Reference: *Biol. Bull.* **223:** 44–65. (August 2012)

Study of Cnidarian-Algal Symbiosis in the "Omics" Age

ELI MEYER*, AND VIRGINIA M. WEIS*

Department of Zoology, Oregon State University, Corvallis, Oregon 97331

Abstract. The symbiotic associations between cnidarians and dinoflagellate algae (*Symbiodinium*) support productive and diverse ecosystems in coral reefs. Many aspects of this association, including the mechanistic basis of host-symbiont recognition and metabolic interaction, remain poorly understood. The first completed genome sequence for a symbiotic anthozoan is now available (the coral *Acropora digitifera*), and extensive expressed sequence tag resources are available for a variety of other symbiotic corals and anemones. These resources make it possible to profile gene expression, protein abundance, and protein localization associated with the symbiotic state. Here we review the history of "omics" studies of cnidarian-algal symbiosis and the current availability of sequence resources for corals and anemones, identifying genes putatively involved in symbiosis across 10 anthozoan species. The public availability of candidate symbiosis-associated genes leaves the field of cnidarian-algal symbiosis poised for in-depth comparative studies of sequence diversity and gene expression and for targeted functional studies of genes associated with symbiosis. Reviewing the progress to date suggests directions for future investigations of cnidarian-algal symbiosis that include (i) sequencing of *Symbiodinium*, (ii) proteomic analysis of the symbiosome membrane complex, (iii) glycomic analysis of *Symbiodinium* cell surfaces, and (iv) expression profiling of the gastrodermal cells hosting *Symbiodinium*.

Introduction

Corals form the trophic and structural foundation of coral reefs, one of the richest and most diverse ecosystems on the planet. Corals are composed of a three-way symbiosis between cnidarian host animals, photosynthetic endosymbiotic dinoflagellates (genus *Symbiodinium*), and a complex and dynamic bacterial flora that reside on and in the host. The symbiosis between corals and dinoflagellates is centered around nutrient exchange, whereby the dinoflagellates provide high amounts of reduced organic carbon from photosynthesis in return for inorganic nutrients such as nitrogen and carbon, a high-light environment, and refuge from herbivory (Muller-Parker and D'Elia, 1997). Despite the importance of corals to coral reefs and the increasing threats that reef ecosystems face due to climate change and other environmental perturbation, the molecular and cellular mechanisms that underlie coral-algal symbioses are just beginning to be understood (Davy *et al.*, 2012). As is the case with the broader field of symbiosis, the genomics era is ushering in a new age in discovery-based, systems-level approaches to the study of coral symbioses that are transforming the field. This review covers the progress to date on "omics" (genomics, transcriptomics, and proteomics) studies of cnidarian-algal symbiosis, provides a comparative compilation of genes of interest from existing cnidarian genomic resources, and discusses future areas of research that emerge from these analyses.

Received 14 February 2012; accepted 30 April 2012.

* To whom correspondence should be addressed. E-mail: meyere@science.oregonstate.edu; weisv@science.oregonstate.edu

Abbreviations: MAMP, microbe-associated molecular pattern; NGS, next-generation sequencing; PRR, pattern recognition receptor; ROS, reactive oxygen species.

Omics Studies to Date on Cnidarian-Algal Symbioses

The study of cnidarian-algal symbiosis is a highly comparative field in which researchers study a diversity of host-symbiont combinations in multiple oceans. In addition, there is a long history of using symbiotic anemones, both temperate and tropical, as model systems for the study of coral symbiosis, due in part to the ease of rearing and manipulating them in the laboratory (Weis *et al*., 2008). Published genomic and proteomic studies (Table 1) span two subclasses within class Anthozoa and include a total of 8 genera and 11 species (Fig. 1). This review focuses on progress on the host component of the partnership. Systems-level studies in the very diverse Symbiodinium lag far

behind progress in the host and will be summarized briefly in the section titled "Omics Research in *Symbiodinium* spp."

Published genomic and proteomic studies of cnidarian symbiosis span 1996 to the present (Table 1). In this time, genomic resources for cnidarians and other early-diverged metazoans have greatly increased in size and quality, and omics technologies have become increasingly sophisticated and affordable. As a result, publication number is increasing yearly, and recent studies are providing richer and more detailed information than earlier ones. Some are expressed sequence tag (EST) studies that tabulate and characterize genes in hosts without any quantitative analysis of expression in the symbiotic state. Others are true functional genomic and proteomic studies that quantify differences between symbiotic and aposymbiotic states in order to identify differential expression as a function of symbiosis. These studies, in the context of existing knowledge of the biology of cnidarian-algal symbiosis, identify a set of candidate genes for future functional investigations.

The first study to quantify differences in expression between symbiotic and aposymbiotic animals was carried out in the temperate anemone *Anthopleura elegantissima* (Weis and Levine, 1996). This study identified numerous protein spots on two-dimensional gels that were differentially upregulated or downregulated as a function of the symbiotic state, and it set the stage for several subsequent proteomic and transcriptomic examinations in *A. elegantissima* and other systems. However, the overall results in subsequent studies were much more nuanced (Barneah *et al.*, 2006; Rodriguez-Lanetty *et al.*, 2006a; deBoer *et al.*, 2007; Voolstra *et al.*, 2009a; Kuo *et al.*, 2010; Schnitzler and Weis, 2010; Yuyama *et al.*, 2011). For example, proteomic studies of a juvenile soft coral *Heteroxenia fuscescens* (Barneah *et al.*, 2006) and larvae of the stony coral *Fungia scutaria* (deBoer *et al.*, 2007) identified almost no differences between symbiotic and aposymbiotic samples. Similarly, microarray studies of three species of coral larvae, *Montastraea faveolata*, *Acropora palmata* (Voolstra *et al.*, 2009a), and *F. scutaria* (Schnitzler and Weis, 2010) found almost no differences in transcriptomic profiles between symbiotic and aposymbiotic larvae. A microarray study comparing symbiotic and aposymbiotic adult *A. elegantissima* found just 2% of the more than 10,000 features to be differentially expressed (Rodriguez-Lanetty *et al.*, 2006a). Furthermore, typical fold-change differences in differentially expressed genes were modest, with most ranging between 1 and 2. Similarly, in a study of the temperate anemone *Anemonia viridis,* of the approximately 14,000 features on a cDNA array, 1% were found to be differentially expressed between the two states (Ganot *et al.*, 2011). The study of *A. viridis*, however, performed additional interesting comparisons by taking advantage of the ability to completely separate aposymbiotic epidermis from symbiotic gastrodermis in these large-tentacled animals. These comparative array studies identified 17 genes that were differentially expressed as a function of the symbiotic state and tissue type and were the subject of further in-depth bioinformatic analyses.

Possible explanations for the absence of a dramatic symbiosis-related protein or gene profile in all of these studies are briefly listed here. First, there is potential for the technical limitation of a very low signal-to-noise ratio present when profiling the proteome and transcriptome of an entire animal, when only a portion of the animal, the gastrodermis, is symbiotic. This problem is potentially amplified in studies of newly colonized animals with very few symbionts and therefore very few symbiotic gastrodermal cells. A colonized *F. scutaria* larva composed of thousands of host cells contains on average only 20 symbionts, and so a differential signal in these few symbiotic cells could be drowned out (Schnitzler and Weis, 2010). Methods to isolate symbiosis-enriched tissue are discussed in the concluding section on areas for research. Another possible explanation for the absence of dramatic symbiosis-specific profiles is the likely involvement of regulatory genes such as transcription factors. These are commonly expressed at low absolute copy numbers (transcripts per cell) (Schwanhausser *et al.,* 2011), or for only a brief period of time (see, for example, Isshiki *et al.*, 2001; Arbeitman *et al.*, 2002). Such subtle differences could be missed in two-dimensional gel proteomic studies, microarrays, and suppressive subtractive hybridization, which favor measuring highly expressed proteins and genes. Recent next-generation sequencing (NGS) techniques such as RNA-Seq could greatly improve the ability to identify these types of transcripts (see ahead to Areas for Future Research).

Another possible factor that could contribute to the observed few differences in expression is high inter-individual variation in gene expression, such as that observed between individuals of *A. viridis* (Ganot *et al.*, 2011). Such high variation has also been described in corals and has been pointed out as an inherent limitation of molecular, genetic, and genomic techniques for the study of uncontrolled, naturally varying populations of animals from the field (van Oppen and Gates, 2006). This variation is only compounded when studies are performed on populations of larvae or juveniles of mixed parentage (Barneah *et al.*, 2006; deBoer *et al.*, 2007; Voolstra *et al.*, 2009a; Schnitzler and Weis, 2010).

An additional compelling biological explanation for the nuanced profile differences in the symbiotic state was first suggested in a microarray study of larvae of *M. faveolata* and *A. palmata* by Voolstra and colleagues (Voolstra *et al.*, 2009a). They found that coral larvae challenged with homologous strains of *Symbiodinium* (strains of algae observed in the host in nature) had very few profile differences from aposymbiotic larvae. However, larvae challenged with heterologous strains (those from other coral species that fail to establish a stable partnership) had dramatically altered

Table 1

Chronological list to date of proteomic and genomic studies examining cnidarian-algal symbiosis

Host organism type	Host organism genus species	Host developmental stage	Technique	Key findings	Genes of interest	Reference
Anemone	*Anthopleura elegantissima*	adults	Proteomics, 2D PAGE	Many differences in profiles between symbiotic and aposymbiotic animals	Carbonic anhydrase Sym32	Weis and Levine, 1996
Anemone	*Aiptasia pulchella*	adults	EST analysis	Many ESTs sequenced, no quantitative analyses performed comparing symbiotic & aposymbiotic states	Ferritin	Kuo *et al.,* 2004
Coral	*Acropora tenuis*	juveniles	Suppression subtraction hybridization	Two unknown genes differentially expressed as a function of symbiotic state	–	Yuyama *et al.,* 2005
Soft coral	*Heteroxenia fuscescens*	juveniles	Proteomics, 2D PAGE	Very few differences in protein profiles between symbiotic and aposymmbiotic animals; differences in profiles with developmental stage	–	Barneah *et al.,* 2006
Anemone	*Anthopleura elegantissima*	adults	Anonymous cDNA microarray	Relatively few differences between symbiotic and aposymbiotic anemones Symbiosis maintained by altering expression of existing genes Differential expressions of transcripts in areas including lipid metabolism, regulation of host cell survival, cell signaling and oxidative stress Proposed model for host-symbiont recognition	SRBI scavenger receptor Sphingosine 1-phosphate phosphatase Lipid metabolism genes	Rodriguez-Lanetty *et al.,* 2006a
Coral	*Fungia scutaria*	larvae	Proteomics, 2D PAGE	Very few differences in protein profiles between symbiotic & aposymbiotic larvae	–	deBoer *et al.,* 2007
Corals	*Montastraea faveolata* *Acropora palmata*	embryos, larvae, adults	EST analysis	A variety of sequences associated with innate immunity identified as possible genes of interest in symbiosis No quantitative analyses performed	Thrombospondin type I repeats LRRs Scavenger receptors Lectins Tachylectins Ferritin	Schwarz *et al.,* 2008
Coral	*Acropora millepora*	embryos, larvae, adults	cDNA microarray	Study focused on developmental stage but some symbiosis-related transcripts described	C-type lectin Carbonic anhydrase	Grasso *et al.,* 2008
Anemone	*Aiptasia pallida*	adults	EST analysis	Genes involved in oxidative stress, apoptosis & autophagy, phagocytosis and immunity identified as possible genes of interest in symbiosis No quantitative analyses performed	Catalase Glutathione pathway components Caspases Complement 3 C-type lectins	Sunagawa *et al.,* 2009
Anemone	*Anemonia viridis*	adults	EST analysis	Identification of genes that have no homolog in non-symbiotic Nematostella vectensis and therefore might be involved in symbiosis No quantitative analyses performed	–	Sabourault *et al.,* 2009

Table 1 *(Continued)*

Host organism type	Host organism genus species	Host developmental stage	Technique	Key findings	Genes of interest	Reference
Corals	*Montastraea faveolata* *Acropora palmata*	larvae	cDNA microarrays	Few changes in larval transcriptome when larvae were colonized with homologous symbiont strain, however significant changes in transcriptome when colonized with heterologous strains Authors suggest homologous symbionts modulate host response, thereby entering host in a stealth manner	–	Voolstra *et al.,* 2009a
Coral	*Fungia scutaria*	larvae	Anonymous cDNA microarray	Very few changes in host transcriptome with onset of symbiosis with homologous symbiont strain Suggests suppression or modulation of host response to colonization by symbionts	–	Schnitzler and Weis, 2010
Anemone	*Aiptasia pulchella*	adults	Suppression subtraction hybridization	Several genes differentially expressed as a function of symbiotic state	Neimann-Pick type C2 Ca2+ binding, EF hand protein	Kuo *et al.,* 2010
Coral	*Montastraea faveolata*	adults	cDNA microarray	Coral transcriptome profile more close correlated to symbiont type than to changes in experimental condition Suggests that host physiology and symbiont type are tightly linked	–	DeSalvo *et al.,* 2010
Anemone	*Aiptasia pulchella*	adults	Proteomics, 2D PAGE, Mass spec	Symbiosome membrane proteins were isolated and purified 17 proteins were identified and assigned to functional categories Evidence that symbiosome functions in cell recognition, cytoskeletal remodeling, ATP synthesis, transport and stress response	ABC transporter ATP synthase HSP 70	Peng *et al.,* 2010
Coral	*Acropora millepora*	adults	cDNA microarray	Diel-rhythmicity in gene expression of clusters of genes involved in light-dependent circadian regulation, antioxidant protection, and metabolism	Cryptochrome Ferritin Catalase Glycolytic enzymes	Levy *et al.,* 2011
Coral	*Acropora tenuis*	juveniles	High coverage gene expression profiling (modified differential display)	Compared expression patterns between aposymbiotic and symbiotic animals colonized with homologous and heterologous symbiont strains Found consistent expression differences in genes involved in lipid and carbohydrate metabolism, signal transduction and membrane transport	–	Yuyama *et al.,* 2011

Table 1 *(Continued)*

Host organism type	Host organism genus species	Host developmental stage	Technique	Key findings	Genes of interest	Reference
Anemone	*Anemonia viridis*	adults	cDNA microarray	Identified 39 genes that are differentially expressed with symbiosis. Many of these had enhanced expression in the symbiotic gastrodermis compared to the non-symbiotic epidermis Identified specific gene duplications with a cnidarian-specific isoform upregulated in the symbiotic state Proposed model of aposymbiotic state upregulating vesicular trafficking and symbiotic state upregulating metabolic exchange between the partners	Neimann-Pick type C2 Calumenin precursor, Ca2+ binding, EF hand protein Sym32 Carbonic anhydrase Collagen	Ganot *et al.,* 2011
Coral	*Euphyllia glabrescens*	adults	Proteomics, 2D PAGE, Mass spec	Lipid bodies from host gastrodermal tissue were isolated, purified and characterized 42 proteins were identified in a variety of functional groups, including metabolism, intracellular trafficking and stress response. Both host and symbiont may be involved in lipid body biogenesis	Lipid metabolism enzymes HSPs	Peng *et al.,* 2011
Coral	*Acropora digitifera*	sperm	NGS (Illumina), genome assembly and annotation	Coral innate immunity repertoire is more complex than non-symbiotic N. vectensis, suggesting that these genes may play a role in symbiosis Host genome contains complete pathway for synthesis of mycosporine-like amino acids, pathway formerly thought to reside in symbiont	TLR pathway NACHT Apoptosis & autophagy pathways MAA pathway	Shinzato *et al.,* 2011

– indicates no data.

transcriptomic profiles compared to aposymbiotic larvae. This result led the authors to suggest that homologous *Symbiodinium* strains enter hosts in a stealth manner and actively circumvent or modulate the host immune response. Indeed, the crucial role of host innate immunity in cnidarian-algal symbiosis is one of the key findings to emerge from both genomic and cell biological studies within the last decade (Miller *et al.*, 2005, 2007; Schwarz *et al.*, 2008; Weis *et al.*, 2008; Dunn, 2009; Weis and Allemand, 2009; Shinzato *et al.*, 2011; Davy *et al.*, 2012;) and is discussed extensively below.

