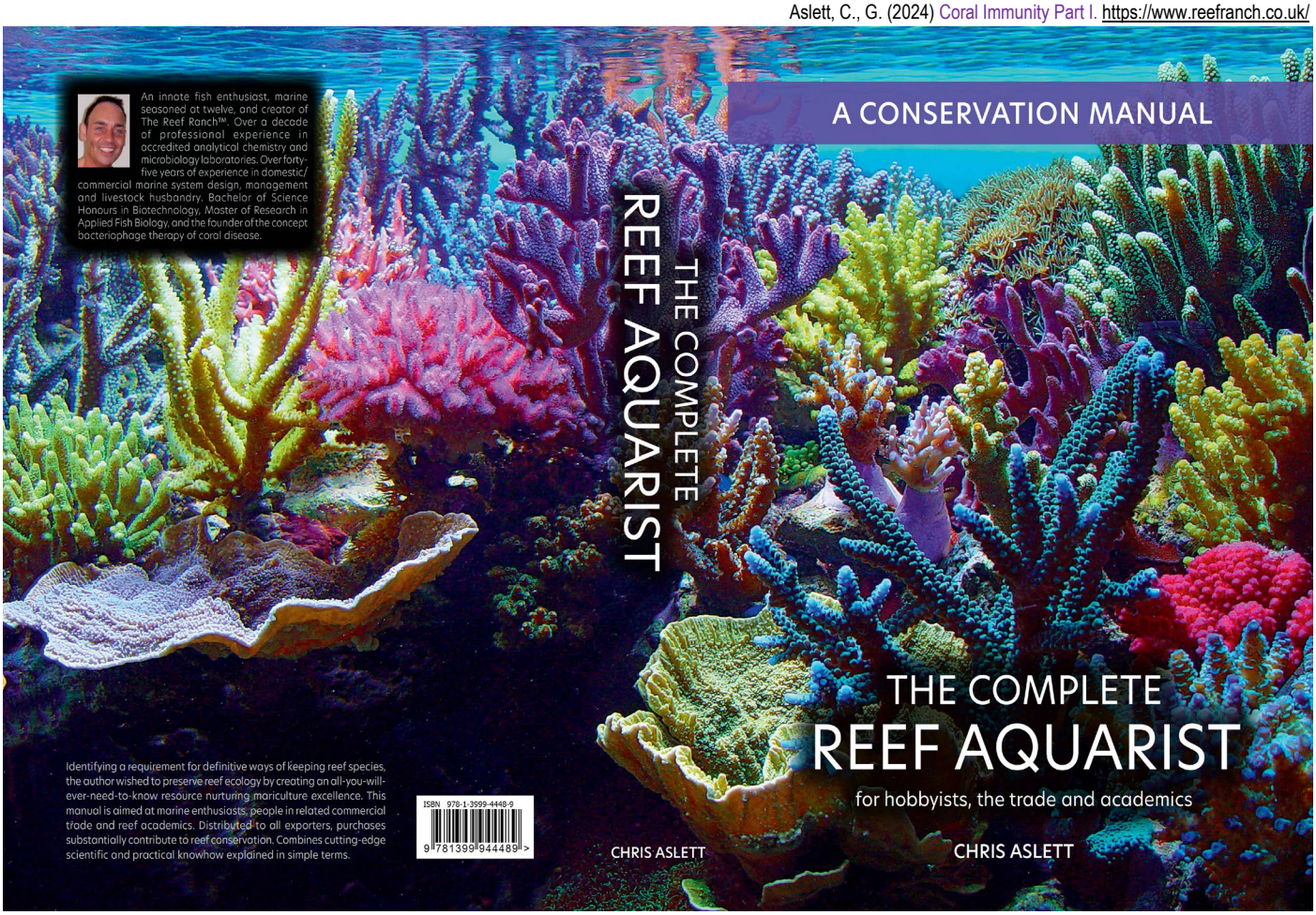

Promised early 2024

Coral Immunity Part I

Chris Aslett

Corals are associated with significantly diverse and rich microbial communities which includes their photosynthetic dinoflagellate “algal” partners (zooxanthellae), whereas prokaryotes (Bacteria and Archaea) and microeukaryotes (chromists; fungi; yeasts) are disseminated throughout their skeletal algal bands, endolithic skeletons, mucosal surfaces, and symbiotic tissues. Equally, these relationships are central to the endurance of mutualism (eubiosis) while dysbiosis and bleaching represent immoderations of adaptive and immunological responses. Coral microclimate niches and their populations (microbiomes) combine to form the host (holobiont) while all affiliates manufacture biocides which can kill other microscopic organisms like pathogens, yet these antimicrobials appear benign to “welcome” commensals and mutualists, where the holobiont differentiates between “friend” or “foe”. Remarkable still, is the discovery that corals have an innate immune response within which antimicrobials are key players. The author set out to unravel these mysteries but nothing could have prepared him for what transpired, while the wealth of studies necessitated the creation of a cutting-edge series advanced in both style and content.

