Integrative and Comparative Biology

Integrative and Comparative Biology, volume 59, number 4, pp. 819–829
doi:10.1093/icb/icz111

Society for Integrative and Comparative Biology

SYMPOSIUM

Responding to Threats Both Foreign and Domestic: NOD-Like Receptors in Corals

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From the symposium "Chemical responses to the biotic and abiotic environment by early diverging metazoans revealed in the post-genomic age" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2019 at Tampa, Florida.

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Synopsis Historically mechanisms with which basal animals such as reef-building corals use to respond to changing and increasingly stressful environments have remained elusive. However, the increasing availability of genomic and transcriptomic data from these organisms has provided fundamental insights into the biology of these critically important ecosystem engineers. Notably, insights into cnidarians gained in the post-genomics age have revealed a surprisingly complex immune system which bears a surprising level of similarity with the vertebrate innate immune system. This system has been critically linked to how corals respond to the two most prominent threats on a global scale, emerging coral diseases and increasing water temperature, which are recognized cellularly as either foreign or domestic threats, respectively. These threats can arise from pathogenic microbes or internal cellular dysfunction, underscoring the need to further understand mechanisms corals use to sense and respond to threats to their cellular integrity. In this investigation and meta-analysis, we utilize resources only recently available in the post-genomic era to identify and characterize members of an underexplored class of molecules known as NOD-like receptors in the endangered Caribbean coral *Orbicella faveolata*. We then leverage these data to identify pathways possibly mediated by NLRs in both *O. faveolata* and the ecologically important branching coral *Acropora digitifera*. Overall, we find support that this class of proteins may provide a mechanistic link to how reef-building corals respond to threats both foreign and domestic.

Introduction

Coral immunity in disease and bleaching

Coral reefs are some of the most biodiverse ecosystems on the planet however, coral reefs are currently in decline as a result of increasing disease prevalence and water temperature (Hughes et al. 2017). Coral diseases involve colonization by potentially pathogenic microbes (Frias-Lopez et al. 2003; Beurmann et al. 2018) and subsequent host pathology, whereas increased water temperature causes cellular dysfunction that can cause the coral to lose its algal symbiont and main source of nutrition in a process termed coral bleaching (Gates et al. 1992; Nielsen et al. 2018). These two stressors therefore represent different points of origin where pathogenic microbes represent an external or foreign threat (Ben-Haim 2003), while elevated temperature causes dysfunctions inside the coral perceived as a domestic threat (Tchernov et al. 2011). Pathogenic bacteria lead to upregulation of components of the coral immune system in an attempt to fight off the foreign invader (Wright et al. 2017) and either regain homeostasis or induce cell death (Fuess et al. 2017). A similar insult to cellular integrity can occur during temperature stress which leads to accumulation of reactive oxygen species (ROS) (Lesser 1996, 2004) and disruption in calcium homeostasis (Fang et al. 1997; Desalvo et al. 2008) that can lead to activation of stress-signaling pathways including apoptosis (Dunn et al. 2007). While our understanding of coral immunity and stress responses has progressed (see reviews by Mydlarz et al. 2016; Palmer and Traylor-Knowles

Advance Access publication June 24, 2019

2018), there are certainly many opportunities to expand our knowledge about specific pathways and how they are affected by pathogen pressure and temperature stress.

In order to properly defend itself against a pathogen, the coral host must be able to recognize a pathogen, signal for a response and execute effector responses (Palmer and Traylor-Knowles 2018). Pattern recognition receptors (PRRs) are essential for pathogen recognition and responding to cellular stress (Pasquier 2006; Tetreau et al. 2017) by recognizing and binding conserved molecular motifs, and inducing subsequent immune responses (Akira et al. 2006). The two primary classes of molecules sensed by PRRs are microbial-associated molecular patterns (MAMPs) (Hargreaves and Medzhitov 2005), and danger associated molecular patterns (DAMPs)(Rosin and Okusa 2011; Newton and Dixit 2012), which can be viewed as foreign and domestic danger signals to the host organism, respectively. PRR signaling pathways consist of a number of intermediate molecules which promote the necessary changes in gene expression and protein activity to generate an effective defense response against potential pathogens (Williams et al. 2018) including production of antimicrobial compounds and the activation of phagocytic cells (Underhill and Ozinsky 2002).