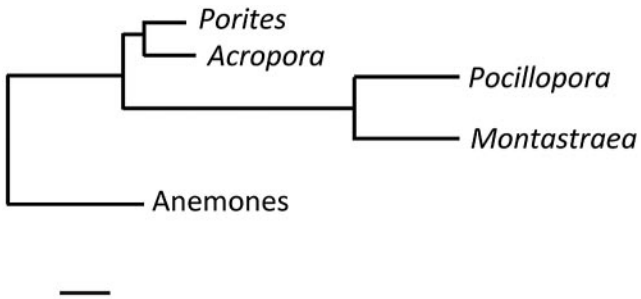

Figure 1. Phylogenetic distances among cnidarian taxa included in sequence comparisons. Redrawn from a previously reported Bayesian tree of 48 cnidarian taxa based on mitochondrial protein-coding genes (Park *et al.*, 2012). Bar represents 0.1 substitutions per site.

A final global pattern of expression that recurs throughout the functional genomic studies is the near absence of true "symbiosis genes": that is, genes that are expressed in an ON/OFF manner as a function of symbiosis (Rodriguez-Lanetty *et al.*, 2006a). This suggests that symbiosis is regulated in the host by existing repertoires of genes that are modulated by the symbiotic state rather than by genes unique to symbiosis. Furthermore, it is now clear that beneficial and negative host-microbe interactions share many

common regulatory mechanisms (Gross *et al.*, 2009; McFall-Ngai *et al.*, 2010; Royet *et al.*, 2011), and these pathways, so well studied in animal-pathogen and animal-parasite interactions, provide a clear way forward for future studies in coral symbiosis (Weis *et al.*, 2008).

Search for Symbiosis-Associated Sequences From Cnidarian Genomic Resources

Many of the proteomic and genomic studies summarized in Table 1 identify and discuss specific genes and pathways that are differentially expressed in symbiosis and their potential roles in symbiosis. From these studies and from other physiological, metabolic, and cellular studies of coral symbiosis, we compiled a list of target genes that form a core of symbiosis-related regulatory mechanisms and pathways. These symbiosis-associated genes are the focus of a search of selected cnidarian sequence databases in this study. The sequence review provides a comparative view of the genetic toolkit available in cnidarian cells to regulate and control symbiosis.

Currently available sequence resources for cnidarians

Until very recently, the search for mechanisms underlying cnidarian-algal symbioses has been constrained by the general lack of data on relevant processes (*e.g.,* immunity) in these non-model organisms. Sequencing the full complement of genes expressed by an organism provides one perspective of its functional capabilities (*e.g.,* biosynthesis or transport). Furthermore, the availability of sequence resources for diverse species of symbiotic anthozoan species opens new comparative possibilities for the study of cnidarian-algal symbiosis. Already, comparative sequence analysis of species pairs has uncovered signatures of selection in acroporid corals (Iguchi *et al.*, 2011; Voolstra *et al.*, 2011). In this context, we reviewed the currently available sequence resources for anthozoans to identify genes that might play a role in symbiosis. These resources should enable functional studies of the mechanistic basis for symbiont recognition, sorting, and maintenance, and for comparative studies of diversity in symbiont specificity.

Although the development of sequence resources for cnidarians has naturally lagged behind that for model systems, considerable sequence resources are now available. The first cnidarian genome completed was a nonsymbiotic anemone, *Nematostella vectensis* (Putnam *et al.*, 2007), followed by a nonsymbiotic hydrozoan, *Hydra magnipapillata* (Chapman *et al.*, 2010). Only recently has a symbiotic anthozoan genome become available, from the scleractinian *Acropora digitifera* (Shinzato *et al.*, 2011). A second, *Acropora millepora*, is expected in the immediate future (Miller *et al.*, 2011).

The majority of sequencing efforts in cnidarian-algal symbiosis over the past decade have focused on the transcriptome. The coral *A. millepora* was first to be sequenced extensively using conventional Sanger technology (Kortschak *et al.*, 2003; Technau *et al.*, 2005). Similar EST resources were developed for Caribbean corals *Montastraea faveolata* and *Acropora palmata* (Schwarz *et al.*, 2008). More recently, the widespread adoption of NGS technologies (primarily 454 pyrosequencing) has fueled rapid resource development for a variety of coral species. Annotated transcriptome assemblies based on NGS technologies are now available for *A. millepora* (Meyer *et al.*, 2009), *Pocillopora damicornis* (Traylor-Knowles *et al.*, 2011), and *A. palmata* (Polato *et al.,* 2011). Unpublished transcriptome assemblies of comparable scale are available for *Acropora hyacinthus* and *Porites astreoides* (Matz Lab, 2011).

Development of sequence resources for symbiotic anemones has followed a similar progression. Assemblies of Sanger ESTs are available for *Aiptasia pulchella* (Kuo *et al.*, 2004), *A. pallida* (Sunagawa *et al.*, 2009), and *Anemonia viridis* (Sabourault *et al.*, 2009). An NGS transcriptomic study of *A. pallida* has recently been completed (Lehnert *et al.,* 2012).

Approach to identifying target symbiosis-associated genes from cnidarian genomic resources

To identify symbiosis-related genes from the variety of genomic resources, first, we obtained gene models from completed genome assemblies (*N. vectensis* and *A. digitifera*), presumably representing a full gene complement (Table 2). Next, we included a selection of transcriptome assemblies that, although incomplete, greatly expand the range of symbiotic anthozoan species represented for comparative analysis. The list includes the anemones *A. viridis* and *A. pallida* and the corals *A. millepora, A. hyacinthus, P. astreoides, P. damicornis, M. faveolata,* and *A. palmata* (Table 2).

To identify candidate symbiosis-associated genes, we reviewed the cnidarian sequence resources for genes and processes implicated by previous studies of cnidarian-algal symbiosis, many of which are summarized in Table 1. Putative homologs for each gene were identified using a reciprocal search strategy with NCBI BLAST. First, representative proteins were compared against each set of transcripts using TBLASTN (bit-score $\geq$ 50). All transcripts were reciprocally compared against UniProt_2010_09 and annotated with gene names of the best match bearing functional annotation (BLASTX, bit-score $\geq$ 50). Transcripts matched by each protein sequence that also matched that same gene (regardless of species) in the reciprocal search were counted as putative homologs. Selected sequences were further characterized to identify conserved domains, signal peptides, and transmembrane helices in the deduced protein sequence using InterproScan ver. 4.8 (Zdobnov and Apweiler, 2001).

Table 2

Cnidarian sequence resources employed in this review

	Species	Resource type[1]	Data source (version)[2]	Sequences (*n*)	Unique BLAST matches
Anemones	*Nematostella vectensis*	G	JGI (v1.0)	27,273	13,851
	Aiptasia pallida	T	Lehnert *et al.,* pers. comm.	60,310	15,272
	Anemonia viridis	T	NCBI	39,996	6,373
Corals	*Acropora digitifera*	G	Marine Genomics Unit, 2011 (v1.0)	23,677	11,208
	Acropora hyacinthus	T	Matz Lab, 2011 (v1)	140,645	11,939
	Acropora millepora	T	Matz Lab, 2011 (v3)	181,031	14,771
	Acropora palmata	T	Polato N.R., 2011	79,859	20,101
	Montastreae faveolata	T	SymBioSys, 2011	11,611	3,882
	Porites astreoides	T	Matz Lab, 2011 (v1)	92,142	11,944
	Pocillopora damicornis	T	PdamBase, 2011	70,786	12,518

[1] G, genome; T, transcriptome.

[2] BLASTX comparison against UniProt (e-value $\leq 10^{-4}$). Public databases: JGI, The Department of Energy Joint Genome Institute; NCBI, the National Center for Biotechnology Information.

Cnidarian Genes Associated With Symbiosis

Symbiosis-associated sequences falling into several broad functional categories are listed in Table 3 and discussed below. Details for all sequences are shown in Supplementary Table 1 (http://www.biolbull.org/content/supplemental), along with sequence data for the publicly available records. To help place these diverse sequences in the context of cnidarian-algal symbiosis, we provide a cellular model of host-symbiont interactions in Figure 2 for reference throughout this section of the review.

Pattern recognition receptors (PRRs)

All animals have innate immune systems that mediate interactions with microbes encountered in the environment (Janeway and Medzhitov, 2002; Gross *et al.*, 2009). Responses include attack and elimination of pathogenic microbes, but also tolerance of beneficial ones. One of the principal means of identifying microbes is by detecting microbe-associated molecular patterns (MAMPs), signature molecules that occur on microbe surfaces, such as glycans and lipopolysaccharide. Recognition is achieved by a broad variety of pattern recognition receptors (PRRs) that bind MAMPs and signal the presence of the microbe by initiating a signaling cascade that can either attack and destroy a pathogenic invader or tolerate and even nurture a symbiont (MAMP-PRR interactions depicted in Fig. 2a). These innate immune processes in cnidarian-algal symbioses have recently been extensively reviewed by Davy and coworkers (Davy *et al.*, 2012).

Lectins are a ubiquitous and diverse group of PRRs with important roles in pattern recognition processes that bind glycans, especially oligosaccharide moieties on glycoproteins and glycolipids. C-type lectins (CTL) comprise a large gene family with roles in cell adhesion, pathogen recognition, and phagocytosis (Lasky, 1992; Weis *et al.*, 1998; Cambi *et al.*, 2005). CTL genes have been implicated in the onset of cnidarian-algal symbiosis (Wood-Charlson *et al.*, 2006) and have been identified in EST studies as candidate symbiosis-associated genes (Table 1) (Grasso *et al.*, 2008; Schwarz *et al.*, 2008; Sunagawa *et al.*, 2009). The conserved CTL domain (CTLD) is found in 67 gene models from the *N. vectensis* genome (Wood-Charlson and Weis, 2009), highlighting the diversity of this gene family in cnidarians. We performed a more restricted analysis, based on a set of 22 *Caenorhabditis elegans* CTLs from the animal lectin database (AnimalLectindb, 2012). Our search identified nine putative CTLs in both the coral (*A. digitifera*) and anemone (*N. vectensis*) genomes, and a smaller number of homologs in each of the coral and anemone transcriptome assemblies (Table 3).

Mannose residues are ubiquitous features of *Symbiodinium* cell surfaces (Logan *et al.*, 2010). Cnidarian sequence resources include numerous homologs of a membrane-bound mannose receptor (MRC1) that represent candidates for binding these cell-surface glycans during the initial contact between host and symbiont (Table 3). Other secreted mannose-binding lectins bind mannose in mammalian serum and form complexes with proteases called mannose-binding lectin-associated serine proteases (MASP), which activate complement components in response to ligand binding (Matsushita *et al.*, 2000). A putative homolog of MASP has been previously identified in the *N. vectensis* genome (Kimura *et al.*, 2009). Our review of current sequence resources identified 1-3 MASP genes in most corals and anemones (Table 3). At least one homolog of the downstream target for this protease, the serum protein complement component 3 (C3), was also found in all coral and anemone resources surveyed here (Table 3). The complement pathway participates in the innate immune response when C3 binds (opsonizes) MAMPs including glycans, thereby labeling them for recognition by the host. C3 is

Table 3

Overview of candidate genes in cnidarian sequence resources

	Corals							Anemones		
Gene name	*A. digitifera*	*A. hyacinthus*	*A. millepora*	*A. palmata*	*M. faveolata*	*P. astreoides*	*P. damicornis*	*N. vectensis*	*A. pallida*	*A. viridis*
PATTERN RECOGNITION										
Lectins: C-type	9	0	1	3	1	3	3	9	7	3
Mannose receptor MR1	8	2	4	3	0	4	4	4	7	2
Mannan-binding serine peptidase 2 (Masp2)	2	2	1	3	1	2	1	0	1	0
Complement component C3	3	2	5	1	1	5	2	2	2	3
Toll/TLR	8	5	8	4	0	3	6	6	3	2
TNFR-associated (Traf6)	11	4	5	13	2	6	11	8	10	7
Scavenger receptor class B	3	3	6	5	0	4	2	2	1	0
Thrombospondin 1	11	11	12	9	1	11	11	20	11	4
NOD-like receptors: NACHT	10	9	6	9	1	7	18	5	3	10
NB-ARC	2	0	1	2	0	2	1	1	1	1
CELL ADHESION										
Fasciclin I (Sym32/periostin)	2	1	2	2	1	2	1	3	1	1
γ-Glutamylcarboxylase	1	2	2	1	2	1	1	2	3	1
Vitamin K epoxide reductase	1	1	1	1	1	1	0	2	0	1
Calumenin	2	4	4	14	5	4	4	7	7	6
VESICULAR TRAFFICKING										
Rab GTPases	26	36	38	55	15	32	16	41	22	17
NADPH oxidase	3	4	4	5	0	2	3	5	10	1
Vacuolar H+ATPase	9	16	15	32	11	16	31	15	15	4
Autophagy-specific (Atg8)	4	4	3	5	3	3	3	4	4	5
REGULATION OF INCOMING LIGHT										
Dehydroquinate synthase	1	0	0	3	0	1	2	2	1	1
O-Methyltransferase	1	3	2	3	1	1	2	2	2	1
ATP-grasp	1	0	0	0	0	0	0	1	1	0
Nonribosomal peptide synthetase	1	2	3	5	0	1	0	3	3	2
Fluorescent proteins	8	2	3	5	1	3	2	2	0	3
APOPTOSIS										
BCL2-associated X (Bax)	7	3	5	8	6	4	6	7	5	4
Caspase 8	9	3	5	11	1	5	11	6	12	4
Sphingosine kinase 1	2	2	1	4	2	2	2	4	6	0
Sphingosine-1-phosphate Phosphatase 1	1	2	5	2	0	3	1	4	3	3
Nitric oxide synthase	2	0	2	2	0	1	1	1	2	0
TGFβ	7	0	1	6	0	2	5	6	6	3
NUTRIENT AND METABOLITE TRANSPORT										
P-type H+ATPase	8	14	13	19	1	14	11	16	20	6
Carbonic anhydrase	8	7	8	14	1	7	11	7	6	5
Aquaporin	2	2	3	4	1	1	8	8	9	0
ABC transporters	34	33	35	30	6	39	32	69	46	33
Glutamate dehydrogenase	3	6	3	12	1	5	12	4	4	9
Glutamine synthetase	1	2	5	4	1	5	3	2	2	2
LIPID STORAGE AND TRANSPORT										
Perilipin (PAT)	0	0	0	0	0	0	0	1	1	1
NPC1	1	2	2	6	4	3	10	3	9	4
NPC2	5	5	3	7	5	2	3	3	6	7
REPONSE TO ROS										
Superoxide dismutase	2	2	2	5	2	1	4	4	3	1
Catalase	1	5	4	3	1	3	3	2	4	2
Peroxiredoxin	3	3	3	7	5	1	9	3	4	5
Glutathione peroxidase	4	3	5	7	4	5	3	9	6	6
Glutathione reductase	1	2	1	6	1	5	1	1	1	2
γ-Glutamylcysteine synthetase	1	3	3	1	1	1	1	1	1	1
Glutathione synthase	2	5	4	2	0	3	3	1	2	0
Glutathione S-transferase	1	4	5	7	5	3	8	8	3	6
Ferritin	2	1	1	5	5	1	8	5	2	3