Convention requires genes to be italicised while their proteinaceous products are not, whereas immune systems must distinguish self from nonself which minimises potentially harmful autoimmunity (Kvennefors et al. 2008). However such concepts may be outdated inasmuch as defence must underpin the health, wellbeing, and the composition of multipartite reef-forming corals (Palmer 2018). Zooxanthellate Scleractinia are considered primitive insofar as they do not have cell-mediated (adaptive) immunity driven by ultra-precise molecular shape recognition, which detects foreign antigens through the unification of exacting complimentary shaped epitopes and paratopes found on antigens and antibodies. Nevertheless these organisms also have a less fastidious arm of defence called innate immunity that relies upon comparatively imprecise pattern recognition receptors (PRRs) and fundamental clotting and biocidal competencies. Such coral defence is both intracellular and extracellular and depends upon the transcription of a suite of immune associated genes (Palmer et al. 2008; Palmer et al. 2010; Palmer et al. 2012; van de Water et al. 2015; Toledo-Hernandez et al. 2023). It was evident from studies of coral disease that the microorganisms in the mucosal microbiome played a role in colony defence which supported the notion that corals were essentially rudimentary, albeit the discovery of innate coral immunity and its multifaceted gene expression is somewhat revelatory (Fig 7.).

The attributes of beneficial microbes include sulphur cycling, antioxidant and biocide manufacture, nitrogen fixation, and quorum quenching (Giambiagi-Marval et al. 1990; Peixoto et al. 2017; Lavy et al. 2018; NCBI 2019; Robbins et al. 2019; Wirth et al. 2020; Maire & van Oppen 2022). Much carbon and sulphur are derived from the bacterial biomineralisation of abundantly ubiquitous dimethylsulphoniopropionate (DMSP) where certain prokaryotes express numerous DMSP-degradative genes and metabolise it differently (Wirth et al. 2020).

Zooxanthellae harbour lysogenic viruses that undergo lytic induction during stress, where coral tissues subjected to nutrient enrichment, increased temperature, and/or irradiance become

Keywords: coral; damage; discolouration; healing; immunity; inflammation; innate; melanin; microbes; phenoloxidase.

abundant in herpes-like virions, and thus lysogeny is a bleaching promoter (Fig 1.; Munn 2019).

Diseased corals alter the expression of hundreds if not thousands of genes (Traylor-Knowles et al. 2021) many of which encode antioxidants (Jin et al. 2016). Coral immune gene products include collagens, peroxidases, bax-like and fibrinogen-like proteins, transforming growth factor beta (TGF-β), and tyrosine kinase (Traylor-Knowles et al. 2021), which is vital for protein-dependent intercellular communication and tissue maintenance because it phosphorylates tyrosine residues using the terminal phosphate of ATP (Hubbard & Till 2000).

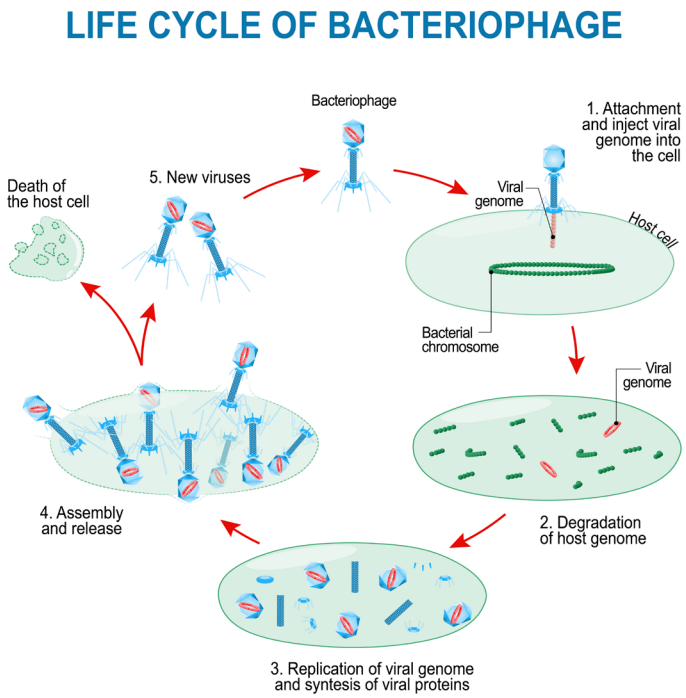

Fig 1. The lytic life cycle of T4-like bacteriophage.