Recently, advances in our understanding of different immune PRRs including the membrane bound Toll-like receptors (TLRs) have shown remarkable homology between coral TLRs (Miller et al. 2007; Shinzato et al. 2011; Wolenski et al. 2011; Poole and Weis 2014) and vertebrate TLRs in their ability to detect threats to cellular integrity in the form of MAMPs (Williams et al. 2018). While TLRs are readily known to be important in corals' responses to extracellular microbes, the cytoplasmic NOD-like receptors (NLRs) have received considerably less attention. NLRs are PRRs which serve as intra-cellular sentinels (Fritz et al. 2006; Rosenstiel et al. 2009) that are capable of detecting both MAMPs such as bacteria and virulence factors (Inohara and Nuñez 2001; Hsu et al. 2008), as well as DAMPs including accumulation of ROS (Bauernfeind et al. 2011; Sutterwala et al. 2014; Liu et al. 2018) and detection of cytoplasmic calcium (Lee et al. 2012), allowing them to respond to threats arising from both foreign and domestic sources. The mechanisms of how biotic and abiotic stress signals are translated into effector responses such as apoptosis, or phenotypes such as symbiont loss and bleaching are still elusive, making NLRs great targets for investigation (Box 1).

Advancements of next generation technology have led to rapid increases in our understanding of coral genomics and transcriptomics. The numerous datasets available to researchers have allowed for novel analyses and development of cellular models. We are now able to take advantage of numerous bioinformatic resources to characterize an underexplored class of receptors in cnidarians known as NLRs as well their possible protein interactions. Our goals of this review and meta-analysis are to investigate the composition of the NLR repertoire of the endangered Caribbean reef-building coral *Orbicella faveolata* and the potential NLR interactome of both our identified *O. faveolata* and previously identified *Acropora digitifera* NLRs (Hamada et al. 2013). Here, we show that the NLR repertoire of both *O. faveolata* and *A. digitifera* possess the molecular machinery to respond to cellular threats both foreign and domestic and may potentially mediate the response of these organisms to both biotic and abiotic stressors.

NLR biology

The intracellular-localized NLRs are a critical portion of the innate immune system (Elinav et al. 2011) and therefore likely evolved as a way to protect an organism from threats both foreign and domestic, because of the ability of these proteins to sense a wide range of both externally derived (MAMPs), and endogenously produced threats (DAMPS) within the cytoplasm (Latz et al. 2013). Initially NLRs were thought to have arisen in teleost fish as model invertebrates such as *Caenorhabditis elegans* and *Drosophila melanogaster* do not possess homologs of NLRs (Ting and Williams 2005; Rosenstiel et al. 2008), this idea was challenged when the purple sea urchin (*Strongylocentrotus purpuratus*) genome revealed a large number of NOD-like proteins (Rast et al. 2006). The evolutionarily basal state of NLRs was further confirmed as NLR homologs were found in early diverging animals like cnidarians (Lange et al. 2011; Hamada et al. 2013). Surprisingly NLRs are not just present in early animals but may actually be expanded compared with their mammalian counterparts, as *A. digitifera* possesses roughly 500 NLR like proteins (Hamada et al. 2013), and the purple sea urchin possesses more than 200 (Rast et al. 2006) compared to the 22 in humans (Sutterwala et al. 2014).

NLRs typically have a tripartite architecture containing: an amino terminus effector domain, a central NACHT/nucleotide binding domain (NBD), and a carboxy terminal repeat region comprised of

Box 1 Role of NLRs in coral–algal symbiosis

As a holobiont, corals must maintain balance between the presence of symbiotic microbes, pathogens, and immunity. In particular, the intracellular symbiosis formed with dinoflagellate algae of the family Symbiodiniaceae is important as these algae provide the coral host with up to 90% of their energy requirements (Mies et al. 2017). These symbiotic dinoflagellates reside within the gastrodermal layer of coral host tissue in an arrested phagosome (termed the symbiosome) (Barott et al. 2015). Immune evasion or suppression by Symbiodiniaceae is a required component of the establishment and maintenance of symbiosis. Due to the intimate association between corals and their microbial symbionts, mechanisms such as NLRs which can both sense molecular patterns and regulate immunity may in be key in facilitating this delicate relationship.

The onset of symbiosis begins with the phagocytosis of the Symbiodiniaceae cell. A critical step is the arrest of phagosome–lysosome fusion (Davy et al. 2012), which prevents digestion of the dinoflagellate cell and allows the phagosome to mature into a symbiosome. Currently, the molecules mediating this arrest remain unknown. However, in mice the interaction of two NLRs (NLRC4 and NAIP5) is necessary for phago–lysosomal fusion, and activation of NLRC4 occurs in response to flagellin (Lage et al. 2014). Interestingly, Symbiodiniaceae undergo a morphological shift when entering a symbiotic state, losing its heterokont flagella and transforming into a coccoid cell (Muscatine et al. 1975). Interestingly, *in situ* hybridization has shown that NLR expression in *Hydra* is limited to the gastrodermal layer (Lange et al. 2011) and future investigations should explore if this expression pattern is conserved as well in symbiotic cnidarians, which may highlight a possible mechanism through which PRRs may play a role in facilitating symbiosis.