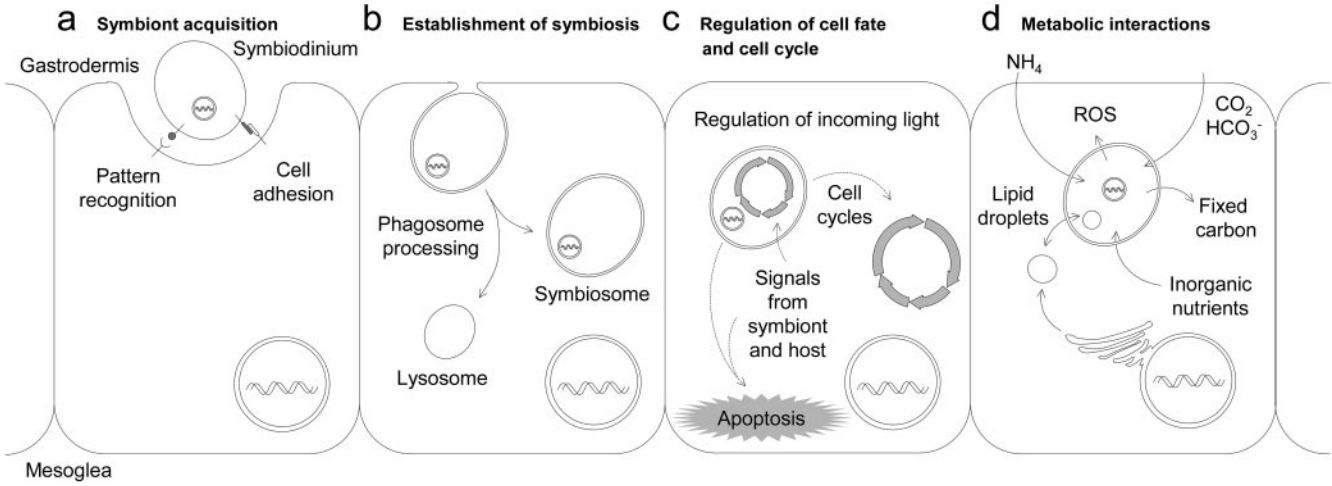

Figure 2. Overview of cellular events and metabolic processes involved in the cnidarian-algal symbiosis. Multiple gastrodermal cells of the cnidarian host are shown for visual clarity, but the processes shown can occur simultaneously within the same cells.

upregulated in *A. millepora* exposed to lipopolysaccharide and peptidoglycan, although its role in symbiosis remains to be determined (Kvennefors *et al.*, 2010).

Toll-like receptors (TLR) are a large family of transmembrane PRRs (10 paralogs in humans) that have been extensively studied (Medzhitov, 2001). TLRs bind a broad range of MAMPs *via* multiple extracellular leucine-rich repeats, activating signaling cascades that result in the production of host defense genes including cytokines and antimicrobial peptides. Previous analyses have identified a diverse repertoire of TLRs in cnidarians, revealing a more sophisticated innate immune repertoire in cnidarians than originally expected on the basis of analysis of model invertebrate species such as worms and flies (Miller *et al.*, 2007; Dunn, 2009; Shinzato *et al.*, 2011). Our review of current sequence resources identified numerous TLRs, including 8 and 6 in the coral and anemone genomes respectively (Table 3). A crucial element in the TLR signaling pathway is TRAF6, a cytoplasmic protein that transduces signals from the membrane-associated TLR to intracellular signaling cascades (NF-κB, and MAP kinase) (Wu and Arron, 2003). Numerous homologs of TRAF6 can be found in cnidarian genes, including 11 in the coral and 8 in the anemone genome (Table 3) (Schnitzler, 2010).

Other PRRs in the cnidarian innate immune repertoire have been identified in genomic studies as putative participants in symbiont recognition and regulation (Table 3). Scavenger receptors are diverse multi-domain cell surface glycoproteins, common in phagocytic cells, that are sometimes referred to as molecular fly paper because of their ability to bind a broad array of MAMPs (McGuinness *et al.*, 2003). A homolog to one of these receptors, scavenger receptor B/CD36, is upregulated in symbiotic *A. elegantissima* (Rodriguez-Lanetty *et al.*, 2006a), suggesting a role in the establishment or maintenance of the symbiosis. Thrombospondins are secreted proteins that include signature TSP-1 domains and are known to mediate target recognition by CD36 (Majai *et al.*, 2006). Intracellular protozoan parasites have developed a wide variety of cellular techniques for avoiding innate immune detection by hosts. For example some gain entry into vertebrate host cells in part by promoting immunosuppressive pathways, modulated by increased expression of thrombospondin (Waghabi *et al.*, 2005; Simmons *et al.*, 2006). Work in progress is finding a role for TSP-1-containing homologs in onset of symbiosis in *A. pallida* (E. Neubauer and V. Weis, unpubl. data). Multiple TSP-1 domain-containing genes have been previously described in coral ESTs (Table 1) (Schwarz *et al.*, 2008), and our review of coral and anemone sequence resources identified TSP-1 domain-containing homologs in all species, with 11 in the coral genome, 20 in the anemone genome, and comparable numbers in anemone and coral transcriptomes (Table 3). Finally, nucleotide oligomerization domain (NOD)-like receptors (NLRs) are intracellular proteins once thought to be unique to vertebrates (Ting and Davis, 2005) that function in detection of inflammatory responses to pathogens (Franchi *et al.*, 2009). Putative homologs bearing the characteristic NACHT or NB-ARC domains have recently been described in nonsymbiotic *Hydra magnipapillata* (Lange *et al.*, 2011), and at least one is upregulated in response to MAMPs (flagellin, lipopolysaccharide) from prokaryotic pathogens. Putative NLRs were found in nearly all cnidarian sequence resources reviewed here (Table 3), with NACHT domains appearing more frequently than NB-ARC domains. These genes are especially promising candidates because of their known roles in regulation of apoptosis in other systems (Franchi *et al.*, 2009), suggesting a link between pathogen detection and the

apoptotic pathways involved with recognition and breakdown of cnidarian-algal symbiosis (Dunn and Weis, 2009).

Cell adhesion genes

Fasciclin I is a homophilic cell adhesion protein originally identified in association with neural development in insects (Elkins *et al.*, 1990). The secreted protein contains four repeating FasI domains. Proteins bearing this domain have subsequently been identified in a wide range of taxa including vertebrates (Coutu *et al.*, 2008) and algae (Huber and Sumper, 1994), suggesting that FasI represents an evolutionarily ancient cell adhesion domain. A proteomic study comparing symbiotic and aposymbiotic *A. elegantissima* was the first to describe what would later be identified as a 32-kDa fasciclin I homolog, named Sym32, as highly differentially expressed in the symbiotic state (Table 1) (Weis and Levine, 1996). Subsequent gene expression studies of Sym32 in the anemones *A. elegantissima* and *A. viridis* confirmed this result (Reynolds *et al.*, 2000; Ganot *et al.*, 2011). Another study localized Sym32 to the symbiosome surrounding resident *Symbiodinium* in the gastrodermis of symbiotic individuals (Schwarz and Weis, 2003), suggesting a functional role in the association (depicted in Fig. 2a).

FasI-containing genes in *A. viridis and A. elegantissima* bear two FasI domains, in contrast to the four found in the vertebrate and insect homologs periostin, βig-h3 and fasciclin I (Ganot *et al.*, 2011). Analysis of conserved domains in other cnidarian sequence resources reveals substantial variation in domain numbers, ranging from 1 to 4 (Fig. 3). Importantly, the fragmented nature of transcriptome assemblies dictates that these represent minimum domain numbers, since additional domains may be missing from the assembled transcripts. Nevertheless, the coral and anemone genomes, which are presumably not subject to this caveat, each contain a two-domain gene and at least one other gene with four domains (Fig. 3). The functional consequences of these alternative gene structures remain an open question and are under active investigation by Ganot and coworkers (P. Ganot, Centre Scientifique de Monaco, pers. comm.).

The vertebrate FasI-containing gene periostin is subject to vitamin K-dependent carboxylation in bone marrow cells, where it is likely to play a role in bone development and repair (Coutu *et al.*, 2008). A similar carboxylation process has been proposed in cnidarians (Fig. 4), where it functions in regulating localization of Sym32 between the plasma membrane (carboxylated form) and the symbiosome (native form) (Ganot *et al.*, 2011). The genes required for this signaling pathway (Coutu *et al.*, 2008) appear to be well conserved across anthozoans. At least one transcript bearing one or more FasI domains was found in all species surveyed (Table 3). Similarly, 13 homologs of γ-glutamyl carboxylase (GGC) and vitamin K_1 2,3-epoxide reductase (VKOR) are present in each cnidarian species reviewed here. Calumenin inhibits VKOR and GGC (Wallin *et al.*, 2001), and multiple homologs for this gene were detected in all cnidarian sequence resources (Table 3), consistent with the hypothesis that calumenin has undergone cnidarian-specific duplication events (Ganot *et al.*, 2011).

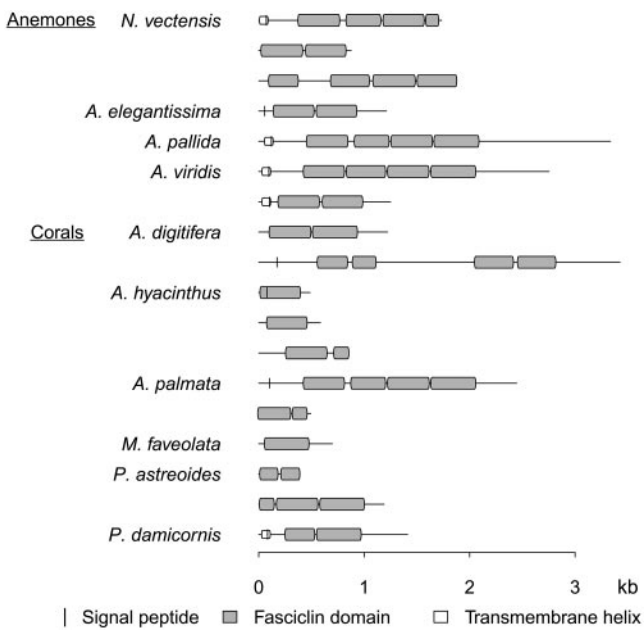

Figure 3. Comparison of cnidarian proteins containing fasciclin-1 (Fas1) domains. Sequences were identified by reciprocal BLAST, and conserved domains, transmembrane helices, and signal peptides were identified using InterProScan. Scale indicates transcript length, and sequences from transcriptome assemblies represent partial transcripts. Sequences used for this figure are listed in Supplementary Table 1 (http://www.biolbull.org/content/supplemental).

Processing of phagosomes and formation of symbiosome

In most host species, *Symbiodinium* cells are initially acquired by phagocytosis. However, instead of being de-

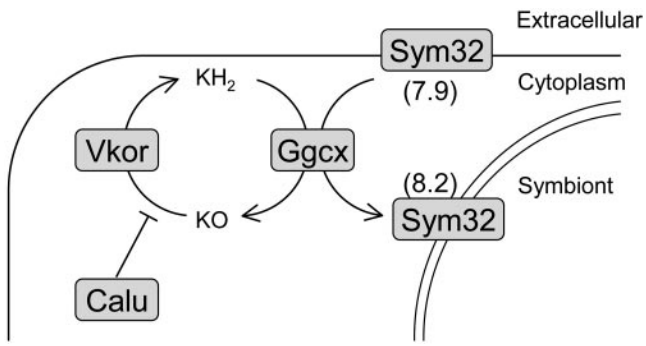

Figure 4. A model describing regulation of the cell adhesion protein Sym32 *via* carboxylation by γ-glutamyl carboxylase (Ggc). 2,3-epoxide reductase (Vkor) reduces vitamin K 2,3-epoxide (KO) to regenerate the vitamin K hydroquinone (KH_2) required for continued function of Ggc, and this process is inhibted by calumenin (Calu). Model redrawn from Ganot *et al.* (2011).

stroyed by phagosomal maturation whereby lysosomes dock onto phagosomes and destroy their contents, symbionts somehow arrest maturation and persist in their vesicular compartments, termed symbiosomes (Davy *et al.*, 2012) (Fig. 2b). Arrest of phagosomal maturation is a common strategy among intracellular parasites to obtain a stable host habitat (Sacks and Sher, 2002; Davy *et al.*, 2012). In mammalian phagocytes, phagosomal maturation involves sequential acidification and acquisition of specific proteins, including Rab GTPases, at precise times onto the phagosomal membrane (Kinchen and Ravichandran, 2008). Several Rab homologs have been characterized in the anemone *A. pulchella* and found to localize in patterns specific to phagosomal maturation arrest, suggesting that *Symbiodinium* somehow modulates phagosomal maturation to persist in the symbiosome (Chen *et al.*, 2004; Chen *et al.*, 2005; Hong *et al.*, 2009a, 2009b). Our search revealed many putative Rab homologs in cnidarian sequence resources, including 26 models in the coral genome and 41 in the anemone genome (Table 3).

NADPH oxidase is a multicomponent enzyme that assembles in phagosomes, generating reactive oxygen species (ROS) in the phagosomal lumen (Segal, 2005). V-type ATPases are recruited to the phagosome late in the process and begin to acidify the lumen by pumping in protons (Kinchen and Ravichandran, 2008). Multiple putative homologs of both of these late phagosomal processing genes were found in both coral and anemone genomes, and were expressed in nearly all transcriptomes reviewed here (Table 3).

The highly conserved process of autophagy is another membrane trafficking phenomenon that serves to degrade organelles and microbial invaders and is a key homeostatic mechanism in eukaryotic cells (Levine, 2005). There is some evidence that autophagy plays a role in removal of symbionts during bleaching (Dunn *et al.*, 2007b; Downs *et al.*, 2009) and therefore could function in host-symbiont recognition. The autophagy-related protein (Atg) employed by eukaryotic cells has been described in detail (Xie and Klionsky, 2007). Atg8 is a ubiquitin-like protein required for autophagosome formation that mediates the fusion of liposomes to form the autophagosome membrane (Nakatogawa *et al.*, 2007) and is a diagnostic marker for autophagy. All cnidarian sequence resources reviewed here include several (range: 3–5) putative Atg8 homologs (Table 3).

