FEMS Microbiology Reviews, 1–26
DOI: 10.1093/femsre/fuad005
Advance access publication date: 7 March 2023
Review Article

The coral microbiome: towards an understanding of the molecular mechanisms of coral–microbiota interactions

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Editor: [Bart Thomma]

Abstract

Corals live in a complex, multipartite symbiosis with diverse microbes across kingdoms, some of which are implicated in vital functions, such as those related to resilience against climate change. However, knowledge gaps and technical challenges limit our understanding of the nature and functional significance of complex symbiotic relationships within corals. Here, we provide an overview of the complexity of the coral microbiome focusing on taxonomic diversity and functions of well-studied and cryptic microbes. Mining the coral literature indicate that while corals collectively harbour a third of all marine bacterial phyla, known bacterial symbionts and antagonists of corals represent a minute fraction of this diversity and that these taxa cluster into select genera, suggesting selective evolutionary mechanisms enabled these bacteria to gain a niche within the holobiont. Recent advances in coral microbiome research aimed at leveraging microbiome manipulation to increase coral's fitness to help mitigate heat stress-related mortality are discussed. Then, insights into the potential mechanisms through which microbiota can communicate with and modify host responses are examined by describing known recognition patterns, potential microbially derived coral epigenome effector proteins and coral gene regulation. Finally, the power of omics tools used to study corals are highlighted with emphasis on an integrated host–microbiota multiomics framework to understand the underlying mechanisms during symbiosis and climate change-driven dysbiosis.

Keywords: coral, microbiome, symbiosis, holobiont, multiomics

Introduction

Corals are metaorganisms that depend on dynamic multipartite symbioses with diverse microbes. These interkingdom interactions between the multicellular eukaryotic coral host and its associated microbiota maintain homeostasis within this complex system and has underpinned its resilience for >500 million years (Jaspers et al. 2019, Robbins et al. 2019, Peixoto et al. 2021). Associations within the metaorganism comprise a large diversity of viruses, prokaryotes, and microeukaryotes that collectively are termed the coral holobiont (Rohwer et al. 2002, Rosenberg et al. 2007, Rosenberg and Zilber-Rosenberg 2018, Zilber-Rosenberg and Rosenberg 2021). Chief among the holobiont microbes, the primary endosymbiotic dinoflagellate of the family Symbiodiniaceae provides the bulk of the required nutritional needs to their coral hosts (Muscatine 1990, Morris et al. 2019). In addition, an increasing body of evidence is unraveling the key roles particular bacterial species in specific and general prokaryotic communities play in maintaining holobiont fitness, potentially via exchanging essential metabolites, recycling nutrient, and providing protection against pathogenic microbes (Bourne et al. 2016). In the Anthropocene era, climate change disrupts these symbiotic relationships, leading to dysbiosis that is characterized by the overgrowth of opportunistic and putatively pathogenic microbes and results in a compromised coral immune system, inevitably causing the onset of coral bleaching and/or disease (van Oppen and Blackall 2019). Most coral microbiome work has been exclusively focused on either endosymbiotic algae or bacteria, while ignoring the other, largely underexplored members of the coral microbiomes due to difficulties associated with studying their role in the holobiont. This imbalance hinders our detailed understanding of the coral holobiont system.

In this review, we provide the latest information on the taxonomic and functional diversity of members of the coral microbiome, focusing on (a) specific microbes that engage in beneficial or harmful interactions with their host, (b) the role these microbes presumably play in coral health or disease, (c) the potential mechanisms of coral–microbiome crosstalk and communication, and (d) new techniques and approaches to further our understanding of the coral holobiont. Our aim is to provide insights into the potential mechanisms through which coral–microbiome interactions occur, and how these mechanisms can be studied to unravel the governing principles of the coral holobiont ecology in a warming ocean.

The coral holobiont

Corals are a reservoir for microbes that includes diverse species of bacteria, archaea, viruses, and microeukaryotes (Bourne et al. 2016), some of which are well-characterized while others are cryp-

Received: October 7, 2022. **Revised:** February 10, 2023. **Accepted:** February 15, 2023

tic (Fig. 1). The best-known coral symbionts are select bacteria and members of the dinoflagellate family Symbiodiniaceae (collectively known previously as the genus *Symbiodinium*). Bacteria colonize all coral microhabitats including the surface mucous layer (SML), different tissue layers, and skeleton (Sweet et al. 2010, Pollock et al. 2018, van Oppen and Blackall 2019), while Symbiodiniaceae inhabit specific host-derived membrane structures called symbiosomes (Davy et al. 2012, Mohamed et al. 2016, Rosset et al. 2021) within the gastrodermis layer (Fig. 1). In addition, a plethora of under-explored microbes are also associated with corals, including newly discovered apicomplexan-related chromerids and corallicolids (Ainsworth et al. 2017, Clerissi et al. 2018, Kwong et al. 2019), endolithic algae, viruses, archaea, and fungi all with mostly unknown function (Fig. 1).

Complex associations with the Symbiodiniaceae family

Symbiodiniaceae were the first and most important symbionts of corals to be recognized (Muscatine and Porter 1977, Bourne et al. 2016, LaJeunesse et al. 2018). They live exclusively in a host-derived compartment known as the 'symbiosome' that originates from the early endosome of the coral host following phagocytosis of these algal symbionts (Fig. 1) (Davy et al. 2012). The symbiosome membrane protects Symbiodiniaceae cells from lysosome degradation by the host (Mohamed et al. 2016) and mediates the mutual transport of nutrients between both taxa (Davy et al. 2012). Considered initially as a single species, *Symbiodinium microadriaticum* (Freudenthal 1962), this group of symbionts now comprises the recently established dinoflagellate family Symbiodiniaceae, which currently includes seven distinct genera (*Symbiodinium*—formerly known as Clade A, *Breviolum*—clade B, *Cladocopium*—clade C, *Durusdinium*—clade D, *Effrenium*—clade E, *Fugacium*—clade F, and *Gerakladium*—clade G) (LaJeunesse et al. 2018). Multiple reference genomes for Symbiodiniaceae are available (Shoguchi et al. 2013, Lin et al. 2015, Aranda et al. 2016, Gonzalez-Pech et al. 2021), including a chromosome-scale genome for *S. microadriaticum* (Nand et al. 2021). These genomes reveal that this family is taxonomically and functionally divergent, a fact reflected in their diverse functional repertoire (González-Pech et al. 2021).

The coral–Symbiodiniaceae symbiosis is likely highly complex as other endosymbiotic associations. Research indicates that metabolite exchange between Symbiodiniaceae and corals involve sugars, lipids, and nitrogen compounds (reviewed in Davy et al. 2012). However, these metabolites may vary in identity and importance among the various associations given the high divergence reported in the Symbiodiniaceae; despite this knowledge, the molecular mechanisms (pathways or molecules) that establish and maintain this interaction is unknown. Few insights into functions and pathways that could enable this symbiotic relationship were established using comparative genomics and transcriptomics (Aranda et al. 2016, Liu et al. 2018, Mohamed et al. 2020a). For example, Aranda et al. (2016) showed that Symbiodiniaceae genomes possess an extensive repertoire of carbon and nitrogen transporters that likely underpin their symbiotic lifestyle and ultimately influence their hosts' physiology. Comparative analysis of four Symbiodiniaceae draft genomes against other dinoflagellate genomes revealed identification of gene families under positive selection that included genes involved in photosynthesis, transmembrane ion transport, amino acid synthesis and transport, and stress responses (Liu et al. 2018). These functions may enable Symbiodiniaceae to be ideal partners to corals. In addition, these processes were shown to be activated during early interactions with coral larvae. Metatranscriptomics revealed up-regulation of specific algal genes involved in carbohydrate, lipid, and nitrogen metabolism, and transport of various metabolites (glycerol, glutamate, choline) during colonization of coral larvae (Mohamed et al. 2020b). More recently, simultaneous transcriptome, metabolome, and proteome data for three ecologically important Symbiodiniaceae isolates have become available (Camp et al. 2022). The availability of such large-scale omics data will inevitably increase our understanding of the molecular characteristics that underpin Symbiodiniaceae responses during their lifestyle changes and environmental stress.

Diverse bacterial symbionts associated with corals

Corals harbour a diverse bacterial microbiome (Blackall et al. 2015), spanning 39 phyla (Huggett and Apprill, 2019), more than one-third of the bacterial phyla found in seawater (Chen et al. 2021). A proportion of these coral-associated bacterial assemblages are thought to support the health and resilience of corals (Bourne et al. 2016, Ziegler et al. 2019, Voolstra and Ziegler 2020, Meunier et al. 2021). Among the numerous bacterial phyla associated with corals, Proteobacteria, Bacteroidetes, Cyanobacteria, and Firmicutes are among the most abundant based on 16S rRNA gene phylogeny of 21 100 sequences derived from a public database (Huggett and Apprill, 2019). Moreover, a recent meta-analysis of 3055 bacterial isolates from 52 coral studies identified that most cultivable bacteria belonged to the Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria phyla (Sweet et al. 2021).

The collective reef microbiome may rapidly respond to environmental stressors, such as ocean warming, eventually leading to reef microbialization. Reef microbialization is characterized not only by a shift in abundance and biomass towards microbes, but more of a shift towards a pathogenic assemblage that can trigger major declines (Haas et al. 2016). Coral-associated bacteria inhabit several compartments within the coral, such as the SML, tissues, gastric cavity, and skeleton (Fig. 1) (Pollock et al. 2018, van Oppen and Blackall 2019, Vanwonterghem and Webster 2020). Distinct physiochemical properties and environmental gradients, including pollution (Wangpraseurt et al. 2016, Pernice et al. 2020) play an important role in shaping the microbial composition within these compartments (Sweet et al. 2010, Leite et al. 2018, Pollock et al. 2018). Bacterial composition varies across these different niche compartments with some bacteria preferentially colonizing specific compartments. For example, bacteria belonging to the genera *Chloroflexi*, *Sphingobacterium*, *Roseobacter*, and *Pseudoalteromonas* were found exclusively in the SML (Sweet et al. 2010), while *Endozoicomonas* were found within aggregates inside coral tissues (Neave et al. 2017). This niche specificity suggests certain bacteria are adapted to the local microenvironment within the coral colony (Ritchie and Smith 2004), which ultimately leads to particular interactions with the host within each microenvironment. More diverse bacterial communities have been reported in the coral skeleton compared to those in the coral tissue or the SML (Pollock et al. 2018).

Nitrogen cycling is common within corals [reviewed in Rädecker et al. (2015)]. Diazotrophs are consistently associated with coral tissues (Rohwer et al. 2002, Lema et al. 2012, Olson and Lesser 2013), particularly in early life stages (larvae and juveniles) (Lema et al. 2014), indicating the potential importance of nitrogen fixation in the coral holobiont. Ammonium generated from nitrogen fixation may be partially oxidized by communities of ammonia oxidizing bacteria and archaea (Beman et al. 2008, Siboni et al. 2008, Yang et al. 2013). Likewise, denitrifying bacteria have also been reported in corals (Kimes et al. 2010, Yang et al. 2013).

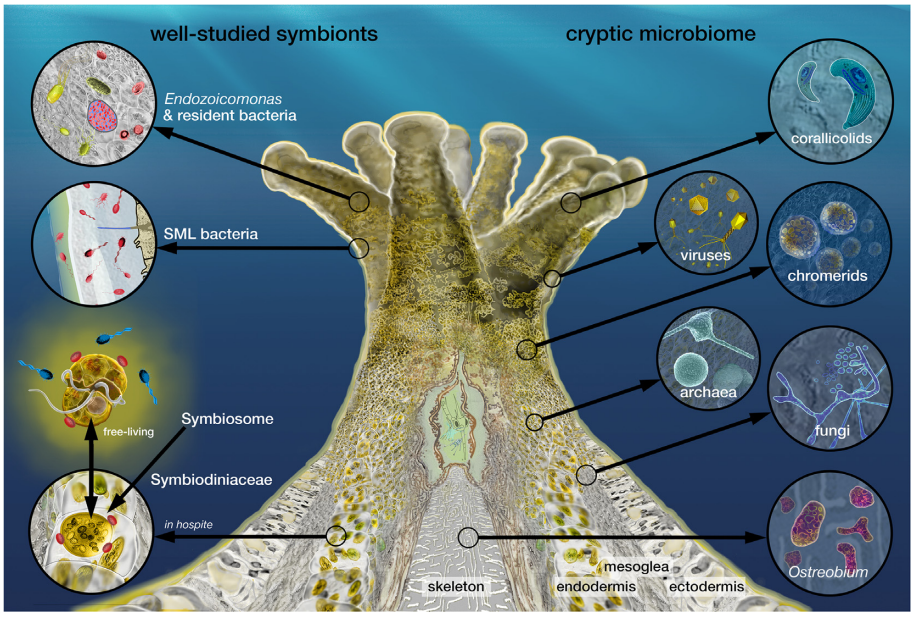

Figure 1. The diverse microbiome of corals. The coral-associated microbiome is distributed across specific locations in a coral colony and is composed of diverse microbes spanning the three domains of life. Symbiodiniaceae and bacteria are among the most-studied coral symbionts (left). Symbiodiniaceae are localized within specialized coral structures called symbiosomes within the gastrodermis layer and are by far the best-studied symbiont of corals. Resident bacteria are found in most coral microhabitats, including the SML, coral tissue, and skeleton. Recent work on resident bacteria focuses on the importance of *Endozoicomonas* spp. as putative obligate coral symbionts. Most of the coral microbiome is considered cryptic, with mostly unknown roles in holobiont homeostasis (right). Some of these members can be endosymbiotic (e.g. corallicolids), while others appear to be epibionts (e.g. Chromerids). Evidence suggests some archaea may be involved in nitrogen cycling, viruses may be important in maintaining microbiome homeostasis, while endolithic communities (*Ostreobium* and fungi) are implicated in primary production.