NLRs are intracellular monitors for the presence of MAMPS and DAMPs, mediating the detection of cellular perturbations that occur during biotic and abiotic stress. If NLRs function in symbiosis as well as damage detection, they may play an integral role in the breakdown of symbiosis under environmental stress. Cellular stress presenting directly as DAMPs like ROS can activate NLRs. In addition, indirect mechanisms of damage can also activate NLRs, where NLRs are bound to resident proteins and activated upon the detection of a conformational change (Jones et al. 2016). Such conformational changes in NLR-bound proteins can occur during changes in cellular redox state as well as alterations in metabolism (Zhou et al. 2010). Well-known roles of Symbiodiniaceae include translocating photosynthetically-derived nutrients and suppressing immunity to "colonize" hosts, supporting the fact that these indirect NLR activation mechanisms may be at play in coral. Further, during stress the cellular redox state is altered as is the flux of photosynthetically derived nutrients; both of which can induce direct and/or off-target effects. In this way NLR activation may be a mechanism that triggers immune activation and symbiont expulsion during stress conditions.

leucine rich repeats (Ting et al. 2008). The amino effector terminus of NLRs usually contains a death domain (DD) fold (Koonin and Aravind 2000) which allows these molecules to directly translate ligand recognition into effector responses through their ability recruit and activate caspases (Martinon et al. 2002; Duncan and Canna 2018). In mammals, the caspase activating effector domains usually comprise either a caspase recruitment domain (CARD), or a Pyrin domain (Inohara and Nuñez 2003), with homotypic interactions (Kersse et al. 2011) occurring between the CARD domain of caspases and CARD containing NLRs directly, or via an adaptor molecule for Pyrin-containing NLR proteins (Vajjhala et al. 2012). The domain which facilities the ligand-detection ability of NLRs is not fully elucidated (Wilmanski et al. 2008), however ligand detection is thought to induce a conformation change, which relieves the auto-inhibitory effects of the C-terminal repeats (Latz et al. 2013). This class of proteins are able to detect a diverse array of signals (Horvath et al. 2011), including lipopolysaccharide (LPS) (Mariathasan et al. 2006), ROS (Martinon et al. 2009), and organelle calcium efflux (Lee et al. 2012) in addition to other signatures of cellular stress (Dostert et al. 2008; Masters et al. 2010; Zhou et al. 2010). The activation of NLRs can be a complex process which can involve both direct as well as indirect mechanisms (Sutterwala et al. 2014). As an example, the detection of Gram-negative LPS by NLRs can occur via recognition through the C-terminal LRRs, though this response can be amplified if other signaling pathways such as TLRs are also concurrently activated (He et al. 2016). Likewise, the ability of these proteins to be activated by ROS can be accomplished directly, as ROS accumulation is essential to increase transcription of some NLRs (Bauernfeind et al. 2011), or indirectly via the redox-sensitive thioredoxin-interacting protein which binds and activates some NLRs (Zhou et al. 2010). These mechanisms demonstrate that in addition to direct ligand binding, indirect sensing mechanisms including protein–protein interactions between NLRs as well as other proteins are (Ferwerda et al. 2008; Hsu et al. 2008; Horvath et al. 2011) important in activation. Thus, NLRs

act as cellular funnels to activate stress responses, in response to a diversity of cellular insults. Interestingly, compared with the well-characterized vertebrate NLRs, cnidarian NLR repertoires are expanded and contain novel domain combinations, both at the amino and carboxy terminus (Hamada et al. 2013; van der Burg et al. 2016).

Regulation of NLR activity occurs at two levels: priming (Bauernfeind et al. 2009) and activation (Miao et al. 2011), which consists of increasing transcription of NLR genes and ligand binding of NLRs, respectively. The relative levels of priming and activation have important phenotypic effects (Lech et al. 2010) as this can dictate whether immune activation and resolution, or cell death occurs (Latz et al. 2013). As the activating agents of NLRs are important in the response of reef-building corals to environmental stress this class of molecules is worth further investigation into their possible role to facilitate or impeded adaptation to increasingly stressful environmental conditions experienced by reef-building corals.