TGF-β is a multifunctional signalling cytokine (Chaudhury & Howe 2009) and its expression appears central to early zooxanthellae infection and immunity, insofar as disrupting *TGF-β* transcription prevents spats (accreted first polyps) recruiting zooxanthellae and generates the cellular toxin (cytotoxin) nitric oxide (NO) in adult colonies (Berthelier et al. 2017). Therefore *TGF-β* inhibition/suppression is prerequisite to an efficacious defence (Fuess et al. 2020).

Far from dormant until an invading pathogen, tissue breach, or abiotic stress is recognised, constituent (background) immunity continuously detects holobiont homeostasis using receptors and responds to disturbances with their downstream molecular cascades, biomolecular signals, and antimicrobial by-products. Microbial network recognition simultaneously adjusts cross-anatomy gene expression which stabilises associations and reinforces eubiosis (Preston 2015; Palmer 2018; Boilard et al. 2020; Santoro et al. 2021).

Coral mucus, tissues, and skeleton are somewhat analogous to microbial biofilms where immeasurable interdependent microorganisms exist in dynamic equilibrium which thrive on the metabolic by-products of their neighbours, where commensals and mutualists perform vital housekeeping. Constituent immunity must optimise holobiont associations through winnowing and recruitment while tolerating healthy microbial communities or while their inaccessibility precludes an efficacious defence. Integrated stress responses (ISRs) protect using extracellular oxidative bursts of reactive oxygen species (ROS) which have until late become biomarkers of thermal stress (Nielsen et al. 2018; Palmer 2018). The danger model of immune activity proposes the release of molecules associated with cellular rupture (lysis) such as heat shock proteins, uric acid, and reactive molecules alert immunity to impairment which facilitates the modulation of an operational response (Palmer 2018).

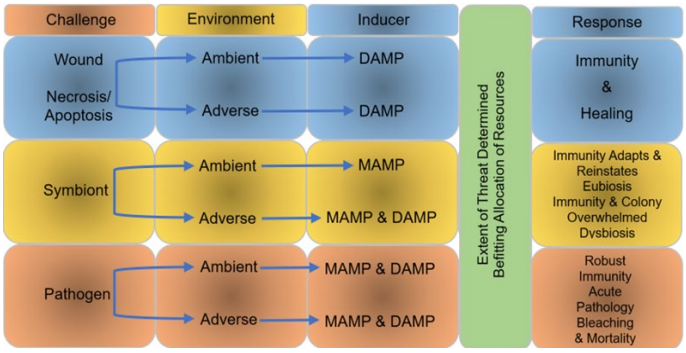

Fig 2. Microbe-associated molecular pattern (MAMP) detection, and/or danger-associated molecular pattern (DAMP) signalling, establish resource allocation and the degree of response. Adapted from Palmer 2018.

