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Cell biology in model systems as the key to understanding corals

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Corals provide the foundation of important tropical reef ecosystems but are in global decline for multiple reasons, including climate change. Coral health depends on a fragile partnership with intracellular dinoflagellate symbionts. We argue here that progress in understanding coral biology requires intensive study of the cellular processes underlying this symbiosis. Such study will inform us on how the coral symbiosis will be affected by climate change, mechanisms driving coral bleaching and disease, and the coevolution of this symbiosis in the context of other host–microbe interactions. Drawing lessons from the broader history of molecular and cell biology and the study of other host–microbe interactions, we argue that a model-systems approach is essential for making effective progress in understanding coral cell biology.

Introduction

Reef-building corals are composed of mutualistic partnerships, or symbioses, between cnidarian hosts, their photosynthetic dinoflagellate endosymbionts and a complex community of microbes. These partnerships form the trophic and structural foundations of the coral reef ecosystem (Figure 1a) [1]. Much is known about the ecology and evolution of corals [1,2]. There has also been significant progress during the past 15 years in understanding the cnidarian–dinoflagellate symbiosis, especially in the areas of host and symbiont diversity, phylogenetics and population structure [3,4]. Furthermore, recently launched functional-genomic studies hold the promise of providing an enormous amount of information about patterns of gene expression both during healthy symbiosis and during stress [5–7]. However, there has been little recent progress in the understanding of host–symbiont interactions at the cellular and molecular levels, such as the mechanisms of recognition and specificity and the modes of inter-partner communication and regulation (e.g. of nutrient exchange and cell division). Gaining detailed insight into these mechanisms has now become critical to our understanding of the response of corals to environmental stresses.

Coral reefs are declining globally, owing to multiple factors that include reduced water quality, over-exploitation of key coral reef species and climate change, which has driven increasingly destructive mass bleaching and mortality events since 1979 [8]. Even in relatively unstressed oceans, such as the Pacific, coral cover has declined by 2% per year over the past 10 years [9]. Climate change is affecting coral reefs in at least two fundamental ways. The first is through ocean acidification, arising from increased concentrations of atmospheric carbon dioxide entering the ocean and decreasing the oceanic concentrations of carbonate ions, and hence reducing coral calcification which is dependent on carbonate [10]. The second way that climate change is affecting coral reefs is via increased sea temperatures that have driven increasingly intense and frequent coral bleaching across thousands of square miles of reefs (Figure 1b) [8]. Coral bleaching occurs when the symbiosis becomes dysfunctional and algal symbionts are lost from the coral tissues [11]. It results in decreased growth, increased susceptibility to disease and dramatically increased mortality [8,11]. We still have very little insight into the underlying cellular mechanisms responsible for bleaching.

Here we argue that we need to develop and use genomic and cell biology resources in the study of cnidarian–dinoflagellate symbiosis and that such an effort will provide insight into the ecology and evolution of this critically important symbiosis. (Our views were crystallized by a recent international workshop that brought coral biologists together with cell biologists and host–microbe interaction biologists to examine the challenges and opportunities in the study of coral cell biology.) We will (i) illustrate how knowledge of the cell biology of cnidarian–dinoflagellate symbioses can inform the study of their ecology and evolution, (ii) explain the value of experimentally tractable model systems in cell biology in general and in host–microbe interactions in particular, (iii) outline the current status of coral cell biology and argue for the adoption of a model-systems approach and (iv) identify key research priorities in the field.

The importance of cell biology for understanding the ecology and evolution of cnidarian–dinoflagellate symbioses

Host–symbiont specificity and flexibility

Thousands of species of cnidarians engage in symbiosis with a large diversity of dinoflagellates from the genus