More recently, Rädecker et al. (2022) have reported the tight relationship between disturbance in nitrogen cycling and coral bleaching. However, the molecular mechanisms by which the nitrogen-related activities of these microbial communities are coupled are largely unknown.

Like all phytoplankton, Symbiodiniaceae associate with bacteria that play a role in their physiology and influence nutrient availability (Seymour et al. 2017). Members of the family Rhodobacteraceae are universally found among many phytoplankton lineages, including numerous Symbiodiniaceae cultures, and have been shown to play major roles in providing essential nutrients, hormones, and cofactors to phytoplankton (Cirri and Pohnert 2019). For example, *Mameliella alba* was shown to enhance the growth of Symbiodiniaceae in co-culture (Varasteh et al. 2020), similar to how other Rhodobacteraceae bacteria enhance the growth of diatoms (Amin et al. 2015) and coccolithophores (Segev et al. 2016). While Symbiodiniaceae–bacteria co-culture experiments are reductive, interactions between both taxa are hypothesized to occur within coral symbiosomes (Garrido et al. 2021). Indeed, nitrogen transfer between bacterial isolates labelled with ^{15}N and Symbiodiniaceae cells has been observed at the single-cell level in the coral *Pocillopora damicornis* (Ceh et al. 2013). Despite these findings, information on specific coral symbionts, opportunists, parasitic, and commensal bacteria and their importance to the coral holobiont is scarce.

Cryptic diversity of the coral microbiome

In addition to Symbiodiniaceae and bacteria, corals are home to a plethora of other microorganisms, including viruses, archaea, fungi (Bourne et al. 2016, Ainsworth et al. 2017, Clerissi et al. 2018), and microeukaryotes, including the apicomplexan-like Chromerids (Moore et al. 2008, Janouškovec et al. 2012) and the recently discovered apicomplexans Corallicolids (Kwong et al. 2019, Keeling et al. 2021). Below is a brief discussion of these mostly cryptic organisms.

Apicomplexans and related organisms

Early work has isolated and identified several lineages of apicomplexans associated with corals. *Gemmocystis cylindrus* was isolated from the gastrodermal cells of multiple corals (Upton and Peters 1986). Further molecular evidence of the existence of related apicomplexans included detection of DNA fragments in Caribbean corals (Toller et al. 2002, Kirk et al. 2013). Analysis of plastid rRNA sequences derived from coral reef environments revealed eight distinct, novel apicomplexan-related lineages associated with corals (Janouškovec et al. 2012). Two of these lineages, *Chromera velia* (Moore et al. 2008) and *Vitrella brassicaformis* (Oborník et al. 2012), comprise photosynthetic alveolates of the phylum Chromerida that are commonly associated with corals worldwide, and are considered the closest known photosynthetic relatives of Apicomplexan parasites (Moore et al. 2008, Janouškovec et al. 2013). More recently, a third apicomplexan taxon has been found ubiquitously associated with corals, potentially being the second most abundant microeukaryotic group in coral tissues after Symbiodiniaceae (Kwong et al. 2019). This taxon belongs to corallicolids (Kwong et al. 2019), a lineage that may be ubiquitous in the oceans including in metagenomes of deep-sea corals (Vohsen et al. 2020). Little is known about the biology of corallicolids or their influence on coral health/fitness, but it is unlikely that corallicolids have a mutualistic relationship with corals (Keeling et al. 2021). A recent transcriptomic study revealed that the coral host response to *C. velia* inoculation was similar to that of parasite or pathogen infection in vertebrates, suggesting that their relationship with corals is not beneficial (Mohamed et al. 2018). Further work is needed to employ inoculation experiments

and subsequent time-series multiomics analyses to elucidate the nature of the coral–corallicolids association.

Endolithic algae and fungi

Endolithic algae form dense bands visible to the naked eye in the skeleton of many coral species and are often dominated by the filamentous green alga *Ostreobium* spp. (Chlorophyta) (Fig. 1) (Kornmann and Sahling 1980). Molecular studies revealed highly diverse communities within this group of green algae (Marcelino and Verbruggen 2016, Del Campo et al. 2017, Verbruggen et al. 2017, Marcelino et al. 2018). More than 120 operational taxonomic units at the near-species level have been reported from 132 coral skeleton samples collected from multiple coral species (Marcelino and Verbruggen 2016). These endolithic communities were shown to substantially vary in identity among coral species. Marcelino et al. (2018) reported a more diverse endolithic community in the massive coral *Porites* spp. compared to the branching species *Seriatopora hystrix* and *Pocillopora damicornis*, suggesting that endolithic algae contribute to the resilience of the former to environmental stress. *Ostreobium* colonizes the skeleton of coral juveniles during their development (Masse et al. 2018) and can interact with the coral tissue through transfer of photosynthates (Schlichter et al. 1995, Fine and Loya 2002, Pernice et al. 2020), particularly after bleaching (Iha et al. 2021), and by enhancing coral recovery post-bleaching via reducing skeletal light reflectance (Galindo-Martinez et al. 2022).

Fungi are known to be associated with many sessile marine invertebrates including corals and sponges (Yarden 2014). In corals, fungi are found in newly deposited coral skeleton along with *Ostreobium* (Le Campion-Alsumard et al. 1995, Golubic et al. 2005), exhibiting rapid growth to match skeletal accretion (Le Campion-Alsumard et al. 1995). Fungi were identified as the most abundant microbes in the metagenome of *Porites astreoides*, contributing more than a third of the total microbial sequences (Wegley et al. 2007). Despite their ubiquitous associations with corals (Yarden 2014), including deep-sea corals (Marchese et al. 2021), their functions remain largely underexplored (Roik et al. 2022). Histological studies show widespread fungal invasion in corals infected with the coral disease white syndrome (Work and Aeby 2006, Howells et al. 2020) [reviewed in Sexton and Howlett (2006)]. In our recent work, reads belonging to the phylum Ascomycota in metagenomes of *Acropora* spp. under heat stress were observed, with higher relative abundance in colonies infected with white syndrome, potentially implicating them in disease manifestation (Amin et al., personal communication). Similarly, more diverse fungal communities were found in *Acropora hyacinthus* colonies living in warm pools compared to colder pools; these communities were also more transcriptionally active in warmer conditions (Amend et al. 2012), implicating them in responses to heat stress.

Archaea

Corals are associated with diverse archaeal species, mainly representatives from the phyla Thermoproteota (also known as Crenarchaeota) and Euryarchaeota. Members of the Thermoproteota are the most commonly reported followed by Marine Group II and Thermoplasma of the Euryarchaeota (Kellogg 2004, Siboni et al. 2008). Archaea can comprise up to half of the prokaryotic community on the SML of some corals (Wegley et al. 2004). Despite their abundance, the functional roles of archaea within the coral holobiont have not been experimentally validated. However, they are often implicated in nitrogen recycling and ammonia oxidation within the SML (Siboni et al. 2008, 2012). Two metagenomically assembled genomes (MAGs) that belong to the Nitrososphaerota (syn. Thaumarchaeota) phylum were assembled from metagenomic reads of the coral *Porites lutea* (Robbins et al. 2019) and revealed the presence of symbiosis-related metabolic pathways, including a reductive tricarboxylic acid cycle and cobalamin biosynthesis, suggesting these archaeal genomes might contribute essential vitamins or dissolved carbon to the host (Robbins et al. 2019).

Viruses

A wide range of coral species and associated microbes are reported to harbour virus-like particles (Wilson et al. 2004, Marhaver et al. 2008, Brüwer et al. 2017). Metagenomic sequencing show a high diversity of coral-associated DNA and RNA viruses (Weynberg et al. 2014). Large metagenomic and metatranscriptomic sequencing efforts towards establishing a 'coral virome' conducted across 101 cnidarian samples from the Red Sea documented DNA and RNA viral assemblages associated with corals (Cardenas et al. 2020) [for a recent review on the roles of viruses in corals see Ambalavanan et al. (2021)]. While the functional roles of coral-associated viruses are still unclear, they likely play important roles in the coral holobiont. The presence of some bacteriophages in the coral SML may regulate the abundance of specific bacteria via targeted infection/lysis (Barr et al. 2013). Viral genes can encode for complementary functions that may be beneficial to the holobiont (Thurber et al. 2017). For example, some coral-associated viruses have genes related to photosynthesis that may alleviate and/or delay damage to Symbiodiniaceae photosystems at higher temperatures (Weynberg et al. 2017). In addition, Knowels et al. (2016) unexpectedly reported a decrease in viral abundance in reefs with high microbial abundance and suggested a lytic-to-lysogenic shift with increased microbial densities. This novel host-viral dynamic has been proposed as a mechanism of reef microbialization (Haas et al. 2016).

However, viruses can also be detrimental to corals. Under various stress conditions, the coral-associated viral consortium exhibits an increase in herpes-like viruses, similar to other cnidarians (Thurber et al. 2008). Temperature-induced latent infection is also suggested to confer virulence to specific coral pathogens that could lead to the onset of coral disease (Weynberg et al. 2014, Work et al. 2021).

Functions of coral-associated microbes

Coral–Symbiodiniaceae symbiosis, the engine of the holobiont

The symbiotic relationship between corals and Symbiodiniaceae enabled the construction of the reef (calcium carbonate skeleton) via bidirectional nutrient exchange (Pogoreutz et al. 2020). The symbiosis relies on reciprocal metabolite exchanges, where Symbiodinaceae share excess photosynthetically derived dissolved organic matter with the coral host in exchange for access to inorganic nutrients and CO_2 generated from respiration (Muscatine 1990, Falkowski et al. 1993, Cunning et al. 2017). Indeed, the sharing of organic photosynthates by Symbiodiniaceae (e.g. glucose) is energetically sufficient for the host to meet 100% of its respiratory requirements (Muscatine and Porter 1977, Bourne et al. 2016).

Although corals are capable of assimilating ammonium to acquire nitrogen, Symbiodiniaceae are responsible for most inorganic nitrogen uptake in the forms of nitrate and ammonium (Pernice et al. 2012). A proportion of this nitrogen is shared with the coral host in the form of dissolved organic nitrogen (e.g. amino acids) (Wang and Douglas 1999, Yellowlees et al. 2008, Reynaud et al. 2009). However, high concentrations of inorganic nitrogen

have been shown to destabilize the symbiosis. Increasing nitrogen fixation leads to an increase in nitrogen availability that subsequently increases cell division rates of the symbiont; this increase alters the N:P ratio within corals and causes phosphate limitation (Wiedenmann et al. 2013). Thus, corals control the growth of their symbionts by regulating access to inorganic nitrogen (Wooldridge 2013). Indeed, this nitrogen-budget balance is critical for the maintenance of the symbiotic relationship and further 'fine-tuning' of its outcome is evident from prokaryotic members of the coral microbiome (see below) (Cui et al. 2019). More recently, Rädecker et al. (2021) showed that coral bleaching can be correlated with disrupted nutrient cycling during heat stress, where the increased energetic demand of the host during heat stress leads to increased catabolism of amino acids, a more rapid release of ammonium concomitant with promotion of the growth of algal symbionts and retention of photosynthates.