Coral NLR repertoire

We investigated the proteome of *O. faveolata* for proteins which contain NACHT/NBD domains, and identified 46 putative NLR-like proteins (Fig. 1) with the protein domain identification program HMMR (Eddy 2011) using the NBD/NACHT consensus sequence obtained from the protein family (PFAM) database (El-Gebali et al. 2019). Canonical NLRs possess a central NBD domain with C-terminal LRRs (Ting et al. 2008) however, as previous reports indicate that NLRs in cnidarians have novel domain combinations (van der Burg et al. 2016) we considered all NBD containing proteins as putative NLRs for this analysis. As our results revealed significantly less NLRs than identified in the reef-building coral *A. digitifera* (Hamada et al. 2013), we performed a subsequent analysis on the *A. digitifera* proteome which yielded similar results as Hamada et al. (2013) confirming our analysis approach. We then investigated the domain composition of the identified *O. faveolata* NLRs by analyzing the peptide sequences with the online PFAM database (El-Gebali et al. 2019). This analysis identified novel domain combinations in the *O. faveolata* NLR repertoire at both termini which reflect the findings in other cnidarians (Hamada et al. 2013; van der Burg et al. 2016). Among the novel domain combinations, we identified NLR-like proteins with N-terminus death effector domains (DED), glycosyl-transferase domains, and HEPN domains (Fig. 1). DEDs belong to the DD fold protein super family as do CARD and pyrin domains seen on the amino-terminus of mammalian NLRs. Interestingly, in contrast to *A. digitifera* we were unable to identify any *O. faveolata* NLRs with N-terminal DDs, which belong to the same protein superfamily as DEDs. Additionally, several of the effector domains in the *O. faveolata* NLR repertoire are associated with immunity, including glycosyl-transferase (Ohtsubo and Marth 2006) and HEPN domains which have been implicated in antiviral responses (Anantharaman et al. 2013). In addition, we also see novel C-terminal repeat domains in *O. faveolata*, including ankyrin, WD40, and tetratricopeptide repeats (Fig. 1).

To support the role of the above proteins during cellular stress conditions we investigated the expression patterns of NLRs in two existing *O. faveolata* transcriptomic datasets, one during treatment with the bacterial MAMP LPS (Fuess et al. 2017) and one during a natural bleaching event (Pinzon et al. 2015). We found that six NLRs were significantly upregulated in LPS treated colonies of *O. faveolata* and three NLRs were upregulated in bleached versus unbleached *O. faveolata* colonies (Supplementary Table S1). Overall, it appears that *O. faveolata* contains NLRs which appear to function in immunity and cellular stress consistent with previous reports of the function of NLRs in cnidarians (Lange et al. 2011; Hamada et al. 2013; van der Burg et al. 2016). These conclusions corroborate a finding by Libro et al. (2016) who determined that increased expression of NLRs confer protection from white band disease in *A. cervicornis*. As NLR effector responses are predicated upon interactions with other proteins and members of the DD-fold superfamily function through homotypic interactions (Valmiki and Ramos 2009), we investigated the potential interaction partners for the DED-containing NLRs of *O. faveolata* as well as the possible interaction partners of the previously identified DED, DD, and CARD-containing NLRs of *A. digitifera* (Hamada et al. 2013).

Coral NLR reactomes

By using the protein domain identification program hmmr (Eddy 2011), we identified 43 proteins in the *O. faveolata* proteome which match the DED consensus sequence obtained from PFAM (El-Gebali et al. 2019) which may serve as potential NLR interaction partners (Fig. 2 and Supplementary Table S2). The identified proteins are involved in several biological processes including: extrinsic apoptosis, immune signaling, and inhibition of intrinsic apoptosis (Fig. 2) indicating that *O. faveolata* NLRs appear capable of