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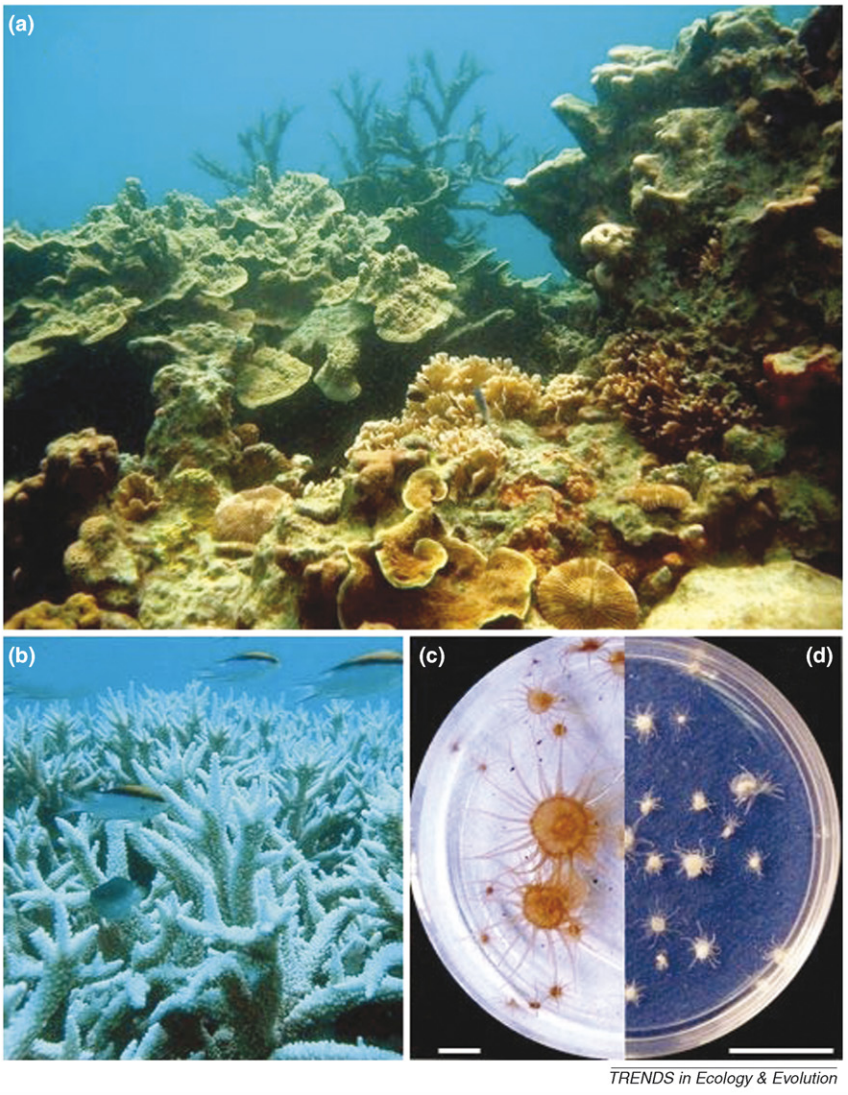

Figure 1. Healthy and bleached corals can be modeled in the laboratory by studying the *Aiptasia–Symbiodinium* symbiosis. **(a)** A healthy coral reef on the coast of Java, Indonesia. The brown color of the corals is caused by the symbiotic dinoflagellates living within their tissues. **(b)** Bleached corals on the southern Great Barrier Reef, January 2002. Thermally induced loss of the symbiotic dinoflagellates and/or loss of photosynthetic pigments results in the underlying white coral skeleton becoming visible through coral tissues. **(c)** Laboratory culture of the sea anemone *Aiptasia* sp, a model organism for the study of coral–dinoflagellate symbiosis. **(d)** Laboratory culture of *Aiptasia* sp., whose symbiotic dinoflagellates have been removed by temperature shock and dark treatment. Such aposymbiotic anemones can be re-infected with symbionts, thus providing a model for studying host–symbiont recognition and symbiosis establishment and maintenance. Scale bar = 1 cm for (c) and (d). (Photos: O.H-G., [a] and [b]; Carlo Caruso, [c] and [d]).