In addition to central metabolites, such as sugars and amino acids, Symbiodiniaceae produce mycosporine-like amino acids, pigments (e.g. fucoxanthin) and carotenoids, which collectively protect against UV radiation and reactive oxygen species (ROS) (Rosic and Dove 2011, Rosic 2019, Roach et al. 2021). Symbiodiniaceae-derived glucosides can serve as energy storage molecules, osmolytes, and antioxidants (Ochsenkühn et al. 2017, Gegner et al. 2019), which may protect photosystem II from free radicals. Other metabolites such as glycerolipids, betaine lipids, and tocopherols that are produced by both host and symbiont are hypothesized to stabilize cellular membranes, assist protein renaturation, and act as antioxidants during heat stress (Hillyer et al. 2017b, Rosset et al. 2017, Roach et al. 2021) and disease (Deutsch et al. 2021). Symbiodiniaceae possess the necessary genes to produce essential steroid precursors like squalenes and lanosterols that corals and other cnidarians either acquire through heterotrophic feeding or through their symbionts (Baumgarten et al. 2015). In addition to these exchanges, it is likely that the coral–Symbiodiniaceae relationship involves dozens to hundreds of metabolites that regulate their complex symbiosis, akin to most well-studied symbiotic systems, for which our knowledge is lacking. Further work is needed to shed light on these chemicals and their role in nurturing healthy corals.

Beneficial coral-associated bacteria can boost coral health and resilience

While the putative symbioses between the coral host and specific bacterial species is obscure when compared to Symbiodiniaceae, recent evidence suggests there are specific bacterial symbionts that benefit their coral host. Mining the literature, we assembled a list of bacteria that are hypothesized to be beneficial to corals based on experiments either *in situ* or in the laboratory (Supplementary Table S1). Interestingly, thus far evidence shows that most bacteria that confer benefit to the holobiont belong mostly to the α- and γ-proteobacteria and to a lesser extent the Actinobacteria, Actinomycetia, Cytophagia, Flavobacteriia, Bacilli, and Oligoflexia classes (Fig. 2 and Supplementary Table S1). The limited number of classes that have been found to be beneficial to corals relative to bacterial orders in seawater suggests there are selection mechanisms that enable corals to form beneficial interactions with such bacteria. Below is a discussion of some of these bacteria and the potential roles they play in benefitting the holobiont.

Tissue-localized members of the coral microbiome, such as bacteria of the genus *Endozoicomonas* (Fig. 2), are hypothesized to be a core symbiont of corals as they are ubiquitously found across a wide range of coral species from diverse geographic locations (Neave et al. 2017, Ziegler et al. 2017, Pogoreutz et al. 2018), including deep-sea corals (Kellogg and Pratte 2021) and form highly stable association with such corals even during bleaching [reviewed in Hernandez-Agreda et al. (2016, 2018, 2019)]. In addition, the relative abundance of *Endozoicomonas* is often strongly correlated to coral health (Bayer et al. 2013, Roder et al. 2015, Neave et al. 2016) with abundance generally high in healthy corals and lower in stressed, bleached, and diseased corals (Bourne et al. 2008, Meyer et al. 2014, Morrow et al. 2015). Based on these observations, it has been suggested that *Endozoicomonas* may be important for coral holobiont health, but its symbiotic exchanges with the holobiont have yet to be identified. *Endozoicomonas* harbour large numbers of genes involved in amino acid synthesis and carbohydrate cycling, prompting suggestions that it is involved in holobiont nutrient cycling (Neave et al. 2017). A recently published *Endozoicomonas* MAG from a *Porites* deep shotgun metagenome study contained genes essential for the biosynthesis of cobalamin, which is a vitamin required for methionine synthesis by both corals and Symbiodiniaceae (Robbins et al. 2019). This MAG also encoded the enzyme DMSO reductase that converts DMSO to DMS, providing a means to recycle dissolved organic sulphur (Robbins et al. 2019). More recently, the genome of *Endozoicomonas acroporae* was shown to encode a DMSP CoA-transferase/lyase gene (dddD), capable of metabolizing DMSP into DMS (Tandon et al. 2020). DMSP metabolism may play a role in structuring the holobiont microbial community (Raina et al. 2013). Finally in response to coral tissue extract additions, *E. marisrubri* was shown to differentially express genes putatively involved in symbiosis establishment, e.g. flagellar assembly, ankyrins, ephrins, and serpins. Proteins involved in vitamin B_1 and B_6 biosynthesis were also upregulated (Pogoreutz et al. 2022).

Many studies have examined the ability of natural bacteria in coral microbiomes to inhibit or prey on coral pathogens. Several strains of *Ruegeria* spp. (Fig. 2) were found to inhibit the growth of the coral pathogen *Vibrio coralliilyticus* and other *Vibrio* spp. (Miura et al. 2019). *Ruegeria* spp. have also been implicated as indicator species in healthy coral microbiomes (Rosado et al. 2019). Reef-building corals were challenged with *V. coralliilyticus* in the presence or absence of the *Vibrio* predator *Halobacteriovorax* sp. PA1 (Fig. 2), which is commonly found at low abundance on coral surfaces (Welsh et al. 2016, Zaneveld et al. 2016). Inoculation of corals with *V. coralliilyticus* induced major changes in the microbiome, especially a large increase in relative abundance of *Vibrio* spp., a reduced microbiome stability and proliferation of opportunists, such as Rhodobacterales and Cytophagales. In contrast, co-inoculation of the corals with both bacteria eliminated the increase in *Vibrio* spp. and prevented the proliferation of opportunists (Welsh et al. 2017). *Pseudovibrio* sp. P12 (Fig. 2) was shown to produce the antimicrobial metabolite tropodithietic acid, potentially through metabolizing coral DMSP, to inhibit the growth of *V. coralliilyticus* and *V. owensii* (Raina et al. 2016). More recently, the beneficial role of degrading excess DMSP during heat stress has been validated (Santoro et al. 2021). Enrichment of a DMSP degrading bacterium is associated with a significant increase of DMSP degradation and a concomitant coral holobiont physiological improvement, resulting in higher survival rates.

Scavenging of ROS is a common mechanism by which bacteria can benefit a eukaryotic host, and has also been recently suggested as a beneficial mechanism for corals (Peixoto et al. 2017). In corals, several bacterial species were shown to detoxify radicals and ROS mainly by producing ROS-reactive pigments. These include *Fabibacter pacificus*, *Paracoccus marcusii*, and *Pseudoalteromonas shioyasakiensis* (Fig. 2) (Varasteth et al. 2021). Six strains of bacteria belonging to *Alteromonas macelodii*, *A. oceani*, *Roseibium aggregata*,

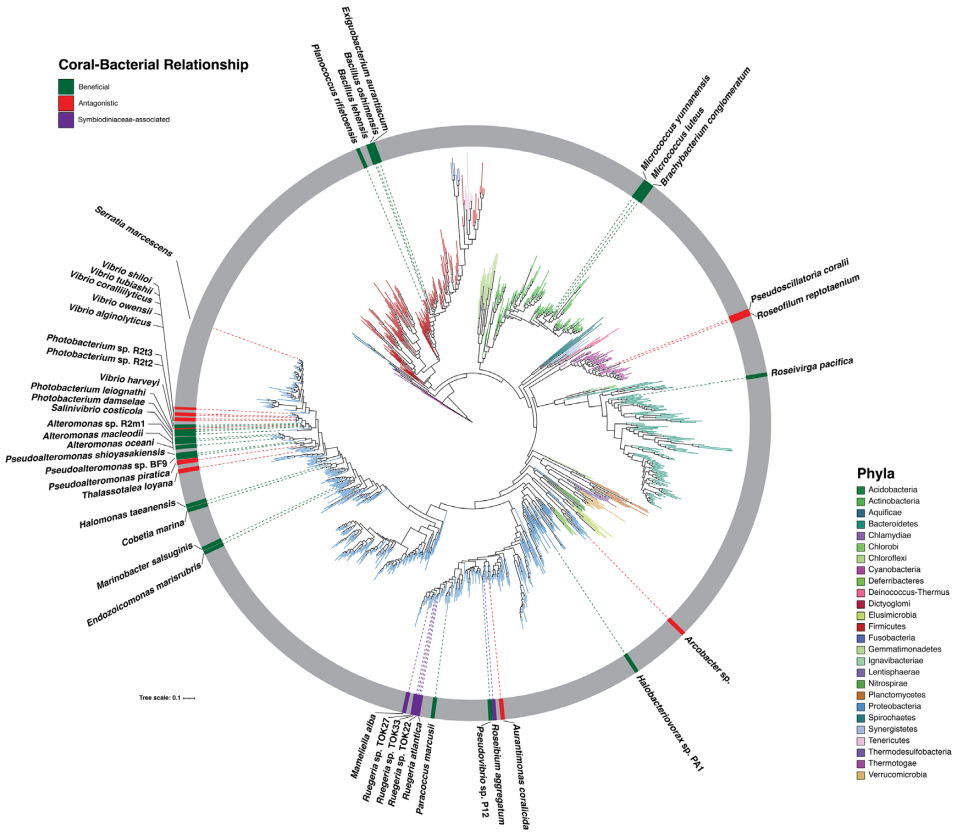

Figure 2. Relationship and diversity of bacterial species associated with corals based on previous research. Coral microbiome data were retrieved from SILVA (Quast et al. 2013) based on a literature search and mapped onto the prokaryotic tree of life (Hug et al. 2016). Additional sequences were merged into the original alignment by Hug et al. (2016) using MAFFT (Katoh et al. 2002). A maximum likelihood tree was constructed using FastTree (Price et al. 2010). In cases where 16S rRNA sequences were not available in the database, the branches were substituted with their closest available neighbour. Only taxa with some experimental evidence as to their relationship to corals were included. Beneficial, antagonistic (opportunists and pathogens), and Symbiodiniaceae-associated bacterial species (highlighted in the outer ring) are clustered in different parts of the tree. The tips of the branches are colour-coded according to the taxonomic classification. Generally, clusters of closely related bacterial species have similar relationships within coral microbiomes. For example, Symbiodiniaceae-associated bacteria mostly belong to the family Rhodobacteraceae, species belonging to the genus *Vibrio* are mostly antagonistic, while species belonging to the genera *Alteromonas* and *Photobacterium* are beneficial. Interestingly, members of the same genus do not always have the same relationship. For example, members of *Pseudoalteromonas* can be beneficial or antagonistic. A full list of bacteria depicted here and their references are provided in Supplementary Table S1.

Marinobacter salsuginis, *Micrococcus luteus*, and *M. yunnanensis* (Fig. 2) were shown to remove oxygen radicals from the coral model *Exaiptasia diaphana* (Dungan et al 2021). SML-assoicated bacteria (Fig. 1) can be an important source of antibiotics that fend off pathogen colonization (Ritchie 2006, Engelen et al. 2018) and isolates of *Pseudaltermonas* strains from *O. patagonica* SML were active against the coral pathogens *V. shiloi*, *V. coralliilyticus*, and *Thalassomonas loyana* (Shnit-Orland et al. 2012).

Symbiodiniaceae–bacterial interactions, a forgotten partnership in a complex symbiotic network

Much of our understanding of the symbiotic relationships within the holobiont stems from host interaction with either Symbiodiniaceae or cultivated bacteria; in contrast, little is known about Symbiodiniaceae–bacteria interactions. Recent work has revealed complex metabolite exchanges between phytoplankton and their associated microbiome occurring within a microscale diffusive boundary layer surrounding phytoplankton cells, known as the phycosphere (Amin et al. 2012, Seymour et al. 2017). Symbiodiniaceae strains in culture harbour different bacterial communities in the phycosphere with abundances exceeding those of the algal cells by almost two orders of magnitude (Ritchie 2012, Lawson et al. 2018). Among these diverse communities, members of the genera *Marinobacter*, *Roseibium* (formerly *Labrenzia*), *Muricauda*, *Hyphomicrobium*, *Methylobacterium*, and members of the families Rhodobacteraceae/Roseobacteraceae (e.g. *Ruegeria*, *Mameliella*) have been consistently detected in Symbiodiniaceae cultures (Ritchie 2012, Lawson et al. 2018, Camp et al. 2020, Varasteh et al. 2020, Maire et al. 2021) [reviewed in Matthews et al. (2020)]. Some of these taxa have been shown to be symbiotic with different phytoplankton lineages. For example, *Marinobacter* spp. provide a bioavailable source of iron to dinoflagellates and some diatom species in iron-limited environments (Amin et al. 2009). In Roseobacteraceae, *Ruegeria pomeroyi* has been shown to provide vitamins to diatoms in exchange for organic sulphur compounds (Durham et al. 2015). *Sulfitobacter pseudonitzschiae* and *Phaeobacter inhibins* convert the diatom- and coccolithophore-secreted amino acid tryptophan to the hormone indole acetate, respectively, which enhances the algal cell division rate (Amin et al. 2015, Segev et al. 2016). *Sulfitobacter pseudonitzschiae* and *Phaeobacter* spp. have also been shown to successfully colonize the phycosphere of diatoms (Fei et al. 2020) by efficiently responding to host secondary metabolites (Shibl et al. 2020).