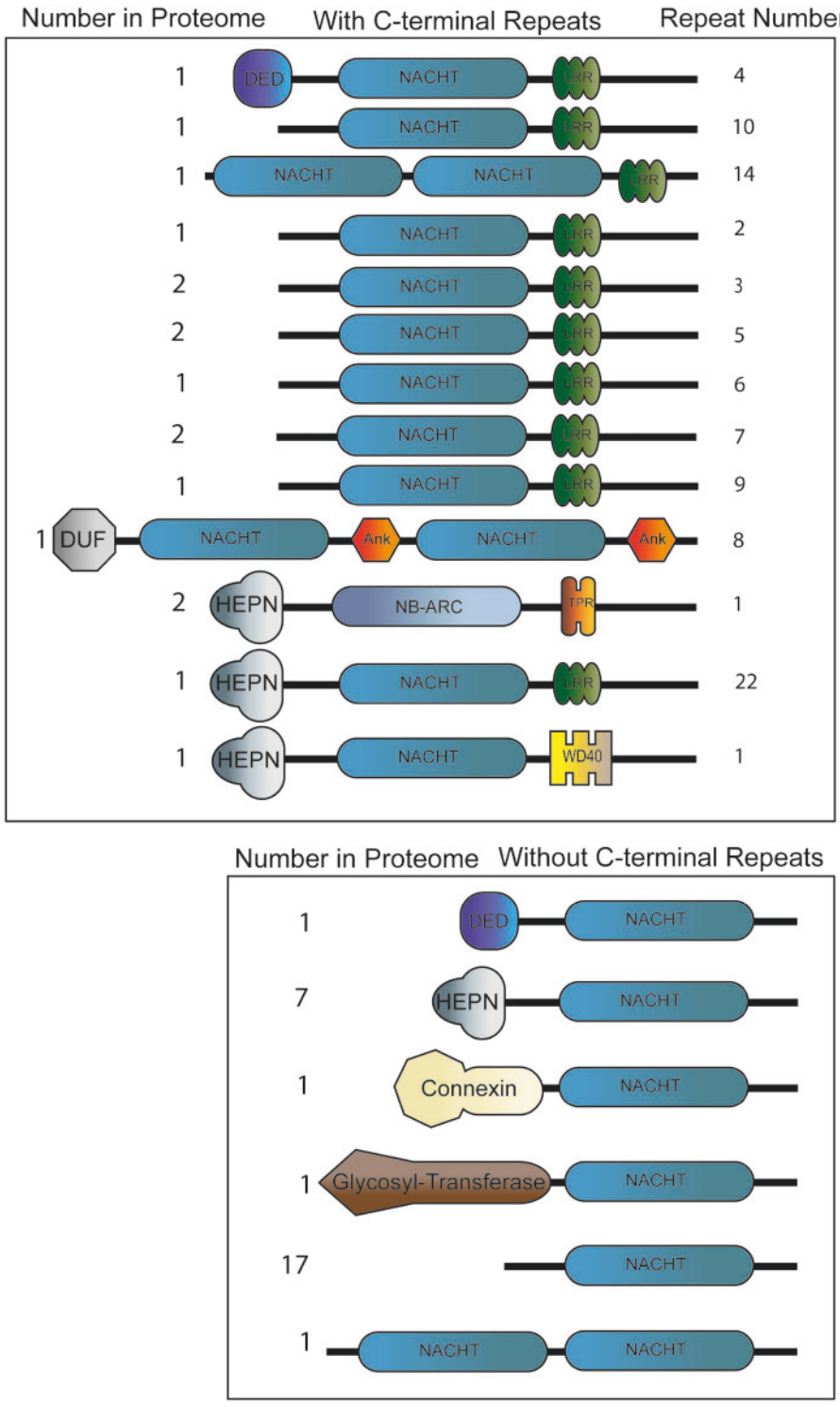

Fig. 1 *O. faveolata*'s NLR repertoire. Forty-six NLR containing proteins identified in the proteome of *O. faveolata*, 18 of which contain variable numbers of C-terminal repeats, and 28 of which do not contain repeats. The N-terminal domain composition of these proteins is variable containing DED (death effector domains), DUF (domain of unknown function), HEPN (higher eukaryotes and prokaryotes nucleotide-binding domain), Connexin, and glycosyl-transferase domains.

playing a role in modulation of both apoptosis and immunity. The *A. digitifera* NLR repertoire is expanded compared with that of *O. faveolata* and likewise has additional N-terminal effector domains including DED, DD, and CARD containing NLRs (Hamada et al. 2013). We used the same methodology to find interaction partners for the expanded domain composition found on *A. digitifera* NLRs