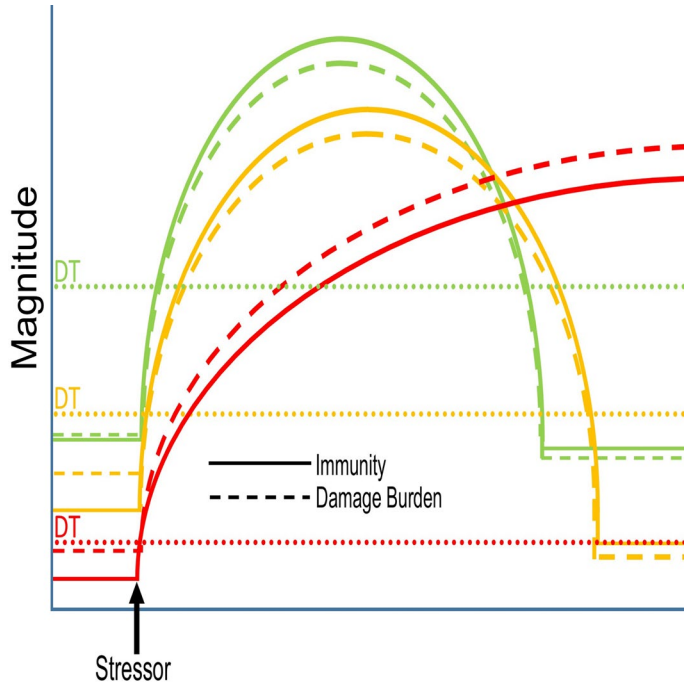

Fig 3. Damage threshold [DT] hypothesis for corals proposed by Palmer 2018. Elevated [green], moderate [orange], and low [red] immune investment strategies leave corals robust, resilient, or highly vulnerable. The stressor has overwhelmed the low investment strategist resulting in fatal pathology. Adapted from Palmer 2018.

Combining the paradigms of self/nonself and the danger model with reference to immune activation may precipitate an authentic perception of immune dynamics. The level of response is likely determined by the profile and degree of signals from microbe- and danger-associated molecular pattern receptors (MAMPs; DAMPs) and holobiont stability. This perspective contextualises the commensal and symbiotic microbial communities and clarifies how the coral can mount a noxious defence against pathogens whilst sustaining a highly diverse and functionally redundant holobiont. For instance MAMPs detect commensals in the absence of DAMPs which informs the coral that all is well. In contrast abiotic conditions may lead to danger molecule release from various microbial symbionts including zooxanthellae where signal intensity expedites coordination and proportion, which reflects the allocation of available resources

and reimburses microbes for incidental harm (Fig 2.). Enlarging this theory provides a dynamic framework whereby corals raise the function and place a ceiling on symbiont diversity, live with commensals, moderate pathogens, and navigate acute environmental disruptions (Palmer 2018).

Defence likely expedites tolerance or resistance (Palmer 2018) which incurs a transitory but significant energetic-, autoimmune-, and dysbiotic-cost, whereas the former curbs and withstands impairment by continually expending nominal resources.

The damage threshold (DT) hypothesis for host-pathogen interactions is influenced by evolutionary, ecological, phenotypic, and resource management factors which proposes continual host vigilance and acquiescence to a specified degree of physiological insult/pathology facilitates tolerance/coexistence with a defined microbiota. The DT is proportional to immunological allocation and inversely related to injury and susceptibility. Hence the background defence and the putative DT observed in species of *Porites* epitomises a tolerant low vulnerability immune strategy (Fig 3.; green; Palmer 2018).

Constituent Immunity ↑
Damage threshold ↑
Damage Burden ↓
Susceptibility ↓

A sensed harm from pathogenic invasion, a physical injury, or a microbiome perturbation that equals or exceeds the DT, triggers resistance which endeavours to obviate the risk, reconstruct tissues, and restore fitness. Raised constituent immunity buffers pathology which curtails disease and bleaching, and thus these species and genotypes/phenotypes endure abiotic and biotic disruptions to which others succumb. Their approach necessitates less upregulation and reverts to baseline levels rapidly compared to low investment highly susceptible strategists. For intermediate tacticians (Fig 3.; orange) there is a smaller buffer between the DT and their damage burden or, colonies that devote moderate resources on background immune vigilance and expression endure more and less continuous harm than those that allocate high and low reserves. These corals remain intermediately vulnerable due to a smaller disparity between their cellular trauma and their DT hence their restorative and corrective response must be greater and takes longer to re-instate homeostasis. Holobionts that dedicate the least to constituent immunity like species of *Pocillopora* and *Acroproa* (Brown et al. 2023) but not all (Toledo-Hernandez et al. 2023) sustain/tolerate damage immediately below their DT and thus pathogens, injury, or environmental conditions pose a more significant threat. Additionally, their microbial communities are less structured and are unaccustomed to the biocidal stressors common to defence. A remedial response that exceeds their DT demands a significant reallocation of resources and is alien to their tissues and microbiota which makes them highly susceptible to autoimmunity, dysfunction, bleaching, and mortality. Furthermore coral energy is finite where chronic stressors such as prolonged elevated temperatures deplete resources which may lead to moribundity and death (Palmer 2018). Nevertheless, the survival of a DT-exceeding chronic stressor, irrespective of immune strategy, would necessitate the recruitment or evolution of resistant microbiota including zooxanthellae which is likely to result in an interim altered homeostatic state and background immunity. Evidence suggests this does occur yet recovered corals revert to their prestressed profiles (Munn 2019; Boilard et al. 2020; Santoro et al. 2021) which may take years.

