its negative consequences for host fitness, modulate gut peristalsis rates to optimize their residence time at the expense of optimal nutrient absorption by the host, and dampen immune responses to favor their persistence (de La Serre et al. 2010; Round et al. 2011; Matsumoto et al. 2012; Maynard et al. 2012).

Counterintuitively, host–microbial conflict over the amplitude of host-signaling pathways can lead to host dependence, but only where the prevalence of the interacting microorganisms in the host population is very high. This important effect was first shown by research on the relationship between a parasitic wasp, *Asobara tabida*, and the vertically transmitted bacterium *Wolbachia*. Several lines of evidence suggest that *Wolbachia* induces oxidative stress and a linked dampening of apoptotic signaling, probably as a result of disruption of iron metabolism (Kremer et al. 2009). As a likely consequence, elimination of *Wolbachia* by antibiotic treatment results in massive apoptosis (programmed cell death) of the ovaries, leaving the wasp reproductively sterile. It has been argued that, over evolutionary time, the wasp apoptotic pathways have gained heightened responsiveness, in compensation for the inhibitory manipulation by *Wolbachia* (Pannebakker et al. 2007). Importantly, this effect is constitutive (presumably because *Wolbachia* is always present under natural conditions), with the consequence that appropriate apoptotic signaling requires the manipulative intervention of the *Wolbachia* bacteria. Analogous host compensation for microbial manipulation may contribute to the multiple demonstrations of interactions between the resident microbiota and the developmental, nutritional, neurological, and immunological health of animals (Stappenbeck et al. 2002; Smith et al. 2007; Bravo et al. 2011; Olszak et al. 2012). Additionally, host dependence on

microbial products for sustained health may be retained in eukaryotic lineages, where the conflict is resolved because of the sheer complexity of signaling networks. Elimination of microbial modulation of one pathway may be selected against because the multiple, knock-on effects on linked signaling pathways are highly deleterious. Ultimately, the symbiotic basis of host health may only be explicable in the context of the deep evolutionary history of interactions between eukaryotes and their resident microbiota.

CONCLUDING COMMENTS

It has taken a long time for biologists generally to appreciate the significance of associations with symbiotic microorganisms for eukaryotes. Despite the discovery of the dual nature of lichens (fungi and algae/cyanobacteria) and near-ubiquitous associations between plant roots and mycorrhizal fungi in the 1800s, symbioses were treated as mere curiosities of nature through much of the last century. The overwhelming molecular evidence for bacterial origins of mitochondria and plastids, together with the realization that the resident microbiota is vital for the human health and productivity of crops and livestock has, at last, placed symbiosis in the mainstream of biology. And yet, multiple questions central to our understanding of the function and evolution of symbioses remain.

Tremendous opportunities are being provided by the latest sequencing technologies. Although currently used mostly to catalog the composition and activities of bacterial communities (Human Microbiome Project 2012b), these methods are starting to be applied to investigate both the diversity of eukaryotic microbial symbionts and how microbial communities are structured (Costello et al. 2012). A key unresolved question is whether the functional traits of resident microorganisms can be predicted from their taxonomic composition. Perhaps such a relationship is frequently confounded by microbe–microbe and microbe–host interactions, resulting in spatiotemporal variation in the traits of microorganisms. Recent advances in single-cell imaging and single-cell sequencing (Pamp et al. 2012; Pernice et al. 2012; Lasken 2013) offer unprecedented opportunities to investigate such heterogeneity.

These ecological issues segue into longstanding evolutionary questions, particularly whether the microbiota can influence the speciation patterns and evolutionary diversification of their hosts (Brucker and Bordenstein 2013). In certain instances, evolutionary change may be facilitated by among-microbe transfer of symbiosis-related gene clusters via "symbiosis islands" (Finan 2002), equivalent to pathogenicity islands in pathogenic bacteria, or by the displacement of one microbial partner by another taxon with different traits (Koga et al. 2013; Toju et al. 2013). With the dramatically improving genomic technologies, it is becoming increasingly feasible to answer these fundamental questions.

We should also recognize that most current research is focused on a tiny subset of the diversity of symbioses, notably a few phyla of Eubacteria associated with animals, especially humans and laboratory mice. A major outstanding issue is the taxonomic and functional diversity of symbioses across the evolutionary radiation of eukaryotes. In particular, relatively little is known about symbioses involving protists as either host or symbiont, even though the protists account for most of the evolutionary diversity of eukaryotes. Some of these associations are apparently without parallel in animals or plants, for example, motility conferred on large protists by spirochete ectosymbionts (Cleveland and Grimstone 1964), and protection from predators by extrusive structures associated with bacterial symbionts of the ciliate *Euplodinium* (Petroni et al. 2000). Reinvigorated research on symbioses involving protists has the potential to expand and modify our concept of the general principles of symbiosis.

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Angela E. Douglas

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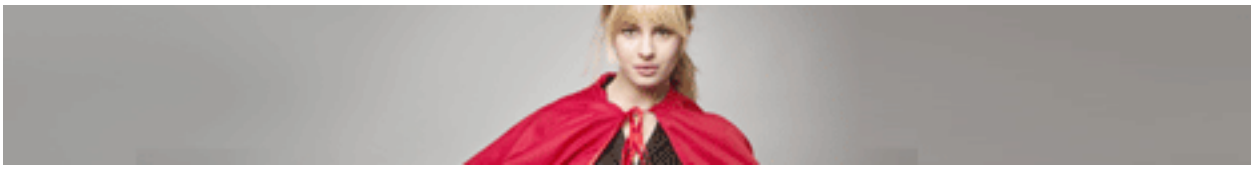

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