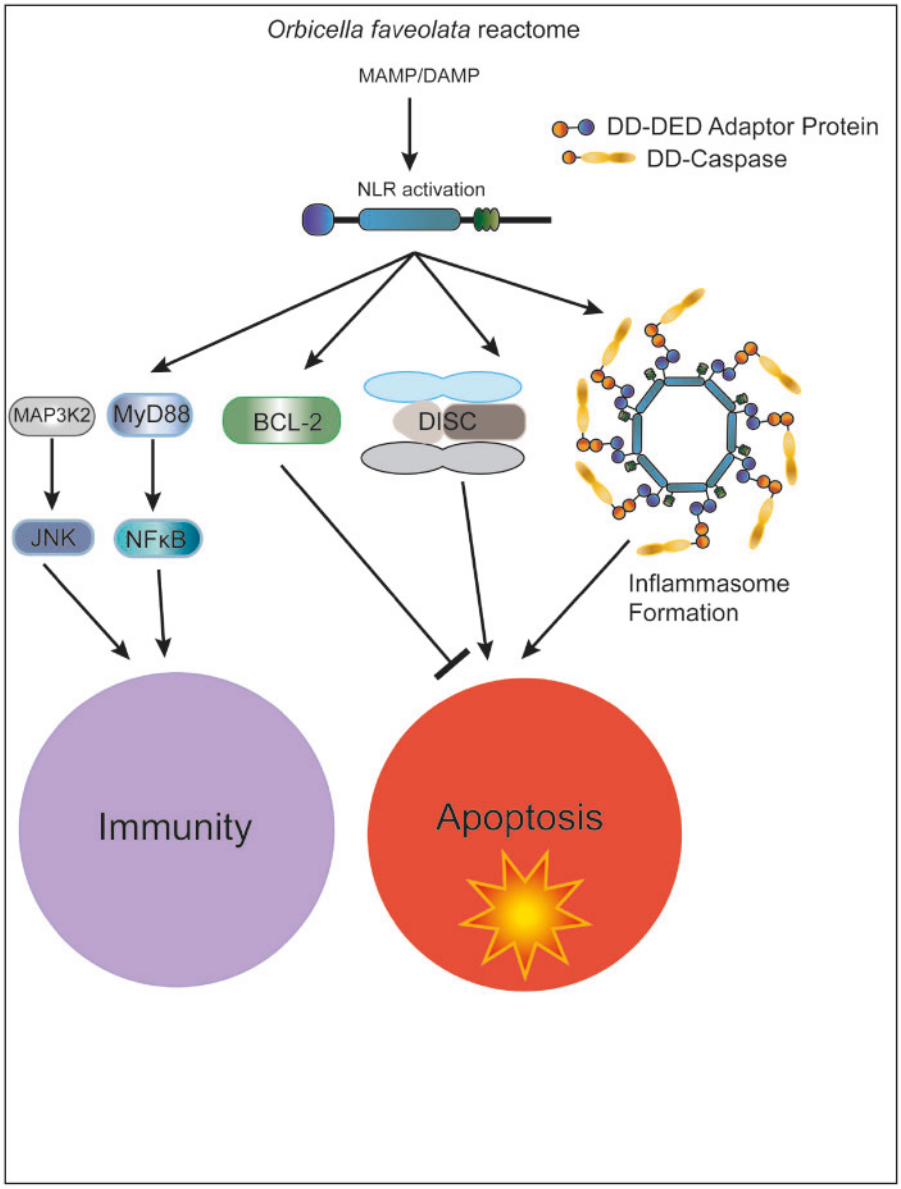

Fig. 2 The reactome of *O. faveolata* NLR proteins: *O. faveolata* contains potential NLR interaction partners involved in apoptosis, and immunity. Four key components of the death-induced signaling complex (DISC) were identified as NLR interaction partners including: Caspase-8, FADD, CFLAR, and Caspase 10 (Davy et al. 2012; Ranjan and Pathak 2016). The DISC which serves to induce apoptosis and has previously been suggested to involve interactions with NLRs (Salvesen and Riedl 2009). Two of the identified NLR interaction partners involved in immune signaling include mitogen activated kinase kinase kinase 2 (MAP3K2) and myeloid differentiation primary response protein (MyD88). MAP3K2 interacts with c-Jun kinase (JNK) (Cheng et al. 2000) which is involved in activation of various immune responses (Huang et al. 2009). MyD88 acts via a signaling pathway leading to the dissociation of the inhibitory Iκb complex from NFκb (Israel 2010) allowing the two subunits of NFκb to translocate into the nucleus and exert their transcriptional activities (Kawai and Akira 2007). Additionally, B-cell lymphoma 2 (BCL-2) was identified as a possible NLR binding partner which can inhibit intrinsic apoptosis through preventing Bak–Bax dependent cytochrome C release (Yang et al. 1997), in support of this interaction other systems have shown BCL-2 to inhibit NLRs (Bruey et al. 2007). An *O. faveolata* DD-DED adaptor protein was identified which may allow DED-containing NLRs to interact with *O. faveolata*'s DD-containing caspases 2 and 3 and possibly function to form an inflammasome.

(Supplementary Table S3). Similar to *O. faveolata* the *A. digitifera* DED-interaction partners involve components of extrinsic apoptosis (Fig. 3). The predicted DD-containing *A. digitifera* NLR interaction partners involve known components of the coral immune system, and apoptotic machinery including the APAF-1 apoptosome which mediates intrinsic apoptosis through activation of executioner caspases (Fig. 3). *Acropora digitifera* CARD containing-NLR appear able to interact with additional apoptotic-activating