Host regulation of incoming light

The light field encountered by *Symbiodinium in hospite* is inevitably influenced by activities of the animal host. Several compounds present in cnidarian cells modulate the incoming light field, which in turn influences symbiont cell survival and division (Fig. 2c). These compounds include mycosporine-like amino acids (MAAs) and fluorescent proteins.

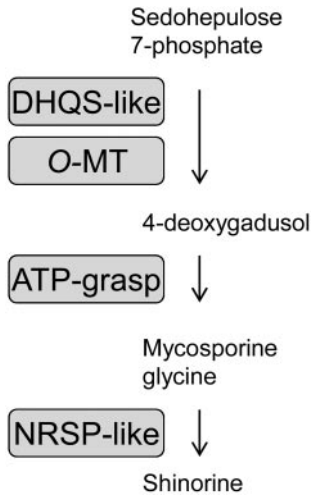

Figure 5. Biosynthetic pathway for the photoprotective mycosporine-like amino acid (MAA) shinorine. All four genes found in both coral and anemone genomes. DHQS-like: dehydroquinate synthase-like; *O*-MT: *O*-methyltransferase; ATP-grasp: adenosine triphosphate (ATP)-grasp superfamily; NRPS-like: nonribosomal peptide synthetase-like.

MAAs are UV-absorbing compounds produced by phylogenetically diverse organisms as an adaptive response to prevent UV damage (Carreto and Carignan, 2011). A variety of MAAs are present in coral holobionts, and their production has long been attributed to the algal partner (Banaszak *et al.*, 2000; Carreto and Carignan, 2011). Sequencing of the anemone genome unexpectedly revealed the complete shikimic acid pathway, an essential precursor for *de novo* MAA biosynthesis (Starcevic *et al.*, 2010). Similarly, the recently sequenced coral genome includes all four genes required for biosynthesis of shinorine (Fig. 5) (Shinzato *et al.*, 2011), a dominant component of coral holobiont MAA pools. Our review of other cnidarian resources confirms that three of these genes are present in most species, while the fourth, ATP-grasp, was found only in the coral *A. digitifera* and the anemones *A. pallida* and *N. vectensis* (Table 3). Although the absence of a gene from transcriptome data might only reflect low expression levels, it would be interesting to explore whether expression-level variation among species correlates with MAA abundance in the holobiont, with environmental factors such as light intensity, or both.

A diverse collection of fluorescent and non-fluorescent proteins homologous to green fluorescent protein (GFP or more generally FP) expressed by cnidarians absorb light at visible and UV wavelengths, and many emit light at a variety of higher wavelengths (Alieva *et al.*, 2008). Multiple homologs of FPs were identified in all cnidarian species we reviewed except for the anemone *A. pallida* (Table 3). Although the sequence data alone are insufficient to allow

the fluorescence properties of these proteins to be inferred, it is clear that multiple FP homologs are present and expressed in symbiotic anthozoans. The functional consequences of variation in FP sequences and expression remain poorly understood, beyond their obvious and dramatic effects on color phenotypes. FPs are primarily expressed in ectodermal tissues of symbiotic corals (Wang *et al.,* 2008; Peng *et al.,* 2008) and anemones (*e.g.,* Leutenegger *et al.,* 2007), and so intercept incident light before it reaches gastrodermal tissues hosting symbionts. This has been widely discussed in the context of shading or photo-protection (Salih *et al.*, 2000; Dove *et al.*, 2001; Roth *et al.*, 2010).

Experiments with *Symbiodinium* freshly isolated from coral tissue (*Euphyllia glabrescens*) demonstrated that the algal cell cycle is stimulated by blue light and is repressed in the presence of GFP (Wang *et al.,* 2008). These findings demonstrate an additional level of complexity (wavelength-specific functions) in modification of the light field beyond simple shading functions. FPs are upregulated in response to elevated light levels in multiple coral species, primarily in response to blue wavelengths (D'Angelo *et al.*, 2008; Roth *et al.*, 2010), which may represent a mechanism for host control of symbiont cell cycles. A diverse array of FPs with varying fluorescence spectra are expressed within corals and among coral species (Alieva *et al.,* 2008). The effects of light-field regulation in general and FP exposure in particular suggest possible functional consequences for this diversity that would be interesting to test.

Regulation of cell fate

One of the mechanisms that confers specificity on the association between cnidarian host and *Symbiodinium* cells is the apoptosis of host cells after uptake of undesirable algal types (Dunn and Weis, 2009). During the breakdown of cnidarian-algal symbiosis resulting from thermal or other environmental stressors, host cells sufficiently damaged by this disruption often undergo apoptosis (Dunn *et al.*, 2002, 2004; Weis, 2008). Reviewing cnidarian sequence resources reveals putative homologs for a number of signaling genes associated with apoptosis (Table 3). Inclusion of a complete set of apoptosis-related genes is outside the scope of this review; instead, we include a sampling of genes here.

Bcl-2 proteins comprise a diverse gene family associated with cell fate and apoptosis in metazoans (Levine *et al.*, 2008) that includes both pro- and anti-apoptotic components. Bcl-2-associated X (Bax) undergoes a temperature-dependent conformational change that results in its insertion in the mitochondrial membranes and initiation of apoptosis (Pagliari *et al.*, 2005). Several putative homologs of this gene (range: 3–8) were found in all cnidarian resources reviewed here (Table 3).

Caspases are a large family of cysteine proteases with central and well-characterized roles in apoptosis (Inoue *et al.*, 2009). As an example, we identified putative caspase 8 homologs (range: 1–12) in all cnidarian resources examined here (Table 3). Caspase 8 transduces signals from tumor necrosis factor (TNF) superfamily proteins called death receptors to initiate apoptosis by an extrinsic pathway (Oberst and Green, 2011).

Another important signaling pathway by which cells regulate apoptosis is the sphingosine rheostat, in which the pro-apoptotic lipid sphingosine (Sph) is converted into the anti-apoptotic sphingosine 1-phosphate (S1P) by sphingosine kinase (SK), with the reverse reaction catalyzed by sphingosine 1-phosphate phosphatase (SPPase) (Spiegel and Milstien, 2011). These genes have been previously identified in expression profiling of symbiotic and aposymbiotic *A. elegantissima* (Table 1) (Rodriguez-Lanetty *et al.*, 2006a). More recently, the anti-apoptotic effects of S1P and the pro-apoptotic effects of Sph have been experimentally demonstrated in *A. pallida* (Detournay and Weis, 2011). Homologs of both enzymes were found in nearly all cnidarian resources examined (Table 3), confirming the presence of this pathway across cnidarian taxa.

Nitric oxide (NO) is a small, highly labile molecule, produced by animal cells *via* nitric oxide synthase (NOS), that promotes apoptosis in some cellular contexts and inhibits apoptosis in others (Chung *et al.*, 2001). In symbiotic anemones exposed to elevated temperatures, production of NO is followed by collapse of the symbiosis (Perez and Weis, 2006). Gene models matching NOS can be found in both the coral and anemone genomes, and most of the transcriptome resources examined here also included 1–2 putative NOS homologs (Table 3).

The transforming growth factor β (TGFβ) gene family is a group of soluble signaling proteins with important roles in cell division, differentiation, apoptosis, and immune tolerance (Heldin *et al.*, 2009). These factors have received little attention in studies of cnidarian-algal symbiosis despite their association with apoptosis and immunity in model systems. Work in progress is showing evidence that the TGFβ pathway is upregulated by the onset of symbioses, thereby promoting a tolerogenic response in host cells harboring symbionts (O. Detournay and V. Weis, unpubl. data). Our review of cnidarian transcriptomes identified multiple putative TGFβ homologs (Table 3), confirming the expression of multiple TGFβ genes in anthozoans as previously reported (Technau *et al.*, 2005).

Metabolic interactions between host and symbiont

The cnidarian-algal symbiosis requires substantial transport of nutrients and metabolites between host, symbiont, and the external environment (Fig. 2d). These metabolic interactions have been extensively reviewed elsewhere (Yellowlees *et al.*, 2008; Gordon and Leggat, 2010b; Davy *et al.*, 2012). Photosynthetically fixed carbon is translocated

from symbiont to host, inorganic nutrients from host to symbiont, and inorganic nitrogen and carbon from the external environment to the symbiont. Here, we focus on a selection of genes thought to take part in these processes with putative homologs in cnidarian sequence resources.

Inorganic carbon (HCO_3^-, CO_2) must be transported from the external environment to support photoautotrophy at a net gain to the host. In anemones, this is accomplished through the combined activity of carbonic anhydrase (CA) and H^+-ATPase genes (Furla *et al.*, 2000). H^+ ions exported into the external environment produce carbonic acid, which is dehydrated by membrane-bound CA, producing CO_2, which diffuses across the plasma membrane, where cytosolic CAs convert it to HCO_3^-. The proton exporter genes underlying this activity have not been identified, although pharmacological experiments suggest a P-type H^+-ATPase (Furla *et al.*, 2000). The activity of this transport system more closely resembles the type IIIA H^+-ATPases expressed in plants and fungi than the H^+/K^+-ATPases expressed in animals (Axelsen and Palmgren, 1998). Cnidarian sequence resources include numerous possible homologs for both ATPase types, but the latter are reported here (Table 3). CA has long been known as an enzyme upregulated in the symbiotic state (Weis *et al.*, 1989), and in several omics studies it is among the most highly differentially expressed proteins or transcripts in the symbiotic state (Table 1) (Weis and Levine, 1996; Grasso *et al.*, 2008; Ganot *et al.*, 2011). Multiple homologs of CA are present in the sequence resources reviewed here (Table 3).

Although CO_2 freely diffuses across plasma membranes, channels produced by aquaporin have been shown to substantially increase CO_2 flux both in heterologous expression (Uehlein *et al.*, 2003) and in human erythrocytes (Endeward *et al.*, 2006). This suggests an alternative CO_2 uptake strategy and possibly a pathway for photosynthate translocation back to the host. Although to our knowledge these genes have not been studied in this context, multiple aquaporin homologs are present in both the coral and anemone genomes and expressed in nearly all transcriptome assemblies reviewed here (Table 3).

ATP-binding cassette (ABC) transporter genes have not been widely studied in cnidarians, but a recent proteomic analysis (Peng *et al.*, 2010) identified at least one ABC transporter as a component of the symbiosome membrane (Table 1). This large gene family acts on a wide range of substrates and serves an important role in uptake of nutrients from the external medium, transport between cellular components, and export of toxins (Higgins, 1992). Although it is tempting to speculate that ABC are involved in nutrient transport in either direction across the symbiosome membrane, these genes remain uncharacterized in cnidarian-algal symbiosis. Putative ABC transporter homologs are abundant in both coral and anemone genomes (34 and 69 gene models respectively) and in all transcriptomes reviewed here (Table 3).

Ammonium metabolism is another area of metabolic interaction between host and symbiont. The coral holobiont accomplishes net uptake of ammonium from seawater (Miller and Yellowlees, 1989), with concentrations as low as 0.6 μmol l^{-1} providing sufficient nitrogen to support metabolic demands of the symbionts (Grover *et al.*, 2002). Both the cnidarian and algal partners assimilate ammonium, so that the supply of ammonium to the symbiont is potentially limited by host assimilation. Glutamine synthase (GS) and glutamate dehydrogenase (GDH), enzymes associated with ammonium assimilation, have been previously described in cnidarians (Yellowlees *et al.*, 2008). Our review of current cnidarian sequence resources identified putative homologs for both genes in all resources surveyed (Table 3).

Some genomics studies found evidence of differential expression of pathways for carbohydrate and lipid metabolism associated with symbiosis (Table 1) (Rodriguez-Lanetty *et al.*, 2006a; Ganot *et al.*, 2011; Levy *et al.*, 2011). Because these large and complex pathways are central elements of cellular metabolism rather than being specific to symbiosis, we deemed these outside the scope of our review. We focused instead on recent developments in the study of lipid bodies (LBs) in cnidarian-algal symbiosis. LBs are the lipid storage organelle of eukaryotic cells, consisting of neutral lipids and sterols enclosed in phospholipid monolayers bearing LB coat proteins. Although traditionally viewed as passive reservoirs, LBs are now recognized as dynamic organelles with characteristic protein components and diverse cellular functions (Beller *et al.*, 2010). LBs interact with other organelles by translocating proteins and lipids between organelles and cellular compartments (Welte, 2007). The metabolism and trafficking of lipids by these organelles in model systems suggests roles in metabolic interactions in cnidarian-algal symbiosis; in fact, LBs were reported in symbiotic anemones and isolated symbionts (Kellogg and Patton, 1983; Patton and Burris, 1983). However, subsequent observations of DNA in apparent LBs cast doubt on the identity and role of these structures (Muscatine *et al.*, 1994).

Recently, several new lines of evidence associating LBs with cnidarian-algal symbiosis have emerged. Luo and coworkers observed changes in the LB composition and morphology associated with symbiotic status in the coral *Euphyllia glabrescens* (Luo *et al.*, 2009). Importantly, the lipid composition analysis in this study demonstrated lipid transport from the symbiont into host LBs. Chen and coworkers demonstrated diel variation in LB abundance, with LBs accumulating in gastrodermal cells in tight association with symbiosomes during daylight, then declining in the dark (Chen *et al.*, 2012). These observations suggest important nutrient transport roles for LBs in the symbiosis.

Proteomic analysis of *E. glabrescens* LBs has recently

been used to characterize the diverse protein components of this organelle (Table 1) (Peng *et al.*, 2011). Although identification of proteins from mass spectrometry data remains challenging in species lacking a completed genome, the authors succeeded in identifying 17 proteins associated with coral LBs. Perilipins (also called lipid storage droplet proteins, LSD), a family of LB coat proteins with important regulatory roles in mammals and insects (Beller *et al.*, 2010), were conspicuously absent from this list. In contrast with these results from a coral, a recent study of the anemone *A. viridis* documented upregulation of a perilipin homolog (termed LSD-2) in symbiotic anemones (Ganot *et al.*, 2011). Our review of cnidarian sequence resources identified a single perilipin homolog in each anemone species, but none in corals, consistent with these previous studies (Table 3). Further proteomic studies will be needed to identify the coat proteins in coral LBs and clarify their roles in the symbiosis.

Cholesterol is an important regulator of the structural integrity and permeability of cellular membranes. One of the mechanisms regulating cellular cholesterol homeostasis is export from endosomes by Niemann-Pick disease proteins (NPC1 and NPC2) (Cheruku *et al.*, 2006). NPC2 mediates transport from internal membranes in endosome membranes to external membranes, where the membrane-associated NPC1 mediates its export to the cytosol. These functions suggest possible roles in the transport of cholesterol from symbionts to host, and two genomic studies found upregulation of an NPC2 homolog consistent with such a function (Table 1) (Kuo *et al.*, 2010; Ganot *et al.*, 2011). We found multiple homologs for both NPC1 and NPC2 in all anemone and coral species included in this analysis (Table 3), identifying candidate genes for future studies of cholesterol transport in cnidarian-algal symbiosis.