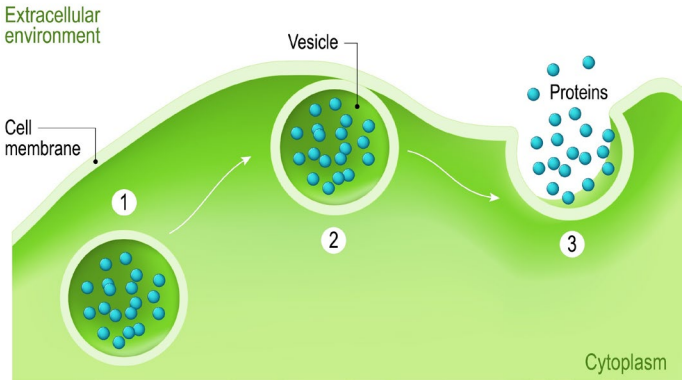

Fig 4. The granulocytes of corals degranulate using exocytosis.

The epigenetic modification and phenotypic plasticity of those that recover may assist retention of potentially heritable immune memory or training (Palmer 2018) which may impart remedial rapidity and efficacy in response to a recurrent danger. Cross-tolerance has been demonstrated in other invertebrates such as plants whereby priming of innate immunity with one stressor confers a resilience to another, while corals demonstrate they are highly adaptable. Efforts are being made to identify genes and robust genotypes that confer an ability to withstand climate change (Palmer 2018; Zoccola et al. 2020).

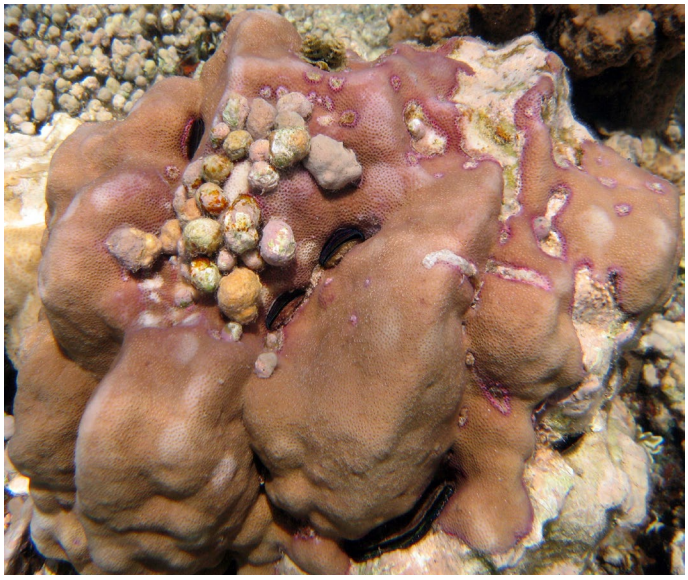
Fig 5. Pink tissue discolouration caused by carnivorous snails of the genus cf. *Coralliophila* (Caenogastropoda: Muricidae) infesting *Porites* species (Hexacorallia: Poritidae).

Wound Healing

Between 21 and 56 percent of wild scleractinian tissues are in a continual state of regeneration, that occurs in four phases coordinated by coral immunity. Initially granular cells exocytose coagulating (clotting) transglutaminase and melanin (phase 1.; Fig 4.; Palmer et al. 2011; Palmer et al. 2012; van de Water et al. 2015). Whereafter the lesion is flooded with microbe and cell debris-phagocytosing eosinophilic granular amoebocytes (phase 2.; Palmer et al. 2008). Fibroblast infiltration and multiplication consolidates and commences to reorganise the clot into epidermis (phase 3.), where scar maturation frequently occurs within 48 hours with sporadic apoptosis (preprogrammed cell

death; phase 4.; Palmer et al. 2011; van de Water et al. 2015). These mechanisms preclude pathogen incursion and colonisation while epidermis is lastly reconstructed into epithelium within the fortnight. However corals like these of the genus *Acropora* grow rapidly compared with other Scleractinia that can take between 60 days and 19 months to heal (van de Water et al. 2015) albeit melaninization responses in Acroporidae are limited compared to Poritidae and Oculinidae (Palmer *et al.* 2012).