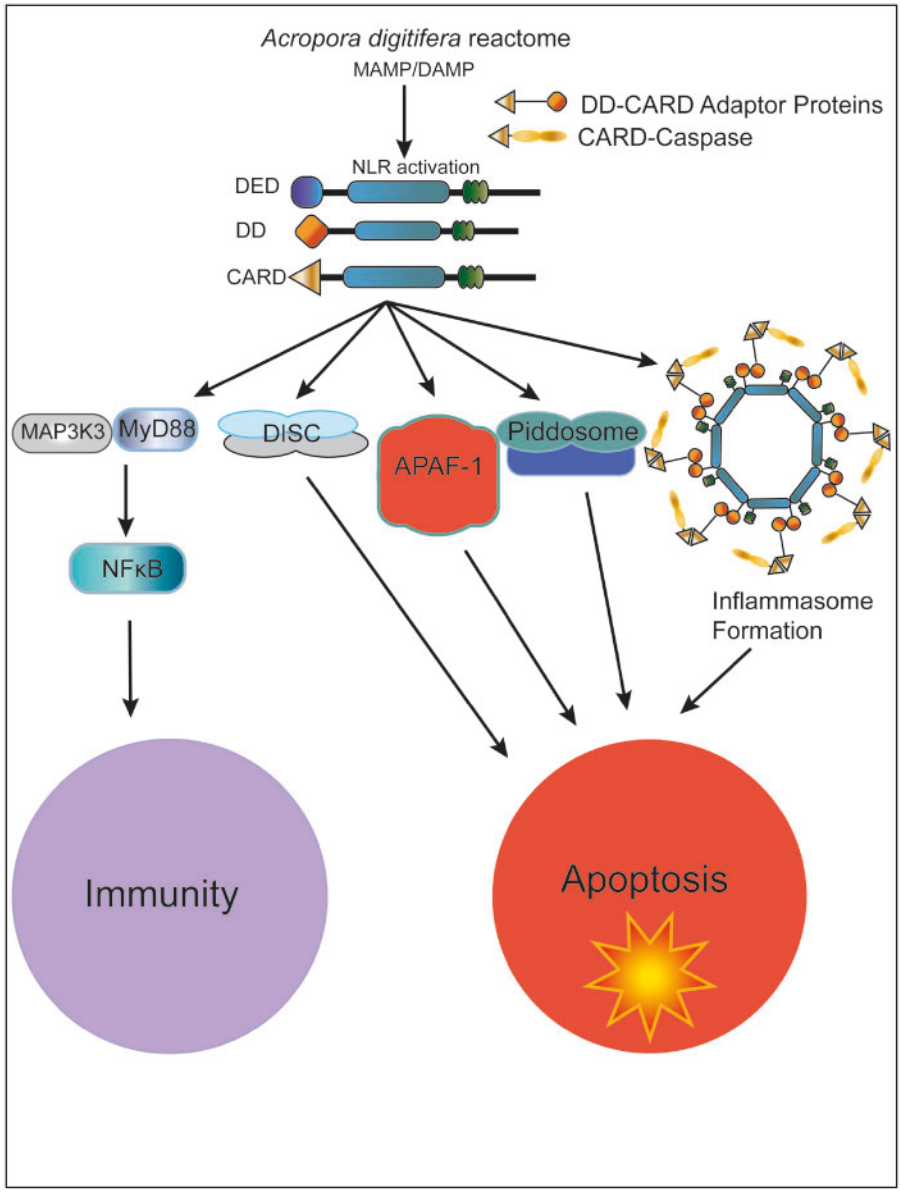

Fig. 3 The reactome of *A. digitifera* NLR proteins: *A. digitifera* DED-interaction partners involve components of extrinsic apoptosis DISC including Caspase-8 and Caspase-10. However, unlike *O. faveolata* other components of the DISC such as FADD and CFLAR do not appear. DD containing proteins in the *A. digitifera* proteome also involve components of apoptosome formation, and immune activation. The apoptosome is a large multi-meric protein complex which activates executioner caspases and relies upon the scaffolding protein APAF-1 (Dorstyn et al. 2018). Homotypic interactions between NLRs and the NFκb p100 subunit as well as the NFκb activating proteins MAP3K3 (Bouwmeester et al. 2004) and MyD88 (Kawai and Akira 2007; Israel 2010) may cause immune activation reflecting work in vertebrate systems which demonstrate that NLRs can activate NFκb (Ogura et al. 2001). CARD containing-NLRs appear able to interact with additional apoptotic proteins, including Caspase-2 and CRADD which are both components of the apoptosis-inducing PIDDomse protein complex (Tinel and Tschopp 2004), as well as APAF-1 (Dorstyn et al. 2018) and capase-8 (Kim et al. 2000). An adaptor protein which contains a DD and a CARD was identified which may expand the caspase repertoire upon which *A. digitifera* NLRs can activate through formation of an inflammasome.

proteins based upon homotypic interactions (Fig. 3). Overall, it appears that both *A. digitifera* and *O. faveolata* NLRs are predicted to have the ability to recruit and activate caspases as well as induce immunity, reflecting work in vertebrate systems (Ogura et al. 2001), and highlighting their probable role in mediating coral stress responses.