Symbiodinium (Box 1). With the advent of molecular tools, the biodiversity of the separate partners has become increasingly well documented [3,12], but the diversity of host–symbiont combinations is astonishingly complex and still poorly understood [13,14]. Considerable effort has been devoted to sampling symbiont types in corals across a variety of spatial and temporal scales [14]. However, we only have a few examples of symbiont shuffling in the wild [15], and we still do not know whether the particular host–symbiont combinations found in nature can change with time and do so rapidly enough to keep pace with the rapidly changing environmental conditions associated with climate change. Indeed, the 'adaptive bleaching hypothesis,' the idea that bleaching is a deliberate strategy that allows corals to swap their symbionts as an adaptation to a changing environment, has been highly controversial [14,16,17]. A better understanding of the mechanisms that confer recognition and specificity between the two partners would help resolve these issues. These mechanisms are likely to include specific interactions during initial host–symbiont cellular contact [18], signals and signal-transduction pathways that allow the symbiont to evade the host's innate immune systems, as well as ecological competition between symbionts for the host intracellular niche [19,20]. In an era of climate change, insight into these cellular events will enhance our ability to predict how and whether cnidarian–dinoflagellate symbioses will cope with rapid environmental change.

Coral bleaching

We are just beginning to understand the proximal cellular events that result in loss of symbionts from host tissues. There is evidence that digestion of symbionts, host-cell apoptosis and necrosis, exocytosis of symbionts and detachment of whole symbiont-containing host cells can all contribute to symbiont loss [21–23], but the relative

Box 1. Systematics and diversity of *Symbiodinium*

For many years, all symbiotic dinoflagellates in corals were considered members of a single pandemic species, *Symbiodinium microadriaticum* [47]. This notion started to be challenged in the 1980s when behavioral, physiological and ultrastructural evidence suggested higher diversity within *Symbiodinium* [48,49]. A decade later, with the advent of molecular phylogenetic techniques, Rowan and Powers revealed considerable diversity within the genus [50]. Many researchers have since contributed to the understanding of the genetic diversity of *Symbiodinium* and have shown that the molecular systematics of the group is complex [3,13]. At present, based on partial regions of 18S rDNA sequence, there are eight clades, A–H, within the genus (Figure I).

Diversity within these clades is very large when more variable ribosomal genes, such as the internal transcribed spacer (ITS), are used [51]. Recent studies indicate that diversity is even more extensive when flanking regions of microsatellite DNA are used to screen for genetic variation [52]. A formal systematic revision of *Symbiodinium* that takes into account the current molecular data has yet to be undertaken, and it will not be surprising if some of the major genetic clades come to represent different taxonomic families or orders.

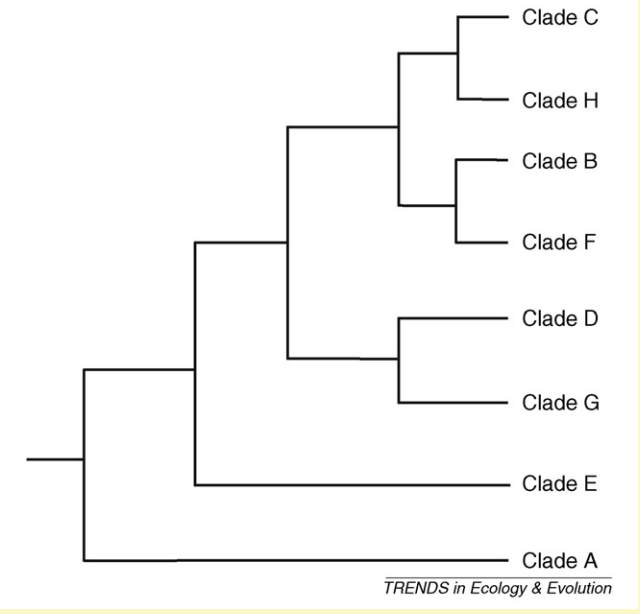

Figure I. The phylogenetic relationships between major clades of *Symbiodinium*. The topology is a consensus cladogram synthesized and generated by Coffroth and Santos [3].

contributions of these different mechanisms, and the factors that might determine their relative importance under different environmental conditions, remain unknown. There is increasing evidence that reactive oxygen species, generated from symbiont photosynthetic dysfunction [24], initiate a host-cell signaling cascade akin to an innate immune response that results in loss of symbionts [25–27], but again the details remain unknown. Clearly, a more complete understanding of the fundamental cellular mechanisms that maintain the symbiosis will contribute greatly to our ability to elucidate and perhaps manipulate the mechanisms that underlie its collapse under stress.