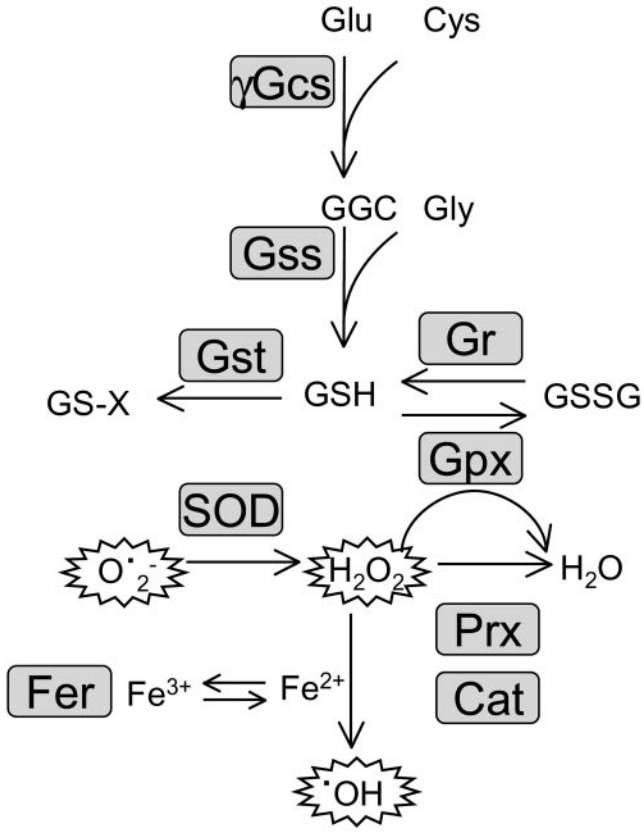

Figure 6. Roles of selected oxidative stress response genes expressed in symbiotic anthozoans. Reactive oxygen species (ROS) produced as byproducts of cellular metabolism and photosynthesis are indicated with white symbols, and genes with grey boxes. Glu: glutamate; Cys: cysteine; GGC: γ-glutamylcysteine; GSH: glutathione; GSSG: glutathione disulfide; GS-X: glutathione S-conjugates; $O^{\cdot}{}_2^-$: superoxide anion; H_2O_2: hydrogen peroxide; $^{\cdot}OH$: hydroxyl radical; γ-Gcs: gamma-glutamylcysteine synthetase; Gss: glutathione synthase; Gst: glutathione S-transferase; Gr: glutathione reductase; Gpx: glutathione peroxidase; Prx: peroxidase; Cat: catalase; Fer: ferritin; SOD: superoxide dismutase.

Responses to reactive oxygen species (ROS)

Reactive oxygen species (ROS) including superoxide anion ($O^{\cdot}{}_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\cdot}$) are inevitable byproducts of cellular metabolism. These molecules cause oxidative damage to lipids, proteins, and DNA if left unchecked, but cells counter these effects with both enzymatic and non-enzymatic antioxidants. Cnidarian cells hosting algal symbionts face elevated ROS levels arising from photosynthesis in the symbiont, and they therefore have a large repertoire of antioxidant genes and pathways to manage these elevated levels (Fig. 2d) (Richier *et al.*, 2005). However, when these antioxidant pathways become overwhelmed during elevated temperature and light stress, ROS figure prominently in the coral bleaching response (Lesser, 2006; Weis, 2008).

Enzymatic antioxidants include superoxide dismutase (SOD), which presents one of the first lines of defense by catalyzing conversion of superoxide anions into O_2 and H_2O_2 (Fig. 6). Symbiotic anthozoans possess multiple SOD genes, with 2 putative SOD homologs in the coral genome, 4 in the anemone genome, and comparable numbers in transcriptome assemblies from other symbiotic anthozoans (Table 3). Catalase (Cat) and peroxiredoxin (Prx) further detoxify H_2O_2, catalyzing its conversion into H_2O (Fig. 6). Multiple putative homologs for both genes are present in symbiotic anthozoans (Table 3).

Glutathione (GSH) is an abundant tripeptide with multiple roles in oxidative stress responses (Dringen, 2000). GSH detoxifies H_2O_2 both directly and as an electron donor in the reaction catalyzed by GSH peroxidase (Gpx) (Fig. 6). The antioxidant GSH is then regenerated as a product of this reaction (glutathione disulfide, GSSG) by the activity of glutathione reductase (Gr). GSH is synthesized from amino acid precursors by γ-glutamylcysteine synthetase (γ-Gcs) and glutathione synthetase (Gss) (Fig. 6). GSH

is consumed by detoxification reactions catalyzed by glutathione S-transferase that enable export of GSH-conjugates from the cell. All of these genes associated with GSH metabolism were found in both anemone and coral genomes, and nearly all were expressed in all transcriptomes reviewed here (Table 3).

Free cellular iron (Fe^{2+}) promotes the formation of hydroxyl radicals ($HO^{\cdot}$), the most reactive form of ROS (Fig. 6). Ferritins are iron-regulated proteins that serve an antioxidant function by binding free iron (Andrews and Schmidt, 2007). Ferritin homologs occur frequently in expression profiling and EST studies of symbiotic anthozoans (Table 1) (Kuo *et al.*, 2004; Schwarz *et al.*, 2008; Levy *et al.,* 2011) and are differentially upregulated during elevated temperature and UV stress (Richier *et al.*, 2008). Both coral and anemone genomes include two putative ferritin homologs, and all transcriptome assemblies included at least one (Table 3).

Omics Research in *Symbiodinium* spp.

Two recent reviews summarize the nascent state of *Symbiodinium* omics approaches (Gordon and Leggat, 2010a; Leggat *et al.*, 2011). There is a single proteomics study comparing cultured and symbiotic *Symbiodinium* spp. from the anemone *Aiptasia pallida* (Stochaj and Grossman, 1997) and three transcriptomic studies of *Symbiodinium* spp. (Leggat *et al.*, 2007; Voolstra *et al.*, 2009b). The paucity of studies is due in part to the very large genomes of dinoflagellates (~2–4 Gb) (LaJeunesse *et al.*, 2005; Hou and Lin, 2009; Leggat *et al.*, 2011), the abundance in these genomes of highly repetitive DNA (Hou and Lin, 2009; Leggat *et al.*, 2011), and a still-emerging understanding of the biodiversity and taxonomy of the algal group.

Symbiodinium is a highly diverse taxon of dinoflagellates with nine clades described to date and with the phylogenetic distance between clades at the order or family level (Coffroth and Santos, 2005; Pochon and Gates, 2010). In addition to the well-known symbiotic clades A–D, microbial ecologists are now describing clades that are likely only free-living and not in symbiosis with host animals (Apprill and Gates, 2007; Pochon and Gates, 2010). Patterns of host-symbiont specificity are highly complex and influenced by a broad array of factors including biogeography, environment, stress, and host ontogeny (Baker, 2003; Coffroth and Santos, 2005). Although a complete discussion of the diversity and functional biology of *Symbiodinium* is well beyond the scope of this review, it is worthwhile to point out two features of *Symbiodinium* functional biology—colonization of hosts and response to environmental stress—to illustrate how future omics approaches will transform our understanding of the symbiosis.

Symbiodinium spp. have varying abilities to colonize hosts, and they elicit differing recognition responses in these hosts (Belda-Baillie *et al.*, 2002; Little *et al.*, 2004; Rodriguez-Lanetty *et al.*, 2006b; Dunn and Weis, 2009). For example, during onset of symbiosis in larvae of the coral *Fungia scutaria, Symbiodinium* types have differing abilities to colonize the host. *Symbiodinium* C1f, the type found in adult *F. scutaria,* persists in larvae and does not elicit an apoptotic response in host cells (Dunn and Weis, 2009). In contrast, type C31, found in adult *Montipora capitata* (co-occurring on the reef with *F. scutaria*) cannot colonize or persist in *F. scutaria* larvae. Furthermore, addition of C31 algae elicits an apoptotic response in gastrodermal host cells, suggesting that the host is mounting an immune response to eliminate the inappropriate *Symbiodinium* type.

Symbiodinium types have highly varying biochemistry, physiology, and responses to environmental stress (*e.g.,* Iglesias-Prieto and Trench, 1997; Warner *et al.*, 1999; Rowan, 2004; Tchernov *et al.*, 2004; Stat *et al.*, 2008). These differential biochemical and physiological qualities have direct impacts on the performance and health of the holobiont. An obvious example is the differential performance of stress-sensitive clade C *versus* stress-resistant clade D symbionts to elevated temperature in corals. Whereas clade C symbionts are the overwhelmingly dominant taxon in healthy IndoPacific corals, they are at least temporarily displaced after bleaching events by clade D types in many coral species (Berkelmans and van Oppen, 2006; Stat and Gates, 2011). In-depth comparative genomic and proteomic characterization of *Symbiodinium* types will help explain such differential performance in colonization of hosts and response to stress from a functional perspective and will add to our understanding of how these differences impact host health and fitness.

Areas for Future Research

Reviewing recent studies of cnidarian-algal symbiosis and the current status of sequence resources, several areas emerge that remain poorly understood, and this suggests directions for future studies.

Directions in Symbiodinium *research*

Improving genomic and proteomic resources for the algal symbiont is the most pressing need in cnidarian-algal symbiosis research. At the time of this article going to press, there are at least two NGS efforts underway for *Symbiodinium* spp. in Saudi Arabia (Voolstra and co-workers, King Abdullah University of Science and Technology, unpubl. data) and Japan (Satoh and co-workers, Okinawa Institute of Science and Technology, unpubl. data). Characterizing the complement of genes expressed by *Symbiodinium* will lead to a better understanding of its metabolic interactions with the host. Sequence resources will facilitate expression profiling of symbionts in pure culture and *in hospite*, as they have in the host (Table 1). Profiling gene expression of

symbionts *in hospite* is complicated by the mixture of host and symbiont transcripts that are inevitably present (Mayfield *et al.*, 2009). Additional sequence information will be useful both for characterizing the scope of the problem on the basis of sequence similarity between host and symbiont homologs, and also for designing solutions that might include gene-specific quantitative PCR assays, or the separation of host and symbiont transcripts in RNA-Seq analysis. Performing sequencing of host and resident symbiont in parallel will enable researchers to look for genomic complementation between the partners—for example, gene or gene family loss in one partner with concomitant gain in the other. These approaches are being pursued by Voolstra and coworkers (unpubl. data) for *Stylophora pistillata* and its clade A symbiont.

The cnidarian-algal symbiosis is based on exchange of nutrients and metabolites across the symbiosome membrane complex. The symbiont is enclosed within a host-derived membrane (Wakefield and Kempf, 2001) across which any translocated substrates must pass. A recent proteomic study of the symbiosome membrane in *Aiptasia pulchella* revealed a diverse protein component in this membrane (Peng *et al.*, 2010). Many of the proteins isolated in that study could not be identified (64%). Sequence analysis suggested both animal and algal contributions and identified multiple cytoskeletal elements and ATP synthase genes. Although our understanding of symbiosome membranes is obviously far from complete with the 17 genes characterized in this study, the involvement of both anemone and algal proteins identified highlights the need for a complete database of gene sequences in both partners. Proteomic studies are especially needed because post-translational mechanisms might be important in the symbiosis. For example, the recruitment of Sym32 homologs from plasma membranes to the symbiosome during early stages of symbiosome formation (Ganot *et al.*, 2011) or the sequential addition of Rab GTPases during phagosome maturation (Chen *et al.*, 2004, 2005; Hong *et al.*, 2009a) may not involve transcriptional changes, but they would potentially be detectable by proteomic analysis of symbiosomes. Functional analysis of proteomic data would be greatly enhanced by the availability of complete genomes for both cnidarian and algal partners, making it possible to identify the origin and putative function of each protein in the symbiosome.

Interactions between host lectins and algal cell-surface glycans play important functional roles in the initiation and maintenance of stable associations (Wood-Charlson *et al.*, 2006; Bay *et al.*, 2011). Although the lectins thought to bind to these glycans are a large and diverse gene family (Wood-Charlson and Weis, 2009), few have been functionally characterized. Nevertheless, substantial sequence resources are now available for exploring the expression, sequence divergence, and subcellular localization of these host components (Table 3). In contrast, the cell-surface glycans in *Symbiodinium* have remained poorly characterized (Logan *et al.*, 2010). Recent technological advances have greatly improved throughput and scope of glycomic analysis (Rakus and Mahal, 2011) and hold promise for use in the study of host-symbiont recognition. Lectin microarrays consist of purified lectins attached to a solid substrate for analysis of fluorescence-labeled glycoprotein-containing samples analogous to traditional nucleic acid microarrays. These approaches may be especially useful for understanding host recognition responses to different symbiont genotypes (Weis *et al.*, 2001; Rodriguez-Lanetty *et al.*, 2004), and differences between generalist clades of *Symbiodinium* associated with multiple coral species *versus* specialists associated with a single species (Baker, 2003).

Applications of existing cnidarian sequence resources

The rapid development of sequence resources for symbiotic cnidarians opens new possibilities for investigating their association with *Symbiodinium*. Unlike other methods for gene expression profiling (*e.g.,* cDNA or oligonucleotide microarrays) that require substantial time and resource investment into platform development, newer methods based on high-throughput sequencing and quantitative analysis of cDNA (RNA-Seq) (Mortazavi *et al.*, 2008) require only a reasonably complete collection of gene sequences, as obtained from transcriptome sequencing (Meyer *et al.*, 2011). The cnidarian sequence resources reviewed here (Table 3) will therefore make it possible to explore across a wide range of taxa and conditions (i) transcriptional responses associated with the acquisition of symbionts, (ii) steady-state differences in expression profiles between symbiotic and aposymbiotic animals, and (iii) expression changes during breakdown of the association. Expression profiles associated with symbiosis have never been reported for many of these species (*A. hyacinthus, P. astreoides, A. digitifera, P. damicornis*). Expanding profiling to additional coral species will likely reveal differences linked to host-symbiont specificity. Whereas the relatively high costs and labor requirements of array methods have limited throughput of gene expression analysis in previous studies to a small number of time points (Rodriguez-Lanetty *et al.*, 2006a; Voolstra *et al.*, 2009a; Schnitzler and Weis, 2010), the low cost of RNA-Seq will allow high-resolution sampling during the time course of symbiont acquisition or expulsion that may provide important insights into the mechanisms involved.