Among the Roseobacteraceae, *Mameliella alba* has been consistently isolated from dinoflagellate cultures (Li et al. 2019, Varasteh et al. 2020, Lin et al. 2021, Ren et al. 2022) and appears to enhance

the growth rate of the dinoflagellates *Symbiodinium* sp., *Alexandrium catanella*, and *Karenia brevis* (Varasteh et al. 2020, Lin et al. 2021, Ren et al. 2022, Amin et al., personal communication), suggesting they produce a growth-promoting hormone. Axenic Symbiodiniaceae cultures originating from the coral *Galaxea fascicularis* have been shown to exhibit a decrease in photosystem II maximum quantum yield and an increased production of ROS. A *Muricauda* sp. (Fig. 2) isolated from xenic Symbiodiniaceae was subsequently shown to protect Symbiodiniaceae Photosystem II from ROS via production of the antioxidant Zeaxanthin (Motone et al. 2020). Despite these advances, no information is currently available on microbial communities associated directly with the Symbiodiniaceae phycosphere within coral symbiosomes [for a recent review see Garrido et al. (2021)]. Matthews et al. (2020) hypothesized that Symbiodiniaceae-associated bacterial consortia regulate Symbiodiniaceae productivity and thus the symbiotic interactions with corals. Future research should exploit recent advances in microfluidics, single-cell sequencing, and metabolomics to uncover the metabolic interaction between Symbiodiniaceae and bacteria within the coral host that are likely central to the holobiont fitness.

Microbial dysbiosis is triggered by environmental stress and could drive the onset of coral disease

During environmental stress, many putative opportunistic and pathogenic taxa increase in abundance, such as members of the Vibrionaceae, Roseobacteraceae, and Rhodobacteraceae, due to the immune-compromised state of the host (Cardenas et al. 2012, Ziegler et al. 2016, Pollock et al. 2017, Certner and Vollmer 2018). It is noteworthy to point out that generalizations about genera or families being beneficial or harmful to corals should be avoided as interspecies interactions rely on a highly specific sets of genes that enable a bacterium to behave one way or another. As pointed out below, some families of bacteria contain species that are both beneficial and harmful to corals. Across coral species, during dysbiosis, specific bacteria have been shown to increase in abundance and activity; concomitantly, larger changes in the coral microbiome that involves one or more groups of bacteria were also shown to change in abundance and some of these have direct repercussions on coral physiology. For example, during coral exposure to elevated temperatures, diazotrophic bacteria were shown to increase in abundance (Santos et al. 2014, Lesser et al. 2018, Mohamed, personal communication) [for reviews see Radecker et al. (2015), Benavides et al. (2017)], which has a direct effect on nitrogen availability. More recent data show that despite an increase in nitrogen fixation that is correlated with an increase in diazotrophs during heat stress, fixed nitrogen is not assimilated by either the coral tissue or the algal symbionts (Rädecker et al. 2022). Below examples of specific parasitic and opportunistic bacteria that have been reported are discussed.

Forty coral diseases have thus far been described (Sweet et al. 2012, Bruckner 2015); however, only few coral pathogens have been described (Pollock et al. 2011) [for a list of putative causative agents of coral disease, see Mohamed and Sweet (2019)]. Among proposed coral pathogens, *V. coralliilyticus* (Fig. 2) is the most well-characterized with direct implication in the onset of both coral bleaching and the infectious disease white syndrome (Ben-Haim et al. 2003, Pollock et al. 2011, Ushijima et al. 2014). Several other *Vibrio* species, such as *V. harveyi*, *V. owensii*, and *V. alginolyticus* (Fig. 2), have also been implicated in white syndrome (Luna et al. 2010, Ushijima et al. 2012, Zhenyu et al. 2013) and *V. tubiashii* (Fig. 2) in white patch syndrome (Sere et al. 2015). *Pseudoalteromonas piratica* (Fig. 2) has also been implicated in white syndrome (Beurmann et al. 2017). Other bacteria have been proposed as causative agents of coral diseases. For example, white pox in *A. palmata* has been proposed to be caused by the enteric bacterium *Serratia marcescens* (Fig. 2) (Patterson et al. 2002). White plague type II in scleractinian corals has been proposed to be caused by *Aurantimonas coralicida* (Fig. 2), a relative of Rhizobiales (Denner et al. 2003), while *T. loyana* (Fig. 2) was proposed to cause a white plague-like disease (Thomposon et al. 2006). Black band disease was originally thought to be caused by the cyanobacteria *Pseudoscillatoria coralii* (Rasoulouniriana et al. 2009) and *Roseofilum reptotaenium* (Fig. 2) (Casamattaet et al. 2012); however, further research described this disease as a lesion of a complex microbial consortium composed of cyanobacteria and other microbes, including the sulphate-reducing bacterium *Desulfovibrio* sp. (Fig. 2), and a diverse array of heterotrophic bacteria, archaea, fungi, and other microeukaryotes (Sato et al. 2016). Finally, *Candidatus* Aquarickettsia rohweri (Fig. 2) is suspected of being implicated in white syndrome type I. This putative parasitic bacterium possesses several tools to benefit from the coral host, including an antiporter to exchange host ATP for ADP, a type IV secretion system, and appears to be using host nutrients, particularly nitrogen (Klinges et al. 2020). Other causative agents and almost all molecular factors of disease remain obscure.

More recently, during the onset of grey-patch disease, a 'microbiome-to-pathobiome' shift occurs that favours multiple specific pathogens that may be involved in degrading coral tissues (Sweet et al. 2019). This shift is hypothesized to be caused by bacterial quorum sensing molecules, such as homoserine lactones (Certner and Vollmer 2015). Homoserine lactones are small molecules produced by many bacteria to regulate their gene expression based on population density. Genes related to pathogenesis in bacteria, e.g. biofilm formation, siderophore production, toxin secretion, are typically regulated by quorum sensing (De Kievit and Iglewski 2000, Winzer and Williams 2001, Visca et al. 2007). Disease symptoms were induced in healthy *A. cervicornis* colonies exposed to bacteria supplemented with exogenous homoserine lactones, which correlated with a 'healthy' to 'disease-causing' microbiome switch and leading to white band disease-like symptoms. Indeed, microbial consortia isolated from white band disease-infected colonies and treated with homoserine lactone inhibitors lost their ability to develop the disease (Crenter and Vollmer 2018). These observations suggest that quorum sensing can modulate bacterial regulatory networks that then reshape the microbial community during disease onset, though the mechanism of how this occurs is still unclear.

Leveraging the coral microbiome to boost resilience of the holobiont

Inoculation of corals with probiotic microbes has been proposed to protect corals from the harmful impact of oil spills. This bioremediation approach was successful in mitigating the impacts of pollution and improved the health of affected corals (Fragoso Ados Santos et al. 2015). Several approaches have been proposed to aid corals in increasing their fitness, such as experimental evolution in coral photosymbionts (van Oppen et al. 2015, 2017) and bacterial probiotics application (Peixoto et al. 2017).

Introducing heat-tolerant Symbiodiniaceae into corals

It is widely accepted that coral thermal tolerance is largely dependent on the physiology of their associated Symbiodiniaceae partners (Berkelmans and van Oppen 2006). *In-vitro* exposure of Symbiodiniaceae cultures to elevated temperatures increases their

thermal tolerance after ~40 generations (Chakravarti et al. 2017, Chakravarti and van Oppen 2018). Despite this acclimation, reintroducing heat-tolerant strains into corals yielded no significant benefit for the holobiont (Chakravarti et al. 2017). In contrast, a small minority of heat-tolerant Symbiodiniaceae strains derived from the same wild-type clone increased the thermal tolerance of coral larvae (Buerger et al. 2020). A mechanistic understanding of how heat tolerance in Symbiodiniaceae occurs and how in turn it influences the coral holobiont is needed to improve the efficacy of this approach.

Using probiotics to help increase corals' resilience to climate change

Bacterial symbionts of corals represent an opportunity to increase the resilience of the coral in response to ocean warming (Zielger et al. 2019, Voolstra and Zielger 2020, Voolstra et al. 2021a). Coral microbiomes, especially those inhabiting the coral SML, are thought to rapidly respond to the surrounding environment and may contribute to the resilience and health of the holobiont (Bang et al. 2018, Ziegler et al. 2017, 2019). Recent efforts have been focusing on coral 'probiotics' applications to boost adaptation to climate change (Peixoto et al. 2017). This approach involves isolation and screening of native bacterial associates of corals for functions beneficial to coral health, and subsequently carry out physiological assays to determine holobiont performance after inoculation with these putatively beneficial microorganisms for corals (BMCs) (Rosado et al. 2019). Experimental manipulation using mixed consortia of native coral bacterial isolates harbouring beneficial genes such as nitrogen fixation (*nifH*) and DMSP-degradation (*dmdA*) genes (Fig. 3) resulted in partial mitigation of coral bleaching compared to controls or corals challenged with the pathogen *V. coralliilyticus* (Rosado et al. 2019). Mesocosm experiments coupled with multiomics revealed an increase in coral resilience following probiotic application and was followed by a reprogramming of coral transcriptional machinery to activate the immune system and stress pathways during the recovery period (Santoro et al. 2021). Despite the successful applications described so far, most marine bacteria remain uncultivable (Lok 2015, Hofer 2018, Jiao et al. 2021) and even with cultured ones, our knowledge of their benefit to the coral is limited. As our understanding of the role bacterial symbionts play expands, e.g. through the use of culturomics (Schultz et al. 2022), more targeted engineering of beneficial microbial communities may be valuable in supporting recovery of coral reefs.

Another approach inspired by fecal microbiota transplantation in humans, called field-based coral microbiome transplantation (CMT) (Doering et al. 2021), has successfully shown the feasibility of microbiome transplantation of homogenized coral tissues from healthy colonies to bleached colonies to increase coral heat tolerance. This approach has many advantages as it circumvents ethical issues associated with introducing new bacterial isolates into the environment, avoids the daunting task of screening bacterial function in the laboratory, and enables the transmission of the large uncultivable fraction of the microbiome. In both probiotic and CMT approaches, the mechanisms underlying the microbiome–host interaction and stress tolerance are yet to be established.

Understanding host–pathogen interactions in the coral-Vibrio system

It is now widely accepted that many coral diseases are caused by a diverse polymicrobial consortium [Roder et al. 2014, Sweet et al. 2019, for a review see (Mohamed and Sweet 2019)] though the mechanisms underlying these infections are largely unknown. During responses to environmental stressors, the microbiome of the reef undergoes shifts towards an increasing microbial diversity (Haas et al. 2016). During this reef microbialization, the coral decline is attributed to the preferential increased abundance of pathogens and their virulence factors. Although most infection experiments examine coral pathogens separately, simultaneous inoculation of *V. coralliilyticus* and *V. mediterranei* in the coral *Oculina patagonica* leads to increased virulence and higher coral tissue damage, suggesting the cumulative effect of both bacteria accelerate pathogenicity (Rubio-Portillo et al. 2014). Recently, Rubio-Portillo et al. (2020) attempted to understand the mechanisms underpinning the interaction between these pathogens and their interaction with corals during infection. When co-cultured together, these bacteria overexpress genes related to virulence factors, such as siderophores, type VI secretion system, and toxins (Fig. 3). These transcriptional responses towards a related competing species suggest these pathogens may favour the colonization of the host when they are present in a mixed population. Moreover, during coral exposure to a coculture of *V. coralliilyticus* and *V. mediterranei*, virulence factors (product of interspecies competition between the two coral pathogens) led to shifts in the coral microbiome favouring specific opportunistic groups. These in turn caused increased production of Lyso-PAFs (Fig. 3) by the coral to fight the pathogens back, which led to increased production of ROS and tissue necrosis (Rubio-Portillo et al. 2020).

Coral–microbiome crosstalk from recognition to gene regulation

Little is known about the mechanisms of coral–microbiome interactions mainly because of the lack of a genetically tractable coral model system that can be manipulated in the laboratory, the lack of cultivable strains of certain coral microbial symbionts (e.g. some bacteria, certain Symbiodiniaceae strains, and cryptic species) and their genomic resources. All these aspects hinder our understanding of the gene regulatory circuits within members of the coral holobiont. Other symbiotic systems, such as human–microbiome interactions, provide an opportunity to learn more about coral holobiont interactions. In this section, identified recognition mechanisms that corals use to interact with its microbiome, the potential for the coral microbiome to produce epigenome-effector proteins, delivery of such microbially derived signals to the coral host, and mechanisms that enable coral–microbiome interactions with a focus on noncoding RNAs (ncRNAs), akin to human–microbiome interactions, are discussed.