Evolution of host–microbe interactions

Recent advances in genomics have revealed unexpected and remarkable conservation of genome organization and content across taxa, as illustrated by the high homology of the genome of *Nematostella* (a nonsymbiotic anemone) and the genes expressed in the coral *Acropora millepora* with those of vertebrates [28,29]. In addition, the dinoflagellates and apicomplexan parasites such as *Toxoplasma* and *Plasmodium* display close phylogenetic placement within the kingdom Alveolata [30], suggesting that these microbes could share common mechanisms for invading and surviving in their hosts. This information should usher in a new period of comparative biology in which knowledge of the cellular mechanisms underlying the coevolution of other host–microbe interactions will provide key insight into the corresponding mechanisms underlying cnidarian–dinoflagellate symbioses.

The crucial role of model systems in cell biology

Model systems for the study of cell biology

The past 50 years have seen enormous progress in molecular, cell and developmental biology. Much of this progress has come from studies in a small number of intensively studied model organisms: the bacterium *Escherichia coli* and its viruses, the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, the nematode worm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, the small mustard plant *Arabidopsis thaliana*, the mouse *Mus musculus* and a few lines of cultured mammalian cells [31]. These organisms were deliberately chosen by far-sighted investigators because they had properties that made them highly amenable to rapid and rigorous experimental investigation, such as quick and easy growth of large numbers in the laboratory, the availability of clonal (or at least inbred) populations and susceptibility to genetic, biochemical, and microscopic analyses. As these properties attracted increasing numbers of investigators, the intrinsic advantages of these organisms were amplified by a rapidly increasing base of knowledge and experimental methodologies, making them even more attractive to new investigators, in a powerful positive-feedback loop.

In light of this history, it seems clear that progress in biology will typically be most rapid when a critical mass of investigators focuses synergistically on one or a very few well-chosen model systems: results will be directly comparable from laboratory to laboratory, effective collaborations will be fostered, new methods will be developed more rapidly and transferred more readily from laboratory to laboratory, the excitement of a rapidly moving field will attract talented and ambitious students and so on. This argument is further strengthened by the fact that the work in earlier model systems has now given us tools (e.g. genomics, vastly improved microscopy methods, gene knockdown by RNA interference [RNAi]) that have removed many of the earlier limitations on what could plausibly be chosen as a model system.

Model host–microbe interactions provide a roadmap for the study of coral–dinoflagellate symbiosis

Intensive examination of cellular interactions of animal cells with protozoan parasites and pathogenic bacteria has been ongoing for many years because of their medical and veterinary importance. Particularly compelling are experimental models that feature unnatural host–pathogen

Table 1. Examples of unanswered questions about the onset of cnidarian–dinoflagellate symbioses that have been well studied in other model host–microbe interactions