Regardless of platform, a major challenge for expression profiling of cnidarian-algal symbiosis is the previously mentioned low symbiosis signal in many types of samples studied (see above), such that subtle signals associated with symbiosis are likely to be overshadowed by background noise from aposymbiotic cells. RNA-Seq allows for profiling of single cells (Tang *et al.*, 2009); therefore, the isola-

tion of suitable samples remains the major bottleneck. Laser-capture microdissection (LCM) allows for sampling at the appropriate (single-cell) scale (Emmert-Buck *et al.*, 1996), but has typically been used in conjunction with microarray platforms that required large amounts of RNA. More recently, this method has been coupled with RNA amplification and RNA-Seq to profile individual cells dissected from tissue slices (Schmid *et al.*, 2012). This class of methods offers the intriguing possibility of profiling cells at specific stages during the acquisition or breakdown of symbiosis (*e.g.,* cells engaged in autophagy of the symbiosome). Fluorescence-activated cell sorting (FACS) is another technology that might allow for isolation of individual cells following enzymatic or mechanical tissue dissociation. This approach has been used to isolate specific cell types from rat brain on the basis of type-specific antigens (Guez-Barber *et al.*, 2011). The presence of an endogenous fluorescent signal in cells containing symbionts (chlorophyll) presents an advantage for applying these methods to the study of cnidarian-algal symbiosis.

Many of the genes expressed in symbiotic cnidarians lack sequence similarity to known proteins. For example, the percent of differentially expressed genes, identified in microarray studies of the anemone *A. elegantissima*, two Caribbean corals, and larvae of the coral *F. scutaria*, that lacked sequence homology were 65%, 26%, and 42%, respectively (Rodriguez-Lanetty *et al.*, 2006a; Voolstra *et al.*, 2009a; Schnitzler and Weis, 2010). This lack of functional annotation obscures the interpretation of these expression profiles. One strategy that might prove useful in resolving the functions of unknown genes is the use of network analyses to identify co-regulated gene modules (Horan *et al.*, 2008). Relationships between the expression levels of unknown genes (*i.e.,* transcripts lacking sequence similarity to known genes) and genes with known functions suggest possible functional relationships (*e.g.,* direct interactions between the gene products, or participation in the same pathways). As additional gene expression datasets become available for cnidarian-algal symbiosis, especially in the context of the NGS experiments made possible by RNA-Seq, correlation-based methods like this will become increasingly powerful for detecting signatures of co-regulation.

Gene annotation is usually accomplished by searching for local sequence similarity between each unknown transcript and a collection of gene sequences with known function. However, extensive sequence divergence is expected between cnidarians and the phylogenetically distant model systems from which most information on gene functions has been obtained, thereby limiting annotation. In the context of this sequence divergence, it may be productive to consider structural comparisons instead, since protein structure is more strongly conserved than protein sequence (Illergard *et al.*, 2009). Although the *de novo* prediction of three-dimensional structures and the comparison of predicted structures with existing databases remain computationally challenging, both are readily available as web services (Kelley and Sternberg, 2009; Holm and Rosenstrom, 2010). Structural predictions may be a useful tool for generating new hypotheses about functional roles of differentially expressed genes associated with symbiosis.

Whether functional annotations are derived from differential expression during symbiosis or from gene sequence or structure comparisons, they remain only hypotheses until confirmed by manipulative experiments. Although gene knockout and knockdown were once confined to model systems, new methods based on RNA interference allow sequence-specific degradation of a target gene in a wider range of organisms (Shan, 2010). These methods have already been used in cnidarians to study developmental processes (Rentzsch *et al.*, 2008; Duffy *et al.*, 2010) and caspase, a protease with roles in apoptosis (Dunn *et al.*, 2007a). The studies of gene expression and sequence resources reviewed here suggest a number of symbiosis-associated genes that would make good candidates for functional testing using these methods.

Concluding Remarks

The rapid growth of sequence resources and the ongoing decline in sequencing costs are bringing new tools to the study of the symbiosis between cnidarians and *Symbiodinium* spp. In the coming years, we expect that functional and comparative studies enabled by these new resources will identify many of the mechanisms underlying initiation, maintenance, and breakdown of these critically important symbioses. These resources will also prove invaluable in efforts to understand how various naturally occurring and anthropogenic abiotic factors affect the health and stability of the partnership. Finally, a comprehensive knowledge of the mechanisms of initiation, regulation, and breakdown of this eukaryote-eukaryote mutualism afforded by omics studies will enhance our broader understanding of eukaryote-eukaryote interactions in general, both mutualistic and parasitic.

Acknowledgments

We thank Erik Lehnert and John Pringle (Stanford University) for sharing NGS transcriptomic data for *Aiptasia pallida*.

Literature Cited

Alieva, N. O., K. A. Konzen, S. F. Field, E. A. Meleshkevitch, M. E. Hunt, V. Beltran-Ramirez, D. J. Miller, J. Wiedenmann, A. Salih, and M. V. Matz. 2008. Diversity and evolution of coral fluorescent proteins. *PloS One* **3:** e2680.

Andrews, N. C., and P. J. Schmidt. 2007. Iron homeostasis. *Annu. Rev. Physiol.* **69:** 69–85.

AnimalLectindb. 2012. Database of animal lectins. [Online]. Available: http://proline.physics.iisc.ernet.in/animaldb/ [2012, April 25].

Apprill, A. M., and R. D. Gates. 2007. Recognizing diversity in coral symbiotic dinoflagellate communities. *Mol. Ecol.* **16:** 1127–1134.

Arbeitman, M. N., E. E. Furlong, F. Imam, E. Johnson, B. H. Null, B. S. Baker, M. A. Krasnow, M. P. Scott, R. W. Davis, and K. P. White. 2002. Gene expression during the life cycle of *Drosophila melanogaster*. *Science* **297:** 2270–2275.

Axelsen, K. B., and M. G. Palmgren. 1998. Evolution of substrate specificities in the P-type ATPase superfamily. *J. Mol. Evol.* **46:** 84–101.

Baker, A. C. 2003. Flexibility and specificity in coral-algal symbiosis: diversity, ecology and biogeography of *Symbiodinium*. *Annu. Rev. Ecol. Evol. Syst.* **34:** 661–689.

Banaszak, A. T., T. C. LaJeunesse, and R. K. Trench. 2000. The synthesis of mycosporine-like amino acids (MAAs) by cultured, symbiotic dinoflagellates. *J. Exp. Mar. Biol. Ecol.* **249:** 219–233.

Barneah, O., Y. Benayahu, and V. M. Weis. 2006. Comparative proteomics of symbiotic and aposymbiotic juvenile soft corals. *Mar. Bioltechnol.* **8:** 11–16.

Bay, L. K., V. R. Cumbo, D. Abrego, J. T. Kool, T. D. Ainsworth, and B. L. Willis. 2011. Infection dynamics vary between *Symbiodinium* types and cell surface treatments during establishment of endosymbiosis with coral larvae. *Diversity* **3:** 356–374.

Belda-Baillie, C. A., B. K. Baillie, and T. Maruyama. 2002. Specificity of a model cnidarian-dinoflagellate symbiosis. *Biol. Bull.* **202:** 74–85.

Beller, M., K. Thiel, P. J. Thul, and H. Jackle. 2010. Lipid droplets: a dynamic organelle moves into focus. *FEBS Lett.* **584:** 2176–2182.

Berkelmans, R., and M. J. H. van Oppen. 2006. The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. *Proc. R. Soc. Lond. B* **273:** 2305–2312.

Cambi, A., M. Koopman, and C. G. Figdor. 2005. How C-type lectins detect pathogens. *Cell. Microbiol.* **7:** 481–488.

Carreto, J. I., and M. O. Carignan. 2011. Mycosporine-like amino acids: relevant secondary metabolites. Chemical and ecological aspects. *Mar. Drugs* **9:** 387–446.

Chapman, J. A., E. F. Kirkness, O. Simakov, S. E. Hampson, T. Mitros, T. Weinmaier, T. Rattei, P. G. Balasubramanian, J. Borman, and D. Busam. 2010. The dynamic genome of *Hydra*. *Nature* **464:** 592–596.

Chen, M.-C., Y.-M. Cheng, M.-C. Hong, and L.-S. Fang. 2004. Molecular cloning of Rab5 (ApRab5) in *Aiptasia pulchella* and its retention in phagosomes harboring live zooxanthellae. *Biochem. Biophys. Res. Comm.* **324:** 1024–1033.

Chen, M.-C., M.-C. Hong, Y.-S. Huang, M.-C. Liu, Y.-M. Cheng, and L.-S. Fang. 2005. ApRab11, a cnidarian homologue of the recycling regulatory protein Rab11, is involved in the establishment and maintenance of the *Aiptasia-Symbiodinium* endosymbiosis. *Biochem. Biophys. Res. Comm.* **338:** 1607–1616.

Chen, W.-N. U., H.-J. Kang, V. M. Weis, A. B. Mayfield, P.-L. Jiang, L.-S. Fang, and C.-S. Chen. 2012. Diel rhythmicity of lipid-body formation in a coral-*Symbiodinium* endosymbiosis. *Coral Reefs* **31:** 521-534.

Cheruku, S. R., Z. Xu, R. Dutia, P. Lobel, and J. Storch. 2006. Mechanism of cholesterol transfer from the Niemann-pick type C2 protein to model membranes supports a role in lysosomal cholesterol transport. *J. Biol. Chem.* **281:** 31594–31604.

Chung, H.-T., H.-O. Pae, B.-M. Choi, T. R. Billiar, and Y.-M. Kim. 2001. Nitric oxide as a bioregulator of apoptosis. *Biochem. Biophys. Res. Comm.* **282:** 1075–1079.

Coffroth, M. A., and S. R. Santos. 2005. Genetic diversity of symbiotic dinoflagellates in the genus *Symbiodinium*. *Protist* **156:** 19–34.

Coutu, D. L., J. H. Wu, A. Monette, G.-A. Rivard, M. D. Blostein, and J. Galipeau. 2008. Periostin, a member of a novel family of vitamin K-dependent proteins, is expressed by mesenchymal stromal cells. *J. Biol. Chem.* **283:** 17991–18001.

D'Angelo, C., A. Denzel, A. Vogt, M. V. Matz, F. Oswald, A. Salih, G. U. Nienhaus, and J. Wiedenmann. 2008. Blue light regulation of host pigment in reef-building corals. *Mar. Ecol. Prog. Ser.* **364:** 97–106.

Davy, S. K., D. Allemand, and V. M. Weis. 2012. The cell biology of cnidarian-dinoflagellate symbiosis. *Microbiol. Mol. Biol. Rev.* **76:** 229-261.

deBoer, M., D. A. Krupp, and V. M. Weis. 2007. Proteomic and transcriptional analyses of coral larvae newly engaged in symbiosis with dinoflagellates. *Comp. Biochem. Physiol. D Genomics Proteomics* **2:** 63–73.

DeSalvo, M. K., S. Sunagawa, P. L. Fisher, C. R. Voolstra, R. Iglesias-Prieto, and M. Medina. 2010. Coral host transcriptomic states are correlated with *Symbiodinium* genotypes. *Mol. Ecol.* **19:** 1174–1186.

Detournay, O., and V. M. Weis. 2011. Role of the sphingosine rheostat in the regulation of cnidarian-dinoflagellate symbioses. *Biol. Bull.* **221:** 261–269.

Dove, S. G., O. Hoegh-Guldberg, and S. Ranganathan. 2001. Major colour patterns of reef-building corals are due to a family of GFP-like proteins. *Coral Reefs* **19:** 197–204.

Downs, C. A., E. Kramarsky-Winter, J. Martinez, A. Kushmaro, C. M. Woodley, Y. Loya, and G. K. Ostrander. 2009. Symbiophagy as a cellular mechanism for coral bleaching. *Autophagy* **5:** 211–216.

Dringen, R. 2000. Metabolism and functions of glutathione in brain. *Prog. Neurobiol.* **62:** 649–671.

Duffy, D. J., G. Plickert, T. Kuenzel, W. Tilmann, and U. Frank. 2010. Wnt signaling promotes oral but suppresses aboral structures in *Hydractinia* metamorphosis and regeneration. *Development* **137:** 3057–3066.

Dunn, S. R. 2009. Immunorecognition and immunoreceptors in the Cnidaria. *Invertebrate Surviv. J.* **6:** 7–14.

Dunn, S. R., and V. M. Weis. 2009. Apoptosis as a post-phagocytic winnowing mechanism in a coral-dinoflagellate mutualism. *Environ. Microbiol.* **11:** 268–276.

Dunn, S. R., J. C. Bythell, M. L. Tissier, W. J. Burnett, and J. C. Thomason. 2002. Programmed cell death and cell necrosis activity during hyperthermic stress-induced bleaching of the symbiotic sea anemone *Aiptasia* sp. *J. Exp. Mar. Biol. Ecol.* **272:** 29–53.

Dunn, S. R., J. C. Thomason, M. D. A. Le Tissier, and J. C. Bythell. 2004. Heat stress induces different forms of cell death in sea anemones and their endosymbiotic algae depending on temperature and duration. *Cell Death Differ.* **11:** 1213–1222.

Dunn, S. R., W. S. Phillips, D. R. Green, and V. M. Weis. 2007a. Knockdown of actin and caspase gene expression by RNA interference in the symbiotic anemone *Aiptasia pallida*. *Biol. Bull.* **212:** 250–258.

Dunn, S. R., C. E. Schnitzler, and V. M. Weis. 2007b. Apoptosis and autophagy as mechanisms of dinoflagellate symbiont release during cnidarian bleaching: every which way you lose. *Proc. R. Soc. Lond. B* **274:** 3079.

Elkins, T., M. Hortsch, A. J. Bieber, P. M. Snow, and C. S. Goodman. 1990. *Drosophila* fasciclin I is a novel homophilic adhesion molecule that along with fasciclin III can mediate cell sorting. *J. Cell Biol.* **110:** 1825–1832.

Emmert-Buck, M. R., R. F. Bonner, P. D. Smith, R. F. Chuaqui, Z. Zhuang, S. R. Goldstein, R. A. Weiss, and L. A. Liotta. 1996. Laser capture microdissection. *Science* **274:** 998–1001.

Endeward, V., R. Musa-Aziz, G. J. Cooper, L. M. Chen, M. F. Pelletier, L. V. Virkki, C. T. Supuran, L. S. King, W. F. Boron, and G. Gros. 2006. Evidence that aquaporin 1 is a major pathway for CO_2 transport across the human erythrocyte membrane. *FASEB J.* **20:** 1974.

Franchi, L., N. Warner, K. Viani, and G. Nunez. 2009. Function of

Nod-like receptors in microbial recognition and host defense. *Immunol. Rev.* **227:** 106–128.

Furla, P., D. Allemand, and M. N. Orsenigo. 2000. Involvement of H^+-ATPase and carbonic anhydrase in inorganic carbon uptake for endosymbiont photosynthesis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **278:** R870.

Ganot, P., A. Moya, V. Magnone, D. Allemand, P. Furla, and C. Sabourault. 2011. Adaptations to endosymbiosis in a cnidarian-dinoflagellate association: differential gene expression and specific gene duplications. *PLoS Genet.* **7:** e1002187.

Gordon, B. R., and W. Leggat. 2010a. *Symbiodinium*-invertebrate symbioses and the role of metabolomics. *Mar. Drugs* **8:** 2546–2568.

Grasso, L. C., J. Maindonald, S. Rudd, D. C. Hayward, R. Saint, D. J. Miller, and E. E. Ball. 2008. Microarray analysis identifies candidate genes for key roles in coral development. *BMC Genomics* **9:** 540.