Recognition mechanisms in coral–microbiome interactions

Strong evidence supports the key roles of the host innate immune system in all aspects of the symbiotic association, from recognition, maintenance, and collapse (dysbiosis) (Weis 2008). Recognition is the first step that enables corals to determine whether a microbe is beneficial or not. Microbial cell membranes (cell walls in the case of Symbiodiniaceae) are decorated with a variety of microbial-associated molecular patterns (MAMPs), including glycans, that are recognized by the coral host via the pattern recognition receptors (PRRs) on phagocyte cell surfaces (Weis 2008). A wide variety of PRRs has been recognized in cnidarians, including toll-like receptors (TLRs), the intracellular pattern recognition receptor nucleotide-binding oligomerization domain 2 (NOD2), complement and its receptor (CRs), scavenger receptors (SRs), and lectins (Fig. 4) (Weis 2008, Davy et al. 2012, Weis 2019). These PRRs can be activated to detect beneficial microbes, e.g. the detection

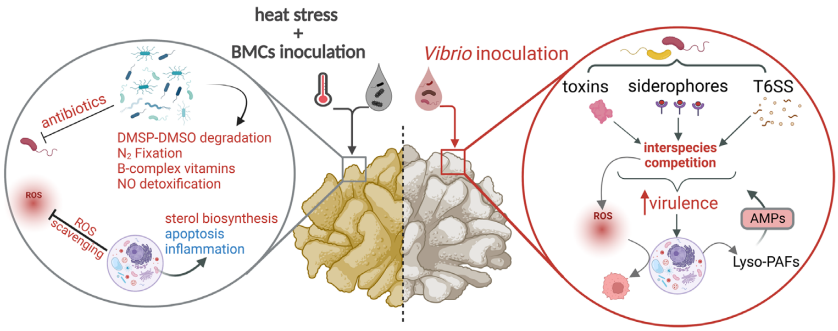

Figure 3. Depiction of the influence of BMCs to reduce mortality after heat stress (left) and pathogenic bacteria of the genus *Vibrio* on the coral host (right). Coral cells are depicted as the eukaryotic cell at the bottom of each side of the figure, while the microbiome or *Vibrio* are depicted by bacterial cells on the top. Function names in red font indicate an increase, while those in blue font indicate a decrease. After being exposed to heat stress and inoculated with BMCs, the coral host increases sterol biosynthesis while decreasing apoptosis and inflammation. BMCs support the coral host via N_2 fixation, DMSP–DMSO degradation, ROS scavenging, proteins related to B-complex vitamins, nitric oxide detoxification, and production of antibiotics. *Vibrio* competes with members of the coral microbiome via production of type VI secretion system (T6SS) proteins, toxins, and siderophores. These interspecies competition mechanisms increase virulence factors that may confer an advantage to these pathogens over resident taxa and induce changes in the coral holobiont. Corals exposed to vibrios show high levels of platelet-activating factors (such as Lyso-PAFs) as a defence mechanism as they have antimicrobial properties. DMSP; dimethylsulfoniopropionate, DMSO; dimethyl sulfoxide, Lyso-PAFs; platelet-activating factors, and AMPs; antimicrobial peptides.

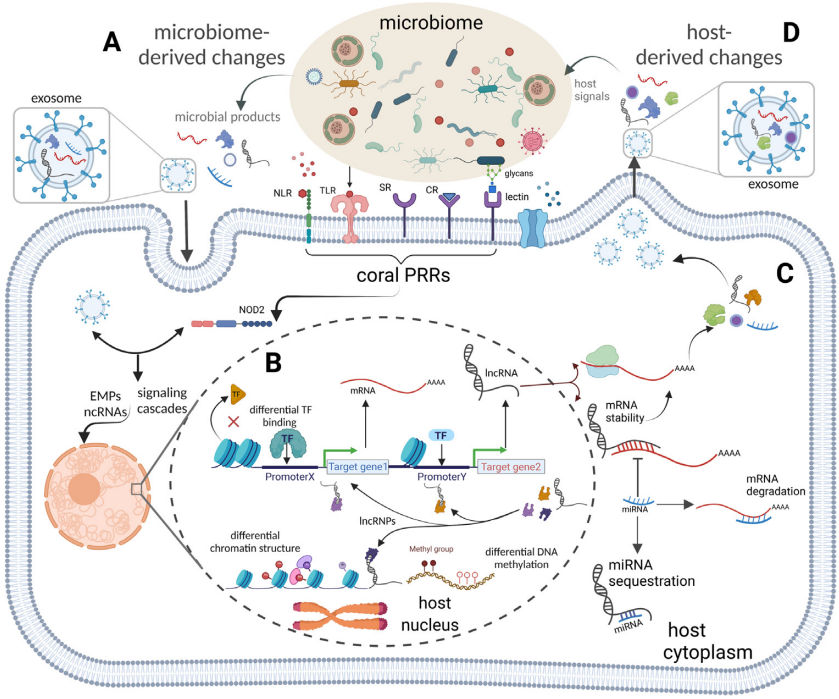

Figure 4. Potential mechanisms underpinning host–microbiota crosstalk in corals. Coral-associated microbes can alter the host gene expression by modifying the host epigenome. The coral host recognizes microbes (lectin–glycan interaction) and their products through interaction with extracellular receptors or through exosome-based delivery of microbiome-derived molecules. (A) These microbial-derived signals may cause specific changes in the host nucleus directly via epigenome-modifying proteins or indirectly through NOD2 signalling, following binding of various pattern recognition receptors to microbial signals (e.g. metabolites). (B) This leads to changes in host gene expression through epigenetic mechanisms, such as changing chromatin accessibility and DNA methylation that will lead to differential transcription factors binding, altered expression of certain genes, transcription factors, and ncRNAs such as long noncoding RNAs (lncRNAs). LncRNAs can further regulate gene activity at the epigenetic, transcriptional, or post-transcriptional levels. (C). The interaction is bidirectional as host-derived signals (proteins, ncRNAs, or metabolites) may be delivered to the coral microbiome (D) leading to differential microbial growth. NLR: NOD-like receptor, lncRNP: lncRNA-protein complex, and miRNA: microRNA.

of Symbiodiniaceae by lectins (Mohamed et al. 2016), while being suppressed during encounters with parasites presumably as a host evasion mechanism (Mohamed et al. 2018). These MAMP–PRR interactions in corals include: lectin–glycan interactions (Wood-Charlson et al. 2006, Bay et al. 2011, Parkinson et al. 2018), scavenger receptors (Neubauer et al. 2016), thrombospondin type 1 repeat proteins (Wolfowicz et al. 2016, Neubauer et al. 2017, Mohamed et al. 2020b), glycoprotein2 (GP2) (Mohamed et al. 2016, 2020b), toll-like/nucleotide oligomerization domain (NOD)-like receptors (TLRs/NLRs) (Hamada et al. 2013, Weiss et al. 2013, Mohamed et al. 2020b), and complement systems (Poole et al. 2016) (Fig. 4).

Once PRRs are activated in the case of Symbiodinaceae, the host facilitates the persistence and proliferation of symbionts

inside symbiosomes via suppression of its immune response and arrest of phagosomal maturation (Davy et al. 2012, Mohamed et al. 2016, 2020b). In contrast, immune response and phagosomal maturation are activated to reject and clear the symbionts out during dysbiosis and bleaching (Downs et al. 2009) and during encounters with parasites (Mohamed et al. 2018). Activation of MAMP–PRR interactions leads to downstream innate immune signalling cascades and production of effector proteins (Fig. 4) such as tolerogenic TGFβ pathway (Detournay et al. 2012, Berthelier et al. 2017), sphingolipid signalling (Kitchen and Weis 2017, Kitchen et al. 2017), and the master immunity regulator NFκB (Mansfield et al. 2017, Jacobovitz et al. 2021). These interactions are well supported in corals and sea anemone by many 'omics studies that implicate innate immune genes (Shinzato et al. 2011, Mohamed et al. 2016, 2020b, Cunning et al. 2017„ Jacobovitz et al. 2021).

Microbially derived host epigenome-effector proteins

Many bacteria, including the pathogens *Helicobacter pylori* and *V. cholerae* deliver effector proteins into a wide range of host cells, including humans, plants, and invertebrates using type IV or VI secretion systems to interfere with host signalling pathways (Green and Mecsas 2016). Not surprisingly, secretion systems have been highlighted as putative mediators of symbiotic associations (Coombes 2009). Interestingly, several secretion systems, including type IV, have been identified in the microbiomes of several coral species (Weber et al. 2019). These secretion systems can be used to deliver effector proteins from the associated microbiomes to the host or other members of the holobiont. Moreover, living cells can send and receive packages of information that are enclosed by cell membranes in the form of extracellular vehicles (EVs) such as exosomes (Fig. 4). EVs are lipid bilayer nanoparticles that act as key messengers in cell-to-cell communication and can be produced by unicellular microbes and multicellular metazoans alike. EVs contain diverse molecules, including effector proteins, such as microbes-derived epigenetic-modifying proteins (Yang et al. 2022). Once delivered to the host, these epigenetic-modifying proteins target the host cell nucleus to affect host responses through epigenetic mechanisms. These epigenetic modulations are hypothesized to directly or indirectly influence phenotypic responses in the host (Barno et al. 2021, Morovic and Budinoff 2021). Barno et al. searched for putative homologs of known epigenome-modifying proteins from other host model systems in 18 bacterial genomes and 52 prokaryotic MAGs associated with two coral species. They identified homologs of the histone modification proteins ankyrin-repeat protein A and internalin B, a histone methyltransferase, and several DNA methyltransferases, suggesting that the coral microbiome has the machinery to modify the host epigenome (Barno et al. 2021).

Molecular mechanisms of host–microbiota crosstalk in corals

The mechanisms enabling the microbiome to influence genetic and physiological responses of the coral host are lacking. Many studies in model organisms, including humans, show an association between the microbiome and host gene expression. However, it is unclear what the direction of causality is with these associations. Disentangling this relationship is crucial for understanding homeostasis of normal symbiosis and dysbiosis, leading to disease etiology (Nichols and Davenport 2021). Upon delivery of microbiome-derived signals, many signalling cascades are likely activated to influence the host epigenome, which ultimately reprograms the host transcriptional machinery towards specific host phenotypes (Fig. 4).

Transcription factor-mediated gene regulation

Gene regulation is usually mediated through transcription factors (TFs) that can link host gene expression and its microbiome (Fig. 4). Host TFs bind to specific DNA motifs (regulatory elements such as promoters and enhancers) to control the transcription of certain genes. Previous research on zebrafish demonstrated interactions between the TF HNF4A and the microbiome promote gene expression patterns associated with inflammatory diseases (Davison et al. 2017). In mice, microbiota colonization of intestinal epithelial cells leads to drastic genome-wide reduction of the HNF4A occupancy, a measure of TF binding to its DNA motif (Davison et al. 2017), suggesting that the microbiota negatively regulate HNF4A. This indicates a conserved role for HNF4A in maintaining homeostasis of the intestine in response to the microbiome. Similarly, in Metazoa, the master regulator of innate immunity, NF-κB (Gilmore and Wolenski 2012) has been implicated during both the onset and breakdown of the coral–Symbiodiniaceae symbiosis. NF-κB activation leads to the upregulation of various effector pathways that drive an innate immune response. Numerous MAMP–PRR interactions that trigger the activation of NF-κB have been a recent focus in cnidarian genomic studies (Poole and Weis 2014, Baumgarten et al. 2015, Williams et al. 2018). The presence of Symbiodiniaceae in *Aiptasia* triggers a strong suppression of the host immune response (Perez and Weis 2006, Detournay and Weis 2011), but the exact mechanism is still unclear. Inoculation experiments in *Aiptasia* suggest that NF-κB is playing a role in this immune suppression, as the addition of symbionts leads to decreases in NF-κB expression in aposymbiotic larvae inoculated with mutualistic Symbiodiniaceae (Wolfowicz et al. 2016, Mansfield et al. 2017). Inversely, NF-κB expression increases during bleaching in adults (Mansfield et al. 2017). These results suggest that during symbiosis establishment, the algal symbionts modulate the host immune response by repressing the expression of NF-κB to enable colonization of the host. However, the link between NF-κB and coral-associated bacteria has not been established yet.

Epigenetic modifications

Other mechanisms of gene regulation include epigenetic modifications that can influence gene activity, including DNA methylation (addition of methyl groups at specific genomic CpG loci) and histone acetylation (addition of acetyl groups at specific histone sites). Epigenetics is a rapidly growing field and of great interest in the context of 'environmental memory' (Eirin-Lopez and Putnam 2019) that may explain phenotypic plasticity and acclimatization (Torda et al. 2017, Liew et al. 2018).