Unanswered questions in the cell biology of cnidarian–dinoflagellate symbioses	Examples of answers to these questions from cell biological studies of other model host–microbe interactions	Refs
What cellular signals are involved in host–symbiont recognition and the establishment of specific symbiotic associations?	Hosts commonly detect the presence of invading microbes by using a diverse array of pattern recognition receptors (PRRs) to recognize equally diverse microbe-associated molecular patterns (MAMPs) on microbe cell surfaces. Examples of very common MAMPs are glycans and lipopolysaccharide.	[53]
	MAMP–PRR interactions also initiate symbiotic host–microbe interactions, such as squid–bacteria and plant–nitrogen-fixing bacteria associations.	[33,34]
	Early studies suggest that MAMP–PRR interactions also occur in cnidarian–dinoflagellate symbioses.	[18]
Does the host mount an innate immune response to invading symbionts?	Animal hosts have a complex and highly conserved innate immune response that is designed to detect and eliminate invading pathogenic and/or parasitic microbes.	[54]
	Homologous mechanisms are used in modulating and regulating the model squid–bacteria mutualism.	[34]
	Recent genomic studies of cnidarians have revealed numerous innate immunity genes in the Cnidaria, suggesting that these responses could be functioning in symbiosis.	[41]
Do symbionts cause changes in host cells that allow the symbionts to persist? For example, is there blockage of phagosome–lysosome fusion or is the host-derived membrane surrounding the symbiont altered by the invading symbiont?	Invading microbes employ a variety of mechanisms to subvert attack by host cells. Many of these mechanisms involve altering the host cell by interfering with cell signaling.	[55]
	For example, the apicomplexan parasite *Toxoplasma gondii* remodels host phagosomes with its own proteins and excludes host proteins that promote phagosome maturation.	[56]
	In another example, *Mycobacterium*, the bacterium which causes tuberculosis, persists in macrophages by arresting phagosome maturation via altering cell-signaling pathways.	[57]
	Early studies suggest that similar mechanisms of persistence are functioning in cnidarian–dinoflagellate interactions.	[58]

combinations such as the injection of *Drosophila* with human pathogens that can be exploited to reveal features of the cellular interactions, such as the types of innate immune responses the human pathogens elicit in a host that would be difficult to decipher in less tractable natural host–pathogen combinations [32]. In addition, there are model systems for the study of symbiosis, including plant–nitrogen-fixing bacteria and squid–*Vibrio* interactions that are also well studied at the cellular level [33,34]. Knowledge of the cellular mechanisms at play in this broad array of host–microbe interactions can directly guide future cellular studies of cnidarian–dinoflagellate symbioses [35]. We illustrate this in Table 1 by providing just a few examples from model host–microbe interactions of answers to questions surrounding the initial interactions between hosts and microbes that are still unanswered in cnidarian–dinoflagellate symbioses. This list could easily be expanded to include other areas of cnidarian–dinoflagellate cell biology.

The way forward: the current status of cell biological study of corals and a move toward model systems

Current status of technique development

We believe that progress in understanding the cellular mechanisms underlying the cnidarian–dinoflagellate symbiosis will depend on the resources and tools that are available at both the molecular (including genomic) and cellular levels and on a move toward the use of model

Table 2. Status of genomic, molecular biological and cell biological resources and methods for study of cnidarian–dinoflagellate symbiosis

Resource/tool	State of development: present (P), under development (U) or absent (A)	Refs
Genomics		
Cnidarian genome	**A** but nonsymbiotic anemone *Nematostella* complete; nonsymbiotic *Hydra* nearly complete	[28]
Cnidarian EST[a] projects	**U** partial EST projects for several corals and symbiotic anemones including *Aiptasia*; more-extensive projects for *Nematostella* and *Hydra*	[6,29,59]
Symbiodinium genome	**A**	
Symbiodinium EST projects	**U** partial EST project for clade C3 from the coral *Acropora aspera*	[7]
cDNA microarrays	**U** partial-genome arrays produced for several corals and symbiotic anemones, and for *Symbiodinium* C3 from *A. aspera*	[5,7,60]
Cell and molecular biology		
Symbiodinium cell culture	**P** many strains	[51]
Host cell culture	**A**	
Release of host cells by maceration of animals	**P** studies in *Aiptasia* and some corals	[61]
In situ hybridizations	**U** limited studies in symbiotic anemones, corals and *Symbiodinium*	[62–65]
Creation of mutants through various forms of mutagenesis	**A**	
Gene knockdown by RNAi	**U** some success in *Aiptasia*	[23,62]
Host-cell transformation with GFP or other reporter-gene constructs	**A** but success in *Hydra*	[66]
Symbiodinium transformation	**A/U** one study	[67]
Immunofluorescence and other imaging techniques	**U** some successes with *Aiptasia* and coral larvae	[26,68–70]

[a]ESTs (expressed sequence tags) are partial sequences of genes that are being expressed in an organism at a given time.

systems. Table 2 presents the current status of genomic and cell biological technique development in cnidarian–dinoflagellate symbioses and Box 2 illustrates how these tools can be used to examine their cell biology. These techniques and resources, now routine in the major organisms used in modern cell and developmental biology, are beginning to be developed for the partners in the cnidarian–dinoflagellate symbiosis.