Gross, R., F. Vavre, A. Heddi, G. D. Hurst, E. Zchori-Fein, and K. Bourtzis. 2009. Immunity and symbiosis. *Mol. Microbiol.* **73:** 751–759.

Grover, R., J. F. Maguer, S. Reynaud-Vaganay, and C. Ferrier-Pages. 2002. Uptake of ammonium by the scleractinian coral *Stylophora pistillata:* effect of feeding light, and ammonium concentrations. *Limnol. Oceanogr.***47:** 782–790.

Guez-Barber, D., S. Fanous, B. K. Harvey, Y. Zhang, K. G. Becker, M. R. Picciotto, and B. T. Hope. 2011. FACS purification of immunolabeled cell types from adult rat brain. *J. Neurosci. Methods* **203:** 10–18.

Heldin, C.-H., M. Landström, and A. Moustakas. 2009. Mechanism of TGF-β signaling to growth arrest, apoptosis, and epithelial-mesenchymal transition. *Curr. Opin. Cell Biol.* **21:** 166–176.

Higgins, C. F. 1992. ABC transporters: from microorganisms to man. *Annu. Rev. Cell Biol.* **8:** 67–113.

Holm, L., and P. Rosenstrom. 2010. Dali server: conservation mapping in 3D. *Nucleic Acids Res.* **38:** W545–W549.

Hong, M.-C., Y.-S. Huang, W.-W. Lin, L.-S. Fang, and M.-C. Chen. 2009a. ApRab3, a biosynthetic Rab protein, accumulates on the maturing phagosomes and symbiosomes in the tropical sea anemone, *Aiptasia pulchella. Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **152:** 249–259.

Hong, M.-C., Y.-S. Huang, P.-C. Song, W.-W. Lin, L.-S. Fang, and M.-C. Chen. 2009b. Cloning and characterization of ApRab4, a recycling Rab protein of *Aiptasia pulchella,* and its implication in the symbiosome biogenesis. *Mar. Biotechnol.* **11:** 771–785.

Horan, K., C. Jang, J. Bailey-Serres, R. Mittler, C. Shelton, J. F. Harper, J. K. Zhu, J. C. Cushman, M. Gollery, and T. Girke. 2008. Annotating genes of known and unknown function by large-scale coexpression analysis. *Plant Physiol.* **147:** 41–57.

Hou, Y., and S. Lin. 2009. Distinct gene number-genome size relationships for eukaryotes and non-eukaryotes: gene content estimation for dinoflagellate genomes. *PloS One* **4:** e6978.

Huber, O., and M. Sumper. 1994. Algal-CAMs: isoforms of a cell adhesion molecule in embryos of the alga *Volvox* with homology to *Drosophila* fasciclin I. *EMBO J.* **13:** 4212.

Iglesias-Prieto, R., and R. K. Trench. 1997. Photoadaptation, photoacclimation and niche diversification in invertebrate-dinoflagellate symbioses. Pp. 1319-1324 in *Proceedings of the 8th International Coral Reef Symposium,* Vol. 2, H. A. Lessios and I. G. Macintyre, eds. Smithsonian Tropical Research Institute, Panama.

Iguchi, A., C. Shinzato, S. Foret, and D. J. Miller. 2011. Identification of fast-evolving genes in the scleractinian coral *Acropora* using comparative EST analysis. *PloS One* **6:** e20140.

Illergard, K., D. H. Ardell, and A. Elofsson. 2009. Structure is three to ten times more conserved than sequence: a study of structural response in protein cores. *Proteins* **77:** 499–508.

Inoue, S., G. Browne, G. Melino, and G. M. Cohen. 2009. Ordering of caspases in cells undergoing apoptosis by the intrinsic pathway. *Cell Death Differ*. **16:** 1053–1061.

Isshiki, T., B. Pearson, S. Holbrook, and C. Q. Doe. 2001. *Drosophila* neuroblasts sequentially express transcription factors which specify the temporal identity of their neuronal progeny. *Cell* **106:** 511–521.

Janeway, C. A., and R. Medzhitov. 2002. Innate immune recognition. *Annu. Rev. Immunol.* **20:** 197–216.

Kelley, L. A., and M. J. E. Sternberg. 2009. Protein structure prediction on the Web: a case study using the Phyre server. *Nat. Protoc.* **4:** 363–371.

Kellogg, R. B., and J. S. Patton. 1983. Lipid droplets, medium of energy exchange in the symbiotic anemone *Condylactis gigantea:* a model coral polyp. *Mar. Biol.* **75:** 137–149.

Kimura, A., E. Sakaguchi, and M. Nonaka. 2009. Multi-component complement system of Cnidaria: C3, Bf, and MASP genes expressed in the endodermal tissues of a sea anemone, *Nematostella vectensis. Immunobiology* **214:** 165–178.

Kinchen, J. M., and K. S. Ravichandran. 2008. Phagosome maturation: going through the acid test. *Nat. Rev. Mol. Cell Biol.* **9:** 781–795.

Kortschak, R. D., G. Samuel, R. Saint, and D. J. Miller. 2003. EST analysis of the cnidarian *Acropora millepor*a reveals extensive gene loss and rapid sequence divergence in the model invertebrates. *Curr. Biol.* **13:** 2190–2195.

Kuo, J., M.-C. Chen, C.-H. Lin, and L.-S. Fang. 2004. Comparative gene expression in the symbiotic and aposymbiotic *Aiptasia pulchella* by expressed sequence tag analysis. *Biochem. Biophys. Res. Comm.* **318:** 176–186.

Kuo, J., Z.-C. Liang, and C.-H. Lin. 2010. Suppression subtractive hybridization identifies genes correlated to symbiotic and aposymbiotic sea anemone associated with dinoflagellate. *J. Exp. Mar. Biol. Ecol.* **388:** 11–19.

Kvennefors, E. C., W. Leggat, C. C. Kerr, T. D. Ainsworth, O. Hoegh-Guldberg, and A. C. Barnes. 2010. Analysis of evolutionarily conserved innate immune components in coral links immunity and symbiosis. *Dev. Comp. Immunol.* **34:** 1219–1229.

LaJeunesse, T. C., G. Lambert, R. A. Andersen, M. A. Coffroth, and D. W. Galbraith. 2005. *Symbiodinium* (Pyrrhophyta) genome sizes (DNA content) are smallest among dinoflagellates. *J. Phycol.* **41:** 880–886.

Lange, C., G. Hemmrich, U. C. Klostermeier, J. A. Lopez-Quintero, D. J. Miller, T. Rahn, Y. Weiss, T. C. G. Bosch, and P. Rosenstiel. 2011. Defining the origins of the NOD-like receptor system at the base of animal evolution. *Mol. Biol. Evol.* **28:** 1687–1702.

Lasky, L. A. 1992. Selectins: interpreters of cell-specific carbohydrate information during inflammation. *Science* **258:** 964–969.

Leggat, W., O. Hoegh-Guldberg, S. Dove, and D. Yellowlees. 2007. Analysis of an EST library from the dinoflagellate (*Symbiodinium* sp.) symbiont of reef-building corals. *J. Phycol.* **43:** 1010–1102.

Leggat, W., D. Yellowlees, and M. Medina. 2011. Recent progress in *Symbiodinium* transcriptomics. *J. Exp. Mar. Biol. Ecol.* **408:** 120–125.

Lehnert, E., M. Burriesci, and J. Pringle. 2012. Developing the anemone *Aiptasia* as a tractable model for cnidarian-dinoflagellate symbiosis: the transcriptome of aposymbiotic *A. pallida. BMC Genomics* **13:** 271.

Lesser, M. P. 2006. Oxidative stress in marine environments: biochemistry and physiological ecology. *Annu. Rev. Physiol.* **68:** 253–278.

Levine, B. 2005. Eating oneself and uninvited guests: autophagy-related pathways in cellular defense. *Cell* **120:** 159–162.

Levine, B., S. Sinha, and G. Kroemer. 2008. Bcl-2 family members: dual regulators of apoptosis and autophagy. *Autophagy* **4:** 600–606.

Levy, O., P. Kaniewska, S. Alon, E. Eisenberg, S. Karako-Lampert, L. K. Bay, R. Reef, M. Rodriguez-Lanetty, D. J. Miller, and O. Hoegh-Guldberg. 2011. Complex diel cycles of gene expression in coral-algal symbiosis. *Science* **331:** 175.

Little, A. F., M. J. H. v. Oppen, and B. L. Willis. 2004. Flexibility in algal endosymbioses shapes growth in reef corals. *Science* **304:** 1492–1494.

Logan, D. D. K., A. C. LaFlamme, V. M. Weis, and S. K. Davy. 2010. Flow-cytometric characterization of the cell-surface glycans of symbiotic dinoflagellates (*Symbiodinium* spp.). *J. Phycol.* **46:** 525–533.

Luo, Y. J., L. H. Wang, W. N. U. Chen, S. E. Peng, J. T. C. Tzen, Y. Y. Hsiao, H. J. Huang, L. S. Fang, and C. S. Chen. 2009. Ratiometric imaging of gastrodermal lipid bodies in coral-dinoflagellate endosymbiosis. *Coral Reefs* **28:** 289–301.

Majai, G., G. Petrovski, and L. Fesus. 2006. Inflammation and the apopto-phagocytic system. *Immunol. Lett.* **104:** 94–101.

Marine Genomics Unit. 2011. *Acropora digitifera* Genome (Version 1.1). [Online]. Available: http://www.irp.oist.jp/satoh/index.php [2011, September 1].

Matsushita, M., S. Thiel, J. C. Jensenius, I. Terai, and T. Fujita. 2000. Proteolytic activities of two types of mannose-binding lectin-associated serine protease. *J. Immunol.* **165:** 2637.

Matz Lab. 2011. Coral transcriptomes. [Online]. Available: http://www.bio.utexas.edu/research/matz_lab/matzlab/Data.html [2011, September 1].

Mayfield, A. B., M. B. Hirst, and R. D. Gates. 2009. Gene expression normalization in a dual-compartment system: a real-time quantitative polymerase chain reaction protocol for symbiotic anthozoans. *Mol. Ecol. Resour.* **9:** 462–470.

McFall-Ngai, M., S. V. Nyholm, and M. G. Castillo. 2010. The role of the immune system in the initiation and persistence of the *Euprymna scolopes—Vibrio fischeri* symbiosis. *Semin. Immunol.* **22:** 48–53.

McGuinness, D. H., P. K. Dehal, and R. J. Pleass. 2003. Pattern recognition molecules and innate immunity to parasites. *Trends Parasitol.* **19:** 312–319.

Medzhitov, R. 2001. Toll-like receptors and innate immunity. *Nat. Rev. Immunol.* **1:** 135–145.

Meyer, E., G. Aglyamova, S. Wang, J. Buchanan-Carter, D. Abrego, J. Colbourne, B. Willis, and M. Matz. 2009. Sequencing and de novo analysis of a coral larval transcriptome using 454 GSFlx. *BMC Genomics* **10:** 219.

Meyer, E., G. V. Aglyamova, and M. V. Matz. 2011. Profiling gene expression responses of coral larvae (*Acropora millepora*) to elevated temperature and settlement inducers using a novel RNA-Seq procedure. *Mol. Ecol.* **20:** 3599–3616.

Miller, D. J., and D. Yellowlees. 1989. Inorganic nitrogen uptake by symbiotic marine cnidarians: a critical review. *Proc. R. Soc. Lond. B* **237:** 109–125.

Miller, D. J., E. E. Ball, and U. Technau. 2005. Cnidarians and ancestral genetic complexity in the animal kingdom. *Trends Genet.* **21:** 536–539.

Miller, D. J., G. Hemmrich, E. Ball, D. Hayward, K. Khalturin, N. Funayama, K. Agata, and T. Bosch. 2007. The innate immune repertoire in Cnidaria—ancestral complexity and stochastic gene loss. *Genome Biol.* **8:** R59.

Miller, D. J., E. E. Ball, S. Foret, and N. Satoh. 2011. Coral genomics and transcriptomics—ushering in a new era in coral biology. *J. Exp. Mar. Biol. Ecol.* **408:** 114–119.

Mortazavi, A., B. A. Williams, K. McCue, L. Schaeffer, and B. Wold. 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat. Methods* **5:** 621–628.

Muller-Parker, G., and C. F. D'Elia. 1997. Interaction between corals and their symbiotic algae. Pp. 96–113 in *Life and Death of Coral Reefs,* C. Birkeland, ed. Chapman Hall, New York.

Muscatine, L., R. D. Gates, and I. LaFontaine. 1994. Do symbiotic dinoflagellates secrete lipid droplets? *Limnol. Oceanogr.* **39:** 925–929.

Nakatogawa, H., Y. Ichimura, and Y. Ohsumi. 2007. Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. *Cell* **130:** 165–178.

Oberst, A., and D. R. Green. 2011. It cuts both ways: reconciling the dual roles of caspase 8 in cell death and survival. *Nat. Rev. Mol. Cell Biol.* **12:** 757–763.

Pagliari, L. J., T. Kuwana, C. Bonzon, D. D. Newmeyer, S. Tu, H. M. Beere, and D. R. Green. 2005. The multidomain proapoptotic molecules Bax and Bak are directly activated by heat. *Proc. Natl. Acad. Sci.* **102:** 17975–17980.

Park, E., D. S. Hwang, J. S. Lee, J. I. Song, T. K. Seo, and Y. J. Won. 2012. Estimation of divergence times in cnidarian evolution based on mitochondrial protein-coding genes and the fossil record. *Mol. Phylogenet. Evol.* **62:** 329–345.

Patton, J. S., and J. E. Burris. 1983. Lipid synthesis and extrusion by freshly isolated zooxanthellae (symbiotic algae). *Mar. Biol.* **75:** 131–136.

PdamBase. 2011. *Pocillopora damicornis* Transcriptomics Database. [Online]. Available: http://cnidarians.bu.edu/PdamBase/cgi-bin/pdamdata.cgi [2011, September 1].

Peng, S.-E., Y. J. Luo, H. J. Huang, I. T. Lee, L. S. Hou, W. N. Chen, L. S. Fang, and C.-S. Chen. 2008. Isolation of tissue layers in hermatypic corals by n-acetylcysteine: morphological and proteomic examinations. *Coral Reefs* **27:** 133–142.

Peng, S.-E., Y.-B. Wang, L.-H. Wang, W.-N. U. Chen, C.-Y. Lu, L.-S. Fang, and C.-S. Chen. 2010. Proteomic analysis of symbiosome membranes in cnidarian-dinoflagellate endosymbiosis. *Proteomics* **10:** 1002–1016.

Peng, S.-E., W.-N. U. Chen, H.-K. Chen, C.-Y. Lu, A. B. Mayfield, L.-S. Fang, and C.-S. Chen. 2011. Lipid bodies in coral–dinoflagellate endosymbiosis: proteomic and ultrastructural studies. *Proteomics* **11:** 3540–3555.

Perez, S., and V. Weis. 2006. Nitric oxide and cnidarian bleaching: an eviction notice mediates breakdown of a symbiosis. *J. Exp. Biol.* **209:** 2804–2810.

Pochon, X., and R. D. Gates. 2010. A new *Symbiodinium* clade (Dinophyceae) from soritid foraminifera in Hawai'i. *Mol. Phylogenet. Evol.* **56:** 492–497.