The host epigenetic profiles are thought to be influenced by its microbiome (Yu et al. 2015, Krautkramer et al. 2017, Miro-Blanch and Yanes 2019). In mice, germ-free animals have lower DNA methylation levels across the genome in the colon cells compared to animals with microbiomes (Yu et al. 2015). Fecal transplants also increase global DNA methylation in germ-free mice (Krautkramer et al. 2017). The microbiome can additionally remodel host responses at the chromatin level (Fig. 4) in intestinal epithelial cells. Profiling chromatin states via ATAC-seq in human colonic epithelial cell culture demonstrated that specific microbes regulate genome-wide accessibility of chromatin and TF binding in the host tissues (Richards et al. 2019). In mice, the presence of the microbiome results in increased levels of chromatin accessibility in intestinal epithelial cells compared to a gnotobiotic mouse (Semenkovich et al. 2016). These microbiome-induced changes to host epigenomes play a major role in various aspects

of health and metabolic disease, including responding to diet, inflammation, obesity, and diabetes [for a review see Sharma et al. (2020)]. However, this microbiome–host epigenome relationship has not been examined in corals yet. Research should be focusing on establishing the link between the coral-associated microbes and the host epigenetic profiles.

While changes in the coral epigenome or microbiome is mostly correlated to environmental stressors (Putnam and Gates 2015, Roder et al. 2015, Dixon et al. 2018, Liew et al. 2018, Ziegler et al. 2019, Tong et al. 2020), similar interaction patterns between the microbiome and the coral epigenome are expected, despite the current lack of such information. Interestingly, a recent shotgun metagenomic study has hypothesized a possible chromatin interaction between corals and their associated microbiomes. MAGs derived from healthy colonies of the coral *P. lutea* were shown to be enriched in genes encoding ankyrin repeat proteins (Robbins et al. 2019). One such protein, AnkA, presumably disrupts host antimicrobial responses against its producer, the intracellular pathogenic bacterium *Anaplasma phagocytophilum* (Garcia-Garcia et al. 2009). AnkA is hypothesized to translocate into the host cell nucleus and bind to regulatory regions of the host chromatin, silencing key host defence genes involved in ROS production. These patterns suggest intracellular pathogens may directly regulate host gene expression by changing host chromatin structure. Similarly, pathogens like *V. coralliilyticus* may be able to modify host responses using similar mechanisms. Intracellular mutualistic bacteria may also use these mechanisms to suppress the host immune response to establish a symbiotic relationship.

ncRNAs as mediators of immune priming and potential communication signals

RNA molecules constitute a common ancient language encoded in all living organisms across all domains of life. Genome-wide transcription in all living cells is responsible for producing two main classes of RNA molecules: coding RNAs (<2%) and ncRNAs comprised of RNA molecules with reduced coding potential that have instead regulatory functions (Hangauer et al. 2013). Among the different classes of ncRNAs, lncRNAs share structural similarities with mRNAs such as polyadenylation at the 3′ ends and the cap structure at the 5′ ends [for a recent review see Statello et al. (2021)]. Mechanisms of lncRNA-mediated gene regulation can happen at the epigenetic (by recruiting or sequestering epigenetic modifiers), transcriptional (forming triple helical-structures with DNA via Hoogsteen base paring), and post-transcriptional (forming duplexes such as lncRNA–mRNA or lncRNA–miRNA duplexes) levels. (Fig. 4). At the molecular level, lncRNAs can sequester or recruit epigenetic modifying proteins, such as DNA methyltransferase and chromatin-remodelling complexes (Yu et al. 2017, Canzio et al. 2019, Liu et al. 2019). They can also stabilize their target transcripts (Geisler and Coller 2013) to increase mRNA expression (Ebert and Sharp 2010) and sequester proteins by forming lncRNA-protein complexes that alter mRNA splicing (Peng et al. 2020) (Fig. 4).

LncRNAs have been proposed as putative regulators of diverse biological processes, including immune responses during host–pathogen interactions in mammals [for a review see Agliano et al. (2019)]. They are also implicated in developing innate immune memory 'priming' (Zhang and Cao 2019) in invertebrates (Gourbal et al. 2018). In sea anemone, pre-exposure to a sublethal pathogen dose enhances short-term survival upon subsequent lethal exposures (Brown and Rodriguez-Lanetty 2015).

Despite the availability of several reference genomes for corals, the mechanistic and genomic basis for immune priming in corals is currently completely unknown. In the context of the coral holobiont, more work is urgently needed in order to understand the functions of lncRNA regulatory networks. The role of ncRNAs in coral holobiont homeostasis is readily teased out from available transcriptomic data, demonstrating the fluctuations in the holobiont transcriptional output during disease progression, bleaching (Libro et al. 2013, Daniels et al. 2015, Pinzon et al. 2015), or host–pathogen/parasite interactions (Burge et al. 2013, Mohamed et al. 2018, van de Water et al. 2018). Preliminary data support the idea that ncRNAs act as mediators of both biotic or abiotic stress responses and symbiosis establishment. Early evidence of the presence of functional ncRNAs in corals was obtained from small RNA-seq experiments performed in *Stylophora pistillata* (Liew et al. 2014), where five microRNAs (miRNAs) among 50 were conserved in other metazoans. Other families of ncRNAs, such as lncRNAs, have also been recently described as putative players in coral responses to microbiome imbalance. In *Palythoa caribaeorum*, transcriptome analysis detected >10 000 expressed lncRNAs, some of which were conserved in higher eukaryotes (Huang et al. 2017). Investigation of differentially expressed lncRNAs in healthy colonies compared to bleached colonies indicated that upregulated lncRNAs in *P. caribaeorum* could act as post-transcriptional modulators of the Ras-mediated signal transduction pathway and components of the innate immune system, as part of the molecular response of corals to bleaching (Huang et al. 2017). ncRNAs have also been implicated in the establishment of endosymbiosis in cnidarians, e.g. miRNAs in the sea anemone *Aiptasia* (Baumgarten et al. 2018) and lncRNAs in the coral *A. digitifera* (Mohamed et al. 2016, Huang et al. 2019). Using high-throughput sequencing cross-linking immunoprecipitation (HITS-CLIP), miRNAs were found to be differentially expressed and subsequently targeted genes implicated in Symbiodiniaceae colonization (e.g. FGFR, TGFβR, and components of the TNFR/TRAF pathways, arrest of phagosomal maturation, and sterol/peptide transporters) in response to endosymbiont infection (Baumgarten et al. 2018). Twenty-one out of 815 lncRNAs were differentially expressed 4 h post-colonization of algal symbionts (Huang et al. 2017) for which gene co-expression networks identified 6395 coral transcripts potentially regulated. Many of these transcripts were involved in the early stages of coral–algal interactions. Cumulatively, these studies provide preliminary evidence that ncRNAs modulate specific pathways related to symbiosis onset and breakdown of the cnidarian–Symbiodiniaceae relationship.

The transfer of communication signals (ncRNA, proteins, metabolites) between cells via exosomes (Fig. 4) was first observed in mouse and human cells (Valadi et al. 2007). Functional transfer of ncRNAs among tissues of the same organism was demonstrated in animal models, where exosome-mediated transfer of miRNA from adipose tissue were released and transported through circulating fluids to their final target distant tissues (Thomou et al. 2017). Exosomes may be microbiota-derived to deliver microbial signals that can control diverse pathways in the host (Ahmadi Badi et al. 2017). Our current understanding of ncRNAs sorting and processing via exosomes stems from miRNAs in human and mammalian systems (Kogure et al. 2019, Lee et al. 2019). However, the mechanisms of ncRNAs processing are not well understood in nonmodel species such as cnidarians and bacteria. ncRNAs are promising communication signals within the holobiont due to their common mechanisms of action among different compartments of the holobiont. The regulatory roles of molecules like miRNAs in eukaryotes and sRNAs in prokaryotes have been previously established (Blenkiron et al. 2016, Viennois et al. 2019).

On the other hand, the regulatory roles of other ncRNAs such as lncRNAs within holobiont context are still unknown.

Integrated host–microbiota multiomics to understand holobiont biology

Over the past decades, technical and computational advances have allowed the collective use of 'omics tools to better understand different aspects of host–microbiome systems. In corals, however, most of these 'omics tools have been used separately to understand a particular molecular level at a time. In this section, we describe the recent omics tools used in the field that shape our understanding of the coral microbiome and discuss the need for integrated coral–microbiota multiomics data to unravel the different layers of the coral microbiota interplay.

Characterizing the structure and functional potential of the coral microbiome

Recent advances in coral holobiont research have been possible due to the rise of 'omics techniques, particularly genomics (Cooke et al. 2019, Jasper et al. 2019, Engelberts et al. 2021). Among the most widely used methods to characterize the diversity of coral microbiomes and understand their role in light of climate change is amplicon sequencing studies (Fig. 5). Most of these efforts focus on examining the diversity of coral-associated microbes using sequencing amplified variable regions of marker genes such as the 16S rRNA gene for prokaryotes or internal transcribed spacer 2 (ITS2) and the 18S rRNA gene for eukaryotes like Symbiodiniaceae and fungi. Amplicon sequencing is affordable, enabling the incorporation of large sample numbers; however, classification of prokaryotes using 16S rRNA amplicons is limited mostly to the family or genus level while species-/strain-level classification is mostly not attainable (Johnson et al. 2019). Shallow whole-metagenome shotgun sequencing has been proposed as a cost-effective tool to study microbial diversity at higher accuracy compared to amplicon sequencing (Xu et al. 2021a). Even when species-level classification is possible using the full-length 16S rRNA gene (Matsuo et al. 2021), connecting such species to functional potential is difficult (Jasper et al. 2019). Inherent biases in PCR that are used in amplicon sequencing is another limitation (Aird et al. 2011). A workaround uses phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt, PICRUSt2) (Langille et al. 2013, Douglas et al. 2020) and Tax4fun (Aßhauer et al. 2015, Wemheuer et al. 2020), which enable prediction of gene function from 16S rRNA gene information based on publicly available reference genomes (Ainsworth et al. 2015, Röthig et al. 2016, Ziegler et al. 2017, Hernandez-Agreda et al. 2018). While this is a useful approach for well-studied and sequenced microbes, prediction of function for microbes with unresolved phylogeny, cryptic microbes, and microeukaryotes is largely limited (Sun et al. 2020). In addition, genomic islands and plasmids in bacteria that are often horizontally transferred among bacteria and typically contain antibiotic resistance genes and other genes for rapid responses to environmental change are not resolved using PICRUSt (Sun et al. 2020).

A handful of studies examined the coral microbiome using metagenomics and the subsequent acquisition of MAGs to study the functional potential of coral-associated microbes (Cai et al. 2017, Meyer et al. 2017, Robbins et al. 2019, Keller-Costa et al. 2021, Wada et al. 2022). Shotgun metagenomics enables the characterization of the diversity and functional potential of microbial communities, including the coral host with less bias than PCR-based amplicon sequencing. The assembly of MAGs enables direct examination of the role specific microbes may play in the holobiont. Metagenomics data can also integrate with other 'omics techniques (discussed below) to provide a more holistic understanding of the holobiont. Despite these advantages, shotgun metagenomic data still suffers from some limitations. Shotgun metagenomics is significantly more expensive, though the cost is constantly decreasing, and computationally more demanding than amplicon sequencing. Due to the larger genomes and biomass of the coral host and Symbiodiniaceae cells compared to other microbial cells, shotgun metagenomics produces mostly coral and symbiont reads, with microbial reads becoming a small fraction of the total output. While *in-silico* separation of these microbial reads from other reads is possible, it is technically challenging and requires sequencing to high depths, especially when lacking reference genomes for the coral and Symbiodiniaceae. Physical size fractionation to enrich prokaryotic cells and DNA has been successfully applied in selected reef species, albeit with limitations (Littman et al. 2011, Robbins et al. 2019). Nonetheless, more metagenomic sequencing is urgently needed to advance our understanding of what coral symbionts contribute to the host and the role of cryptic microbes in this relationship.

Metatranscriptome profiling to study holobiont transcriptional responses

The availability of draft genomes for several coral species paved the way to a wave of coral transcriptome-wide sequencing studies (Fig. 5). RNA-seq is the most widely used among the 'omics methods to understand coral responses during exposure to disease (Libro et al. 2013, Wright et al. 2015, Anderson et al. 2016, Frazier et al. 2017), establishment of coral–algal symbiosis (Mohamed et al. 2016, Yuyama et al. 2018, Mohamed et al. 2020b, Yoshioka et al. 2021), adaptation to the deep sea environment (Yum et al. 2017), responses to natural bleaching (Pinzon et al. 2015, Rose et al. 2015, Seneca and Palumbi 2015), and heat stress (Savary et al. 2021, Voolstra et al. 2021b). In addition to characterizing the transcriptional responses of coral hosts via mRNA differential analysis, metatranscriptomics has been scarcely utilized in corals to study the transcriptional responses of the microbiome primarily because of the need to overcome the relatively high abundance of host RNA relative to the microbiome. Studies that utilized metatranscriptomics have discovered new patterns in the holobiont. For example, Daniels et al. (2015) identified shared and distinct transcriptional responses to disease among different holobiont compartments, where innate immunity, cytoskeletal integrity, cell adhesion, and oxidative stress characterized the coral response, heat shock proteins, genes related to oxidative stress, and DNA repair characterized the bacterial response, and photosynthesis, and metal transport characterized the algal symbiont's response. These results highlight a functional integration across the holobiont in response to disease. Metatranscriptome data from three different coral species identified host and algal symbiont genes that exhibited different changes in gene expression in a lineage-specific way (among the Robust and Complex coral clades) and showed higher bacterial diversity, bacterial metabolic capabilities, and transcriptional activity in the thermo-tolerant to -susceptible species suggesting potential roles for the bacterial microbiome in supplementing the metabolic needs of the holobiont during heat stress (Avila-Magaña et al. 2021). Metatranscriptomic data have also been generated from *in-hospite* Symbiodiniaceae in both adult and larval stages (Bellantuono et al. 2019, Maor-Landaw et al. 2020, Mohamed et al. 2020b) highlighted a genealized transcriptome-wide suppression that includes photosynthesis and protein synthesis during symbiosis onset.