Inflammasomes

Mammalian NLRs possess the ability to oligermize (Inohara and Nuñez 2001) into large multi-protein complexes called inflammasomes which provide a scaffold to activate inflammatory caspases, potentiate immune cascades, as well as lead to apoptosis.

The formation of these complexes is dependent on the ability of NLRs to interact with caspases either directly or through adaptor proteins (Stutz et al. 2013; Van Opdenbosch et al. 2014). To investigate the possibility of coral NLRs to have an analogous function we searched for proteins which may function as NLR-caspase adaptors in both species. We identified a previously undescribed *O. faveolata* adaptor protein which contains a DED and a DD which may allow *O. faveolata*'s NLRs to interact with additional caspases (Fig. 2). The *A. digitifera* proteome likewise contains an adaptor protein featuring both a DD and CARD domain which may also facilitate additional caspase interactions (Fig. 3). These adaptor proteins could potentially expand the caspase repertoire upon which NLRs may interact in both *O. faveolata* and *A. digitifera* by forming inflammasome-like complexes. There is precedent for inflammasome-like complexes in basal animals as the cnidarian *Hydra magnipapillata* contains an NLR adaptor protein termed DODE which has been suggested to function like mammalian inflammasome adaptor proteins (Lange et al. 2011), and our analysis may have identified the coral versions of this protein. Overall, we show that both species of coral investigated may have the required molecular machinery to form inflammasome like complexes through the action of their NLRs, which would allow corals to potentiate immune and stress signaling when responding to threats both foreign and domestic.

Conclusion

Our investigation revealed the presence of an NLR repertoire in the coral *O. faveolata* which may be important in the regulation of both immunity and apoptosis. Cnidarian immune systems demonstrate remarkable similarity to vertebrate innate immunity given the high level of divergence between these clades (Mansfield et al. 2017), which we see reflected in NLRs. Given the key role of immune proteins in allowing organisms to survive, conservation and convergence of key elements of immune pathways are not surprising (Meunier and Broz 2017). In support of this our results are in agreement with other investigations which have highlighted a conserved function of NLRs to activate immunity as well as modulate apoptosis near the base of animal evolution (Lange et al. 2011; van der Burg et al. 2016).

NLRs have been suggested to functionally converge (Zhang et al. 2010) and our findings indicate that both coral species have the necessary machinery to be able to form inflammasome-like complexes, similar to NLRs from more well-studied systems (Martinon et al. 2009; Latz et al. 2013). While direct detection of inflammasomes in cnidarians is lacking, our results corroborate the study by Lange et al. (2011) which demonstrated formation of an inflammasome like complex in the cnidarian *Hydra*. In addition, our data indicate that complex apoptotic mechanisms may be related to NLR activity in corals. As apoptosis has been demonstrated to be critically-linked to coral disease and bleaching (Richier et al. 2006; Tchernov et al. 2011; Fuess et al. 2017), NLRs may act as cellular mediators to translate both biotic and abiotic threats into apoptosis activation in corals.

Studies into the mechanisms which corals use to respond to both immune challenge and elevated temperature have highlighted overlapping pathways indicating that these stressors may not act via entirely distinct mechanisms (Palmer 2018). Given the known battery of NLR-activating compounds this class of molecules may play a role in the observed overlap, as these proteins are capable of sensing both externally derived danger signals (MAMPs) and endogenously derived danger signals (DAMPS) (Mariathasan et al. 2006). Furthermore, when disease and temperature interact there is a synergistic effect on coral mortality (Bruno et al. 2007) indicating common mechanisms may be at play. NLR activity exists on a continuum where lower levels of activation can potentiate an immune cascade leading to immune promotion and resolution (Kayagaki et al. 2011; Broz et al. 2012), whereas higher levels of activation can lead to inflammatory cell death (Latz et al. 2013). If both temperature and disease stress converge on NLRs, this could tip the balance toward cell death and be a cellular mechanism underling the synergistic interaction of biotic and abiotic stress in leading to coral declines. Overall, NLRs are a mechanism to sense and overcome threats both foreign and domestic, and functional characterization of the NLR repertoire of corals may shed light on mechanisms corals use to cope with increasingly stressful environments.

Funding

National Science Foundation-IOS award number: 1831860 Principal Investigator: Laura Mydlarz.

Supplementary data

Supplementary data are available at *ICB* online.

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