Polato, N. R., J. C. Vera, and I. B. Baums. 2011. Gene discovery in the threatened elkhorn coral: 454 sequencing of the *Acropora palmata* transcriptome. PLoS One 6:e28634.

Putnam, N. H., M. Srivastava, U. Hellsten, B. Dirks, J. Chapman, A. Salamov, A. Terry, H. Shapiro, E. Lindquist, V. V. Kapitonov, *et al.* 2007. Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science* **317:** 86–94.

Rakus, J. F., and L. K. Mahal. 2011. New technologies for glycomic analysis: toward a systematic understanding of the glycome. *Annu. Rev. Anal. Chem.* **4:** 367–392.

Rentzsch, F., J. H. Fritzenwanker, C. B. Scholz, and U. Technau. 2008. FGF signaling controls formation of the apical sensory organ in the cnidarian *Nematostella vectensis. Development* **135:** 1761–1769.

Reynolds, W. S., J. A. Schwarz, and V. M. Weis. 2000. Symbiosis-enhanced gene expression in cnidarian-algal associations: cloning and characterization of a cDNA, sym32, encoding a possible cell adhesion protein. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **126:** 33–44.

Richier, S., P. Furla, A. Plantivaux, P.-L. Merle, and D. Allemand. 2005. Symbiosis-induced adaptation to oxidative stress. *J. Exp. Biol.* **208:** 277–285.

Richier, S., M. Rodriguez-Lanetty, C. E. Schnitzler, and V. M. Weis. 2008. Response of the symbiotic cnidarian *Anthopleura elegantissima* transcriptome to temperature and UV increase. *Comp. Biochem. Physiol. D. Genomics Proteomics* **3:** 283–289.

Rodriguez-Lanetty, M., D. A. Krupp, and V. M. Weis. 2004. Distinct ITS types of *Symbiodinium* in clade C correlate with cnidarian/

dinoflagellate specificity during onset of symbiosis. *Mar. Ecol. Prog. Ser.* **275:** 97–102.

Rodriguez-Lanetty, M., W. Phillips, and V. Weis. 2006a. Transcriptome analysis of a cnidarian-dinoflagellate mutualism reveals complex modulation of host gene expression. *BMC Genomics* **7:** 23.

Rodriguez-Lanetty, M., E. Wood-Charlson, L. Hollingsworth, D. A. Krupp, and V. M. Weis. 2006b. Dynamics of infection and localization of dinoflagellate endosymbionts in larvae of the coral *Fungia scutaria* during the onset of symbiosis. *Mar. Biol.* **149:** 713–719.

Roth, M. S., M. I. Latz, R. Goericke, and D. D. Deheyn. 2010. Green fluorescent protein regulation in the coral *Acropora yongei* during photoacclimation. *J. Exp. Biol.* **213:** 3644.

Rowan, R. 2004. Coral bleaching: thermal adaptation in reef coral symbionts. *Nature* **430:** 742.

Royet, J., D. Gupta, and R. Dziarski. 2011. Peptidoglycan recognition proteins: modulators of the microbiome and inflammation. *Nat. Rev. Immunol.* **11:** 837–851.

Sabourault, C., P. Ganot, E. Deleury, D. Allemand, and P. Furla. 2009. Comprehensive EST analysis of the symbiotic sea anemone, *Anemonia viridis. BMC Genomics* **10:** 333.

Sacks, D., and A. Sher. 2002. Evasion of innate immunity by parasitic protozoa. *Nat. Immunol.* **3:** 1041–1047.

Salih, A., A. Larkum, G. Cox, M. Kuhl, and O. Hoegh-Guldberg. 2000. Fluorescent pigments in corals are photoprotective. *Nature* **408:** 850–853.

Schmid, M. W., A. Schmidt, U. C. Klostermeier, M. Barann, P. Rosenstiel, and U. Grossniklaus. 2012. A powerful method for transcriptional profiling of specific cell types in eukaryotes: laser-assisted microdissection and RNA sequencing. *PloS One* **7:** e29685.

Schnitzler, C. E. 2010. Temperature stress, gene expression, and innate immunity at the onset of cnidarin-dinoflagellate symbiosis. Ph.D. dissertation, Oregon State University.

Schnitzler, C. E., and V. M. Weis. 2010. Coral larvae exhibit few measurable transcriptional changes during the onset of coral-dinoflagellate endosymbiosis. *Mar. Genomics* **3:** 107–116.

Schwanhausser, B., D. Busse, N. Li, G. Dittmar, J. Schuchhardt, J. Wolf, W. Chen, and M. Selbach. 2011. Global quantification of mammalian gene expression control. Nature **473:** 337–342.

Schwarz, J. A., and V. M. Weis. 2003. Localization of a symbiosis-related protein, Sym32, in the *Anthopleura elegantissima-Symbiodinium muscatinei* association. *Biol. Bull.* **205:** 339–350.

Schwarz, J. A., P. Brokstein, C. Voolstra, A. Terry, D. Miller, A. Szmant, M. Coffroth, and M. Medina. 2008. Coral life history and symbiosis: functional genomic resources for two reef building Caribbean corals, *Acropora palmata* and *Montastraea faveolata. BMC genomics* **9:** 97.

Segal, A. W. 2005. How neutrophils kill microbes. *Annu. Rev. Immunol.* **23:** 197.

Shan, G. 2010. RNA interference as a gene knockdown technique. *Int. J. Biochem. Cell Biol.* **42:** 1243–1251.

Shinzato, C., E. Shoguchi, T. Kawashima, M. Hamada, K. Hisata, M. Tanaka, M. Fujie, M. Fujiwara, R. Koyanagi, T. Ikuta, A. Fujiyama, D. J. Miller, and N. Satoh. 2011. Using the *Acropora digitifera* genome to understand coral responses to environmental change. *Nature* **476:** 320–323.

Simmons, K. J., P. N. Nde, Y. Y. Kleshchenko, M. F. Lima, and F. Villalta. 2006. Stable RNA interference of host thrombospondin-1 blocks *Trypanosoma cruzi* infection. *FEBS Lett.* **580:** 2365.

Spiegel, S., and S. Milstien. 2011. The outs and the ins of sphingosine-*1*-phosphate in immunity. *Nat. Rev. Immunol.* **11:** 403–415.

Starcevic, A., W. C. Dunlap, J. Cullum, J. M. Shick, D. Hranueli, and P. F. Long. 2010. Gene expression in the scleractinian *Acropora microphthalma* exposed to high solar irradiance reveals elements of photoprotection and coral bleaching. *PloS One* **5:** e13975.

Stat, M., and R. D. Gates. 2011. Clade D *Symbiodinium* in scleractinian corals: a "nugget" of hope, a selfish opportunist, an ominous sign, or all of the above? *J. Mar. Biol.* **2011:** e730715.

Stat, M., E. Morris, and R. D. Gates. 2008. Functional diversity in coral-dinoflagellate symbiosis. *Proc. Natl. Acad. Sci.* **105:** 9256–9261.

Stochaj, W. R., and A. R. Grossman. 1997. Differences in the protein profiles of cultured and endosymbiotic *Symbiodinium* sp. (Pyrrophyta) from the anemone *Aiptasia pallida* (Anthozoa). *J. Phycol.* **33:** 44–53.

Sunagawa, S., E. Wilson, M. Thaler, M. Smith, C. Caruso, J. Pringle, V. Weis, M. Medina, and J. Schwarz. 2009. Generation and analysis of transcriptomic resources for a model system on the rise: the sea anemone *Aiptasia pallida* and its dinoflagellate endosymbiont. *BMC Genomics* **10:** 258.

SymBioSys. 2011. *Montastraea faveolata* transcriptome. [Online]. Available: http://sequoia.ucmerced.edu/SymBioSys/index.php [2011, September 1].

Tang, F., C. Barbacioru, Y. Wang, E. Nordman, C. Lee, N. Xu, X. Wang, J. Bodeau, B. B. Tuch, and A. Siddiqui. 2009. mRNA-Seq whole-transcriptome analysis of a single cell. *Nat. Methods* **6:** 377–382.

Tchernov, D., M. Y. Gorbunov, C. de Vargas, S. Narayan Yadav, A. J. Milligan, M. Haggblom, and P. G. Falkowski. 2004. Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proc. Natl. Acad. Sci.* **101:** 13531–13535.

Technau, U., S. Rudd, P. Maxwell, P. M. K. Gordon, M. Saina, L. C. Grasso, D. C. Hayward, C. W. Sensen, R. Saint, and T. W. Holstein. 2005. Maintenance of ancestral complexity and non-metazoan genes in two basal cnidarians. *Trends Genet.* **21:** 633–639.

Ting, J. P. Y., and B. K. Davis. 2005. CATERPILLER: a novel gene family important in immunity, cell death, and diseases. *Annu. Rev. Immunol.* **23:** 387–414.

Traylor-Knowles, N., B. R. Granger, T. Lubinski, J. R. Parikh, S. Garamszegi, Y. Xia, J. A. Marto, L. Kaufman, and J. R. Finnerty. 2011. Production of a reference transcriptome and a transcriptomic database (PocilloporaBase) for the cauliflower coral, *Pocillopora damicornis. BMC Genomics* **12:** 585.

Uehlein, N., C. Lovisolo, F. Siefritz, and R. Kaldenhoff. 2003. The tobacco aquaporin NtAQP 1 is a membrane CO_2 pore with physiological functions. *Nature* **425:** 734–737.

van Oppen, M. J., and R. D. Gates. 2006. Conservation genetics and the resilience of reef-building corals. *Mol. Ecol.* **15:** 3863–3883.

Voolstra, C. R., J. A. Schwarz, J. Schnetzer, S. Sunagawa, M. K. Desalvo, A. M. Szmant, M. A. Coffroth, and M. Medina. 2009a. The host transcriptome remains unaltered during the establishment of coral–algal symbioses. *Mol. Ecol.* **18:** 1823–1833.

Voolstra, C. R., S. Sunagawa, J. A. Schwarz, M. A. Coffroth, D. Yellowlees, W. Leggat, and M. Medina. 2009b. Evolutionary analysis of orthologous cDNA sequences from cultured and symbiotic dinoflagellate symbionts of reef-building corals (Dinophyceae: Symbiodinium). *Comp. Biochem. Physiol D Genomics Proteomics* **4:** 67–74.

Voolstra, C. R., S. Sunagawa, M. V. Matz, T. Bayer, M. Aranda, E. Buschiazzo, M. K. DeSalvo, E. Lindquist, A. M. Szmant, and M. A. Coffroth. 2011. Rapid evolution of coral proteins responsible for interaction with the environment. *PloS One* **6:** e20392.

Waghabi, M. C., M. Keramidas, J. J. Feige, T. C. Araujo-Jorge, and S. Bailly. 2005. Activation of transforming growth factor beta by *Trypanosoma cruzi. Cell. Microbiol.* **7:** 511–517.

Wakefield, T. S., and S. C. Kempf. 2001. Development of host- and symbiont-specific monoclonal antibodies and confirmation of the origin of the symbiosome membrane in a cnidarian-dinoflagellate symbiosis. *Biol. Bull.* **200:** 127–143.

Wallin, R., S. M. Hutson, D. Cain, A. Sweatt, and D. C. Sane. 2001. A molecular mechanism for genetic warfarin resistance in the rat. *FASEB J.* **15:** 2542.

Wang, L.-H., Y.-H. Liu, Y.-M. Ju, Y.-Y. Hsiao, L.-S. Fang, and C.-S. Chen. 2008. Cell cycle propagation is driven by light-dark stimulation in a cultured symbiotic dinoflagellate isolated from corals. *Coral Reefs* **27:** 823–835.

Warner, M. E., W. K. Fitt, and G. W. Schmidt. 1999. Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. *Proc. Natl. Acad. Sci.* **96:** 8007–8012.

Weis, V. M. 2008. Cellular mechanisms of cnidarian bleaching: stress causes the collapse of symbiosis. *J. Exp. Biol.* **211:** 3059–3066.

Weis, V. M., and D. Allemand. 2009. What determines coral health? *Science* **324:** 1153–1155.

Weis, V. M., and R. P. Levine. 1996. Differential protein profiles reflect the different lifestyles of symbiotic and aposymbiotic *Anthopleura elegantissima,* a sea anemone from temperate waters. *J. Exp. Biol.* **199:** 883–892.

Weis, V. M., G. J. Smith, and L. Muscatine. 1989. A "CO_2 supply" mechanism in zooxanthellate cnidarians: role of carbonic anhydrase. *Mar. Biol.* **100:** 195–202.

Weis, V. M., W. S. Reynolds, M. D. deBoer, and D. A. Krupp. 2001. Host-symbiont specificity during onset of symbiosis between the dinoflagellate *Symbiodinium* spp. and the planula larvae of the scleractinian coral *Fungia scutaria. Coral Reefs* **20:** 301–308.

Weis, V. M., S. K. Davy, O. Hoegh-Guldberg, M. Rodriguez-Lanetty, and J. R. Pringle. 2008. Cell biology in model systems as the key to understanding corals. *Trends Ecol. Evol.* **23:** 369–376.

Weis, W. I., M. E. Taylor, and K. Drickamer. 1998. The C-type lectin superfamily in the immune system. *Immunol. Rev.* **163:** 19–34.

Welte, M. A. 2007. Proteins under new management: lipid droplets deliver. *Trends Cell Biol.* **17:** 363–369.

Wood-Charlson, E. M., and V. M. Weis. 2009. The diversity of C-type lectins in the genome of a basal metazoan, *Nematostella vectensis. Dev. Comp. Immunol.* **33:** 881–889.

Wood-Charlson, E. M., L. L. Hollingsworth, D. A. Krupp, and V. M. Weis. 2006. Lectin/glycan interactions play a role in recognition in a coral/dinoflagellate symbiosis. *Cell. Microbiol.* **8:** 1985–1993.

Wu, H., and J. R. Arron. 2003. TRAF6, a molecular bridge spanning adaptive immunity, innate immunity and osteoimmunology. *Bioessays* **25:** 1096–1105.

Xie, Z., and D. J. Klionsky. 2007. Autophagosome formation: core machinery and adaptations. *Nat. Cell Biol.* **9:** 1102–1109.

Yellowlees, D., T. A. V. Rees, and W. Leggat. 2008. Metabolic interactions between algal symbionts and invertebrate hosts. *Plant Cell Environ.* **31:** 679–694.

Yuyama, I., H. Hayakawa, H. Endo, K. Iwao, H. Takeyama, T. Maruyama, and T. Watanabe. 2005. Identification of symbiotically expressed coral mRNAs using a model infection system. *Biochem. Biophys. Res. Commun.* **336:** 793–798.

Yuyama, I., T. Watanabe, and Y. Takei. 2011. Profiling differential gene expression of symbiotic and aposymbiotic corals using a high coverage gene expression profiling (HiCEP) analysis. *Mar. Bioltechnol.* **13:** 32–40.

Zdobnov, E. M., and R. Apweiler. 2001. InterProScan—an integration platform for the signature-recognition methods in InterPro. *Bioinformatics* **17:** 847.