The anemone Aiptasia as a model for corals

A model-systems approach has not previously been widely embraced by the coral-biology field, most of whose practitioners have come from ecological and comparative-biology backgrounds and who have tended to study corals and other reef organisms that are ecologically important in one or more parts of the world. With an understanding of the success of other model systems in the study of cell biology, we argue here for a shift to intense focus on one or a few models. (The conclusions reached at the international workshop were striking: after five days of often-intense discussion that included detailed comparisons of the pros and cons of various model systems, even the most die-hard coral biologists had agreed not only that there was a need to focus on model systems but also on what the model systems should be.) For most aspects of the cell biology, the model cnidarian host on which investigators should concentrate is the small tropical anemone *Aiptasia* sp., an anthozoan (like the major reef-building corals) that is also symbiotic with dinoflagellates of the genus *Symbiodinium* (Figure 1c). The advantages of *Aiptasia* as a model are brought out very clearly by a direct comparison with corals (Table 3). Of particular note, and a huge advantage for the study of many aspects of the symbiosis, is that *Aiptasia* can be fully cleared of symbionts by temperature shocks (Figure 1d) [19,26], maintained in the symbiont-free state for months or years [36] and re-infected at will with a variety of *Symbiodinium* strains [19]. *Aiptasia* is not a complete newcomer to the field: it has been used for studies of cnidarian–dinoflagellate symbiosis in areas such as nutrient dynamics between partners [37], recognition and specificity [19] and bleaching [22] for more than 30 years.

Box 2. The use of genomic and cellular tools for the study of coral cell biology

Both genomic and cellular resources and tools are required for the study of modern cell biology and together they will improve our understanding of cnidarian–dinoflagellate symbiosis cell biology. Genomic resources can be mined to identify genes of interest in the symbiosis. One way to do this is to examine other model host–microbe interactions, and hypothesize which genes and pathways are involved in the symbiosis (Table 1). These genes can be searched for in annotated genomes and expressed sequence tag (EST) databases of model hosts and symbionts. Another way is to perform microarray experiments which can examine changes in gene expression of thousands of genes with changing conditions, such as a change in symbiotic state, through host development or with the onset of environmental stress. Once identified by genome searches or with microarrays, target genes and their associated pathways can be subjected to further empirical studies at the cellular level, such as those outlined below, to uncover their role in symbiosis.

An understanding of the cellular interactions between cnidarian host and dinoflagellate symbiont, such as inter-partner signaling, coordination of cell division and control of nutrient transport, is reliant in part on the ability to manipulate genes and gene products and to document resulting changes in the organisms. Manipulative techniques include (i) gain-of-function or loss-of-function gene mutations, (ii) gene knockdown by RNAi, (iii) introduction of genes by transformation with an accompanying reporter gene such as GFP (green fluorescent protein) and (iv) inhibition of gene products by the addition of pharmacological agents or antibodies. Documentation of these manipulations is possible using imaging techniques, such as confocal microscopy or flow cytometry, that track fluorescent probes. The ability to generate mutations, introduce genes or inhibitors and visualize experimental outcomes can be greatly enhanced when working with individual cells in culture or generated by maceration.

Because *Aiptasia* lacks a skeleton and the corresponding complex surface area of corals, it is not an appropriate model for some types of study, most obviously studies of the coral skeleton, calcification and surface and endoskeletal microbes [38–40]. For such studies, the organism of choice is the Indo-Pacific coral *Acropora millepora*, selected because its branching growth allows relatively easy collection and laboratory maintenance of coral fragments and because it is already widely studied, especially at the genomic level [29,41]. Other coral species such as the