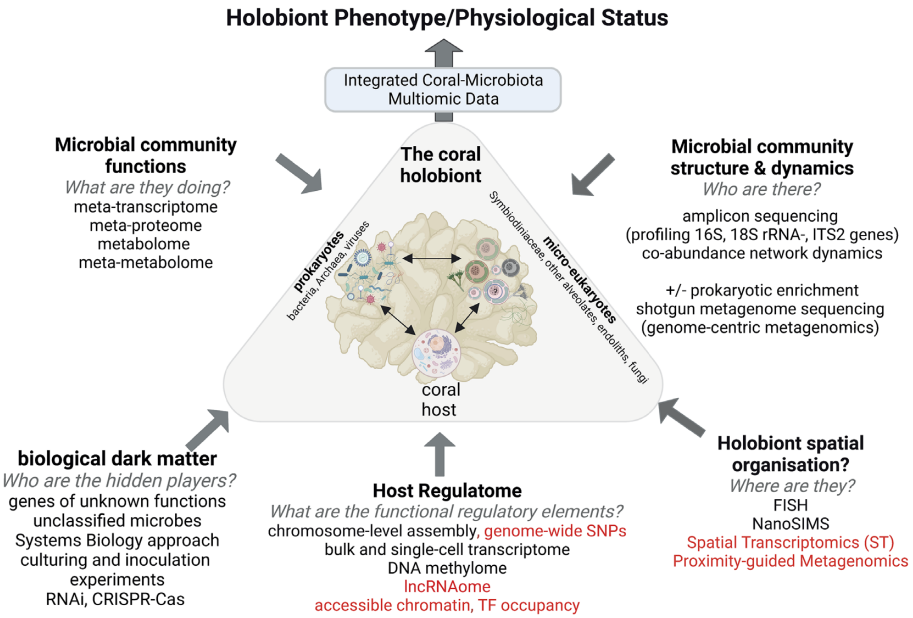

Figure 5. The different omics approaches deployed for studying the coral holobiont. An integrated coral–microbiota multiomics approach is needed to fully understand the biology of the coral holobiont. New approaches yet to be explored are highlighted in red.

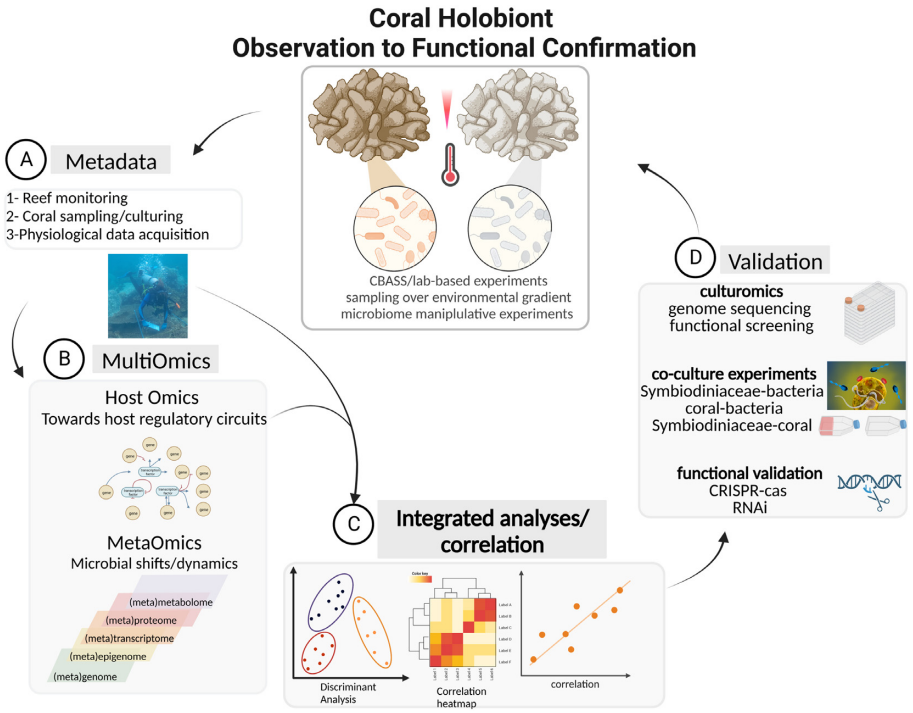

Figure 6. Strategies for conducting experiments aimed at understanding coral holobiont functions. The collection of multiomics data representing both the coral (host omics) and its microbiome (metaomics). The integrated multiomics analyses need to be paired with accurate metadata measurements for correlations. Hypotheses drawn from the integrated multiomics data should be tested by further validation experiments.

Coupling gene co-expression networks with identifying key regulators in metatranscriptomics

Gene networks have been recently utilized to identify gene expression patterns in corals using weighted gene coexpression network analysis (WGCNA), that quantifies the co-expression patterns among DE genes, to identify clusters of highly correlated genes (gene modules) (Langfelder and Horvath 2008). These efforts have mainly focused on coral gene expression data to identify expression modules or 'clusters of co-expressed genes' (Rose et al. 2015). Indeed, WGCNA-inferred gene networks revealed a potential adaptive mechanism named 'transcriptional frontloading', which means the constitutive higher baseline of expression levels of stress response genes in well-adapted 'stress resilient' corals compared to less-adapted 'stress susceptible' counterparts (Brashis et al. 2013). WGCNA analyses were implemented to study the response to experimental heat stress of *A. hyacinthus* colonies

that had been transplanted between two differing reef environments (Rose et al. 2015, Bay and Palumbi 2017). These experiments identified modules of coexpressed genes where some of which correlated strongly with the bleaching responses of individual colonies, hence called 'bleaching modules'. These genes were proposed as potential biomarkers for predicting coral survival under environmental stress. However, the applied WGCNA approach is best for identifying expression modules but has not been used to pinpoint to master regulators that could control the transcriptome remodelling due to a given perturbation.

Other information can be readily extracted from coral transcriptomes beyond differentially expressed genes, such as the regulatory potential of TFs and ncRNAs including lncRNAs. Despite the availability of the needed coral transcriptomic data, these analyses are not widely performed in the coral field. Amongst other promising network approaches, the Partial Correlation and Information Theory (PCIT) algorithm (Reverter and Chan 2008) aims at identifying key regulatory factors within the gene network by applying. PCIT combines partial correlation coefficients with information theory to explore all the correlations between possible triplets of genes within the dataset prior to the identification of significant correlations. This approach has been coupled with the concept of differential networks (the difference in connections per node from one network to another) (Ideker and Krogan 2012, Hu et al. 2016) to understand various traits in other species and identify master regulators (Cánovas et al. 2014, Wouters et al. 2020, Botwright et al. 2021, Mohamed et al. 2022). Master regulators undergo substantial changes in connectivity to genes during the transition between physiological states so that differential connectivity may identify highly differentially connected genes between the networks (Hudson et al. 2012). These master regulatory genes within a network may act as key regulatory components of transcriptional networks that could be used further in functional assays (e.g. gene editing) to understand their functions beyond correlations. A shift from differential expression to differential networks in coral molecular studies would allow an understanding of the gene regulatory circuits underlying various traits in corals.

Proteomics and metabolomics potential to uncover the metabolic activity of the holobiont

While metatranscriptomics alone has proven useful in the few examples it has been applied, integrating with other meta-'omics techniques can further confirm gene expression patterns, quantify their impact and shed light on new metabolic and symbiotic patterns. Particularly, mass-spectrometry based techniques, like proteomics and metabolomics (Fig. 5), are elucidating new discoveries in holobiont research. Proteomics was recently successfully used to distinguish between host and symbiont-related responses due to heat stress, showing downregulation of symbiosis signals in the host, and photosynthesis breakdown in the symbiont (Mayfield et al. 2021, McRae et al. 2021, Petrou et al. 2021). Proteomics also shed light on the potential role of bacterial symbionts, such as vitamin supply by *Endozoicomonas* to their host (Pogoreutz et al. 2022). New molecules such as the dipeptides lysine–glutamine and arginine–glutamine have been identified as molecular biomarkers for coral thermal stress (Williams et al. 2021a).

Although proteomic data correlates well with physiological data, there is a temporal delay in transcript-to-protein responses in most organisms that often renders proteomic data in apparent disagreement with transcriptomic data if this delay is not taken into consideration (Mayfield et al. 2018). However, proteomic data are considered more representative of observed phenotypes, unlike transcriptomics where epigenomic regulation and post-translational modifications may skew transcriptome–phenotype comparisons (Manzoni et al. 2018). Therefore, proteomic studies need to become a larger component of coral studies.

Compared to other 'omics techniques, metabolomics (Fig. 5) has largely lagged due to major limitations in detection of low-abundance metabolites and the large number of cryptic metabolites that are often observed in biological systems (Vohsen et al. 2019). Therefore, most studies resolve to compare overall metabolic profiles at various conditions, while highlighting only putative identifications (Sogin et al. 2017, Hillyer et al. 2017a, Lohr et al. 2019). Several metabolomics studies have shed light on new mechanisms of interactions within the coral holobiont, such as increases in platelet activation factors at coral–algal interfaces (Quinn et al. 2016). The correlations of various lipid classes like betaine–lipids and diacyl–glycerides were able to indicate previous bleaching events of coral colonies (Roach et al. 2020), can be markers of disesases (Deutsch et al. 2021). Likewise, dipeptides were reported as indicators of heat stress (Williams et al. 2021a). By sampling coral colonies at different distances from the coral animal surface, dozens of metabolites and chemical features were found to form a gradient around coral colonies; these metabolites were composed of diverse chemical classes that may be important in structuring the SML microbiome (Ochsenkühn et al. 2018), such as the hormone estrogen (Vilela et al. 2021), which can restructure the microbiome in addition to controlling stress responses (Stien et al. 2020). *In-silico* analysis of mass shifts in holobiont mass spectrometry datasets, like the addition/substraction of e.g. hydroxy (−OH) or methyl groups (−CH3), can be used to reveal genetic differences and even can be correlated to transcriptomic variances of closely related organisms (Hartmann et al. 2017). Beyond predicting metabolites, examination of elemental exchanges within the holobiont using stable-isotope labelling (e.g. ^{13}C, ^{15}N) of metabolites or nutrients has been useful in determining the role of diazotrophs in the holobiont as a source of nitrogen in a stable symbiotic state but disregards an oversupply of microbial derived N during heatstress (Rädecker et al. 2021). Isotope labelling of coral fragments in ^{13}C-bicarbonate enriched media of e.g. amino acids, fatty acids or lipogenesis intermediates confirmed under severe heat stress conditions decreases of *de novo* biosynthesis of fatty acids in the symbiont leads to a consequent decrease in fatty acids in the host (Hillyer et al. 2017b).

Despite its promises and relatively low cost compared to sequencing, metabolomics suffers from several limitations. The immense diversity of chemical formulae and resulting structures poses the biggest challenge in mass spectrometry-based metabolomics, where annotations can only be confirmed reliably with comparisons to known standards. Further, extraction protocols, e.g. differences in solvent mixtures, disruption techniques, or even cooling, have an influence on detected molecules as some metabolites are prone to degradation (Lu et al. 2020), while others are not efficiently extracted (Andersson et al. 2019), which calls for standardization. In the context of the holobiont, a major challenge is linking metabolites with their producing organism. Statistical correlation of metabolite and amplicon sequencing data enables linking of an organism with its metabolites (Jorissen et al. 2021); however, most central metabolites are shared across different organisms within the holobiont and so this approach only applies to unique secondary metabolites. Using metagenomic, metatranscriptomic, or proteomic data can enhance metabolomics significantly since enriched organisms/genes/proteins from a specific organism is often indicative of a corresponding increase in

metabolite abundance. For example, steroids depend on the presence of algal symbionts, which cnidarians are unable to produce. Therefore, the expulsion of symbionts during bleaching, consequently leads to a decrease of steroids in the host (Jiang et al. 2021). Another approach relies on isolating and culturing microbes from the host, characterizing metabolites from these microbes, and combining this information with data acquired from coral samples. This approach identified antioxidants and osmolytes, like betaine–lipids or glucosides from the coral microbiome, which are hypothesized to increase holobiont stress resistance (Gegner et al. 2017, Ochsenkühn et al. 2017, Roach et al. 2021). Despite its limitations, metabolomics is steadily gaining traction with improvements in protocols, instrumentation and analysis and is becoming a complementary tool with high predictive power (Lu et al. 2017, Greene et al. 2021, Wegley Kelly et al. 2021).

Multiomics data are urgently needed for a holistic understanding of the holobiont

A single layer of omics is not usually adequate to understand a complex system such as the coral holobiont. An integrated host–microbiota multiomics framework has been developed and proposed to understand other holobiont systems (Nyholm et al. 2020). Nyholm et al. recently coined the term 'holo-omics' to describe experiments that aim to obtain multiple omics data from both host and microbiota domains. This holistic approach has been recently applied to study the plant microbiome (Zolti et al. 2020; for a review, see Xu et al. 2021b), the human microbiome (Heintz-Buschart et al. 2016, Lloyd-Price et al. 2019, Park et al. 2022) [for a review, see Zhang et al. (2019)]. For example, Lloyd-Price et al. (2019) provided a comprehensive multiomics data during the functional dysbiosis in the human gut microbiome upon the progression of the inflammatory bowel disease. In the Lloyd-Price paper, metagenomic, metatranscriptomic, and stool metabolomic profiles were combined to show a unique microbiome restructure characterized by an increase in facultative anaerobes at the expense of obligate anaerobes and identify biochemical and host factors central to this dysregulation.

In corals, most of the omics data generated were obtained solo; there have been attempts to attain multiomics data (Cziesielski et al. 2018, Maruyama et al. 2021, Santoro et al. 2021, Voolstra et al. 2021b, Williams et al. 2021b, Pogoreutz et al. 2022). However, most of these studies have exclusively relied on descriptive microbiome tools such as amplicon sequencing (Table 1), that have been significant in shaping our understanding of the coral microbiome composition; but limited in providing mechanistic insights into mechanisms of coral–microbiome interactions. However, a few examples showed the power of integrating metatranscriptomes and metagenomes to understand microbial processes during onset and progression of the black band disease (Arotsker et al. 2016, Sato et al. 2017). Williams et al. (2021) combined polar metabolomics with host transcriptomics to investigate gene–metabolite interactions in the coral *Montipora capitata* exposed to a 5-week period of thermal stress. The gene–metabolite integrated analysis revealed thermal stress affects reproductive activity evidenced by the downregulation of CYP-like genes and the irregular production of sex hormones. Despite these data being focused on the coral host, they provided a set of genes and metabolites that can be used as markers of coral thermal stress. Indeed, leveraging the integrated metatranscriptomic and metagenomic data would greatly enhance our understanding of onset of coral disease that will help in coral disease prognostics and coral bleaching management. Santoro et al. (2021) was a clear example that made use of manipulative experiments, physiology to define the phenotype before collecting multiomics data, 16S rRNA amplicon sequencing, host gene expression, and metabolomes that were well correlated with physiological responses associated with health status. However, we currently lack comprehensive functional insights into coral–microbiome interactions, despite the recent advances in coral microbiome research. To reach a more comprehensive, systems-level view of coral–microbiome interactions, experiments should focus on pairing the host-centred omics data such as host transcriptome, epigenome, and metabolome/proteome with microbially centred data such as shotgun metagenomes, metatranscriptomes, and meta-metabolome/meta-proteome (Fig. 5).

Current limitations and new directions

Despite years of significant work on cnidarian symbiosis [for reviews see Weis (2019), Rossett et al. (2021)], insights into the onset of coral–algal symbiosis (Davy et al. 2012, Mohamed et al. 2016, 2020b, Yoshioka et al. 2021), and more recently the role of nutrient cycling in the breakdown of coral–algal symbiosis during heat stress (Rädecker et al. 2022), a comprehensive mechanistic understanding of the synthesis, homeostasis, and demise of symbiosis due to coral bleaching is still lacking. Many of the current insights stem from studies conducted on other cnidarian species such as *Aiptasia* [see e.g. Celeves et al. (2020)] as a coral model, due to difficulties in experimentally manipulating corals in the lab. Only recently, the tropical stony coral species *Galaxea fascicularis* was proposed as a candidate coral model system (Puntin et al. 2022). This will indeed help us understand the molecular underpinnings of coral symbiosis in the near future.

Despite the high potential of multiomics data, the presence of cryptic biological information ('biological dark matter', e.g. the noncoding part of the genome (Eisenstein 2021) and hypothetical proteins with unknown function (Stephens et al. 2018) and cryptic or undescribed microbes (Lok 2015)) is a major limiting factor for understanding the coral holobiont. On the other hand, traditional molecular biology and biochemistry techniques to characterize gene/protein function are time consuming and are low throughput. Gene networks have been proven to detect hypothetical proteins with presumable importance (Cleves et al. 2020). By applying the 'guilt by association' principle, these genes/proteins or unclassified microbes connected with other genes/microbes of known function (Fig. 5) may shed light on their importance. A few such genes were co-expressed along other genes in the same module that was upregulated shortly after thermal stress (Cleves et al. 2020). These hypothetical proteins of interests can be then folded using Alpha-fold to gain more insights into their functionality (Ma et al. 2022). More methods such as RNA interference and CRISPR/CAS9-mediated genome editing that enable identification and characterization of microbial and gene functions are needed to overcome this major hurdle in biology.

Recent advances in genomics have allowed the construction of detailed cell type atlases for a soft coral (*Xenia* sp.) and a stony coral (*Stylophora pistillata*) using single-cell RNA sequencing (scRNA-seq) (Hu et al. 2020, Levy et al. 2021), chromosome-level genome assemblies (Fig. 5) for the soft coral *Xenia* sp. and the hard corals *A. millepora* and *M. capitata* (Fuller et al. 2020, Hu et al. 2020, Stephens et al. 2021), and the coral endosymbiont *S. microadriaticum* (Nand et al. 2021). Recent initiatives such as the Aquatic Symbiosis Genomics (ASG) project (McKenna et al. 2021) will provide chromosome-level genomes for ~500 symbiotic systems including corals and their microbiomes. Within the ASG project, a

Table 1. Recent coral studies adopting multiomics to investigate the molecular basis of disease onset, thermal tolerance, and symbiosis. The coral species, the 'omics approaches utilized, and the scope of the study are shown.

Study	Coral species	'Omics approach	Scope
Daniels et al. 2015	*Orbicella faveolata*	Metatranscriptomics Host, algal/bacterial symbionts	White plague disease
Sato et al. 2017	*Montipora hispida*	Metatranscriptomics Shotgun metagenomics	Black band disease
Meyer et al. 2017	*Montastraea cavernosa*	16S rRNA amplicon sequencing Shotgun metagenomics	Black band disease
Cziesielski et al. 2018	*Aiptasia pallida*, a coral model	Host transcriptomics Proteomics	Thermal stress
Cleves et al. 2020	Review article	Multiomics and reverse genetics	Thermal stress
Roach et al. 2020	*Diploria strigosa* and *O. faveolata*	Shotgun metagenomics Metabolomics	Coral–turf algal interactions
Williams et al. 2021b	*M. capitata*	Host transcriptomics, metabolomics	Thermal stress
Santoro et al. 2021	*M. hispida*	16S rRNA amplicon sequencing Host transcriptomics Metabolomics	Microbiome-enabled thermal tolerance
Voolstra et al. 2021b	*Stylophora pistillata*	Host + algal transcriptomics and ITS2/16S rRNA amplicon sequencing	Thermal tolerance
Savary et al. 2021	*S. pistillata*	Host + algal transcriptomics and ITS2/16S rRNA amplicon sequencing	Thermal tolerance
Maruyama et al. 20212021	*Acropora tenuis*	Host + algal transcriptomics, 16S rRNA amplicon sequencing, and bacterial genome sequencing	Coral–microbiota interactions
Pogoreutz et al. 2022	*A. humilis*	16S rRNA amplicon sequencing, host transcriptomic, proteomics, and bacterial genome sequencing	Coral–*Endozoicomonas* symbiosis

combination of long-read and long-range genomic data will be generated using the Pacific Biosciences sequencing platform to generate high fidelity reads in the 15–20 kb range, along with Oxford Nanopore long-read technologies. Transcriptome data will be generated to help annotate those genomes. Chromatin conformation capture sequencing, known as 3C or Hi−C, will be used to link sequences within chromosomes and organelles (Belton et al. 2012). The same proximity ligation strategy has been developed recently to study the microbiome (meta3C). The ProxiMeta platform is designed to deconvolute chromosomes and plasmids in a mixed microbial sample into complete genomes (Stadler et al. 2019). More recently, spatial transcriptomics (based on 10× Genomics data) has been developed to couple gene expression data with spatial information (spatially resolved transcriptomes) that enable measuring all the gene activity in a given tissue sample and mapping where the activity is occurring (Larsson et al. 2021, Rao et al. 2021). These new approaches will enable disentangling functions of different microbiome members within the holobiont. These data will provide baseline genomic resources that will revolutionize the way researchers look at and design symbiosis experiments to understand the molecular mechanisms underlying homeostasis of the coral holobionts and their responses to climate change.

The Assay of Transposase Accessible Chromatin sequencing (ATAC-seq) has become increasingly popular for detecting chromatin accessibility (Yan et al. 2020). The quest of identifying regulatory elements across different cell types and developmental stages has led to large international efforts mostly focusing on model organisms, such as the human Encyclopedia of DNA Elements (ENCODE; Moore et al. 2020), and the Functional Annotation of Animal Genomes (FAANG; Giuffra et al. 2019), to unravel the regulatory elements in model/nonmodel organisms. Spatiotemporal changes in the epigenome at the chromatin level are crucial to development, cellular differentiation, health, and disease (Gorkin et al. 2020, Fang et al. 2021). In corals, epigenetic mechanisms have been exclusively studied through DNA methylation. The study by Liew et al. (2018) reported that DNA methylation levels in corals are highly sparse, where only 9% of genome-wide CpG loci were methylated, most of which are co-located within gene bodies, in contrast to higher-level genome-wide methylation levels in promoters and enhancers in vertebrates. ATAC-seq data, paired with RNA-seq, was utilized for the first time to study the cnidarian–dinoflagellate model *Exaptasia pallida* to reveal a role of chromatin dynamics in response to thermal stress (Weizman and Levy 2019). Compared to DNA methylation, chromatin accessibility data (Fig. 5) hold a great promise towards understanding epigenetic mechanisms and their role in regulating gene expression in corals as shown for other non-model species (Alexandre et al. 2021, Mohamed et al. 2022). Indeed, defining the coral regulatory vocabulary would allow understanding many aspects of coral biology including responses to climate change at an unprecedented level.

The use of the forementioned omics technologies and their integration will be only valuable when combined with specific manipulative experiments with accurate physiological and/or environmental monitoring to be correlated with the molecular and/or microbial data. The correlation of the multiomics data with the holobiont's physiological status (phenotype) would provide valid hypothesis to be developed to understand coral thermal thresholds, their response to stress, susceptibility to pathogens, and

resilience. Finally, these hypotheses have to be validated with further genetic or microbial manipulative experiments to confirm these sequencing-based findings (Fig. 6).

Conclusion

Despite the recent development of molecular tools for understanding the diversity and function of coral holobionts, a mechanistic knowledge of the coral microbiome and its role in coral evolution and adaptation is still missing. Here, we charted an overview of the taxonomic diversity and function of microbiota associated with corals, the latest updates on coral microbiome research, and insights into the possible mechanisms through which coral–microbiota interactions could occur. We highlight that while most 'omics techniques developed for corals have been powerful, integrated 'coral–microbiota' multiomics data are needed for holistic and systems-level understanding of the mechanisms underpinning the symbiotic and dysbiotic interactions within the coral holobiont. We also point the importance of the coral regulatory circuits and elements in responding to the microbiome and environmental change and how this knowledge can be used in an integrated multiomics framework. These advances, especially when combined with specific manipulative experiments and/or field samples and correlated with physiological status, promise to push the boundaries of knowledge of coral–microbiome research and may help global efforts to preserve corals in the future.

Acknowledgements

We would like to thank Mr. Glynn Gorick for his help creating Fig. 1. Figures 3–6 were created with BioRender.com.

Supplementary data

Supplementary data is available at *FEMSRE*online.

Conflict of interest statement. The authors declare no conflict of interest.

Funding

This work was supported by Tamkeen under the NYU Abu Dhabi Research Institute Award to the NYUAD Center for Genomics and Systems Biology (ADHPG-CGSB) and the NYU Abu Dhabi grant [grant number AD179] to S.A.A.

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