Table 3. Advantages of *Aiptasia* as a model system

Property	Corals	*Aiptasia*	Refs
Size	Large colonies of many polyps	Individual polyps of variable size (0.5 mm–1 cm oral disc diameter)	[71,72]
Growth rate	Slow	Fast[a]	[71,72]
Growth conditions	Finicky, require very specific growth conditions	Tolerant, hard to kill[a]	[40,72]
Availability	Mediocre to poor: hard to grow, hard to collect (protected; only in tropical oceans), different species in different parts of world	Excellent: can be grown in large numbers in any laboratory using artificial sea water with fluorescent lights and pet-store brine shrimp as food	[71,72]
Genetic uniformity	Variable	Clonal populations available	[12,72]
Accessibility of cells for microscopy	Poor because of calcium carbonate exoskeleton	Excellent: by examination of whole polyps or cells in macerates	[26]
Accessibility for biochemical and molecular biological procedures	Mediocre because of calcium carbonate exoskeleton	Excellent: whole anemones can be homogenized or extracted	[59]
Susceptibility to genetic methods, including RNAi and transformation	Poor, except for some larval systems	Uncertain but has potential (and some promising early results)	[26,62]
Tolerance of bleaching	Poor: typically dies after bleaching	Excellent: can be fully bleached, maintained for months, and re-infected	[8,19,26]

[a]These properties cause *Aiptasia* to be considered a 'pest species' by salt-water aquarists.

Caribbean coral *Acropora palmata* [6] (because of its endangered species status) and the cosmopolitan Indo-Pacific *Stylophora pistillata* (because it is relatively easily cultivated in aquaria [40]) might also be considered as models.

We further argue that studies of *Symbiodinium* should focus on those symbionts found to occur naturally in the model hosts *Aiptasia* and *A. millepora*. These are clade B1, one of two types found in *Aiptasia* [42] and clade C3, which is found in *A. millepora* [43]. B1 is already in culture [42]. Both are considered generalists, that is they are found in many different hosts in nature. B1 is one of the dominant strains in a variety of anthozoan hosts in the Caribbean, whereas C3 is one of the dominant strains in corals in the Indo-Pacific [44,45]. Studies of symbionts that occur in a large number of host species could lend insight into mechanisms that are at play across a broad range of partnerships.

Future research priorities

In summary, we believe that rapid progress in the study of coral cell biology will greatly contribute to an understanding of coral ecology and evolution. Below we outline key priorities for future research.

Genome sequencing of Aiptasia and Symbiodinium clade B1

The genome sequences of the experimental models will give us information about genes that are present in the organisms, allowing us to examine their roles in the symbiosis. This genomic information is central to accelerating the pace of research in cell biology.

Initial host–symbiont interactions

The study of innate immunity and symbiont invasion, as outlined in Table 1, and their role in establishment, regulation and breakdown of the symbiosis, are key to understanding host–symbiont recognition and specificity.

Host–symbiont regulation, integration and nutrient exchange

Insight into the integration of the two partners, including coordination between cell division in the partners and the role of the host cytoskeleton and symbiosome membrane (the host membrane surrounding the symbiont), is fundamental to understanding the relationship. Genomic and metabolomic (broad-spectrum profiles of cellular metabolites) data from both symbiont and host will greatly increase our understanding of nutrient exchange and its role in the maintenance of the symbiosis.

Cellular processes involved in bleaching

The progress made in the cellular study of bleaching has uncovered just a few pieces of a very complex cellular puzzle [24–27]. Continued examination of the proximal cellular pathways involved in bleaching, both in the symbiont and host, is required to gain a full understanding of the phenomenon.

Study of bacterial symbionts of corals

There is growing evidence that the corals include populations of epi- and endosymbiotic bacteria [39,46] and nothing is known about the cellular role that they play in corals. Cellular studies of these host–bacteria and dinoflagellate–bacteria interactions, such as localization of the symbionts, nutritional contribution to the whole association and dynamics during bleaching and disease, are required to better understand the role of these associations in corals.

The field of cnidarian–dinoflagellate symbiosis cell biology is on the verge of a breakthrough. We are beginning to take advantage of knowledge gained from other host–microbe interactions and of tools developed in other model systems. With these, we are poised to develop model systems of our own that we believe will accelerate progress in coral cell biology and greatly enhance our understanding of coral ecology and evolution.

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