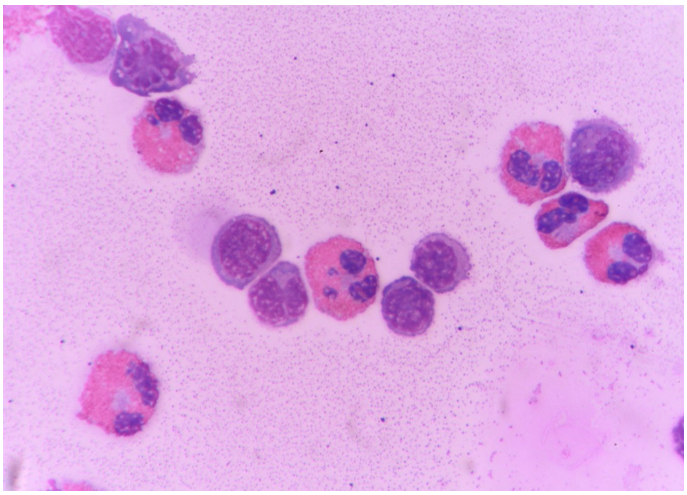

Fig 6. Rare eosinophils with pink (eosin) stained cytoplasm with purple haematoxylin-stained lobular nuclei which look like the granular eosinophilic amoebocytes of corals, yet they lack their finger-like projections called pseudopodia.

A three-phase transcriptome response to injury occurred over the study period at two, four and 10 days. Phase one initiated within 24 hours and was toll-like receptor (TLR)-dependent where immune genes like *TRAF6, MEKK1*, and *ERK2* were upregulated at two days after TLRs detected MAMPs. Antimicrobial peptide (AMP) transcription is modulated by TLRs which is redolent of wound-disinfecting AMP production in *Acropora aspera*. Positive feedback loops were identified in other invertebrates whereby TLR-stimulation induces signalling cytokines which reinforce the expression of TLRs and their downstream signals. There was an upregulation in the expression of TLR-associated signalling genes like *TIR-1*, *TRAF6*, *MEKK1*, *ERK2*, *cFos* and *NF-jB*, and compliment immune effector genes like *Bf* and *C3* in phase II (day four). *cFos* and *ERK2* TLR-pathway, *Bf* complement system, and *HL-2* coagulation system genes were upregulated in phase III at near-complete wound regeneration on day 10. *cFos* and *ERK2* have a tissue regenerative role, however the timely combined expression of these TLR, compliment, and coagulation system genes may conserve bacterial community structures (van de Water et al. 2015). Continued in part III.

Innate Immunity

The repertoire of coral defence includes AMPs which like perforin and granzyme in higher organisms create holes in the membranes of pathogens and enter to initiate apoptosis (Fig 8.; Liu et al. 1995; Vidal-Dupiol et al. 2011; Hlongwane et al. 2018). The expression of genes associated with immunity, cell endurance, and proliferation are upregulated via the JNK, MAPK p38, and NF-jB pathways after host pattern recognition receptors (PRRs) such as TLRs or nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) detect MAMPs. Coral haemolectin and transglutaminase homologues are indicative of sophisticated coagulation mechanisms (Palmer et al. 2012; van de Water et al. 2015). Transglutaminase-mediated plasma protein cross-linking forms an insoluble coagulative gel that hardens into a clot (Palmer et al. 2012).

MAMP detection induces the melanisation cascade (proPO system) whereby inactive prophenoloxidase (proPO) is cleaved into active phenoloxidase (PO) which expedites the deposition of wound-sealing and pathogen-sequestering melanin (Palmer et al. 2008; Cerenius et al. 2010, cited in Pollock et al. 2019), while highly unstable and toxic quinone intermediates flood and sanitise the lesion (Cerenius et al. 2010, cited in van de Water et al. 2015). Moreover melanin has antimicrobial properties which may explain the immediate loss of zooxanthellae observed in *Porites cylindrica* postinjury, albeit algal symbionts increased, there was no coagulation plug, and the cells were a disorganised mass in the sea fan *Plexaurella fusifera* (Meszaros & Bigger 1999, cited in Palmer et al. 2011; Palmer et al. 2011).

Three PO pathways generate different enzymes competent at metabolising monophenols, ortho-diphenols, or para-diphenols. Monophenoloxidases synthesise melanin by hydroxylating monophenols like tyrosine to diphenols, while diphenoloxidases oxidise ortho-diphenols like dopamine to chromatic compounds and quinones which are precursors for the non-enzymatic manufacture of melanin. In contrast laccases likely oxidise para-diphenols and dopamine-like molecules to spicule-like sclerites which sclerotizes, which may be overexpressed during aspergillosis and algal galls of Caribbean sea fans (Pathways 1. to 3.; NOAA 2014; Andras 2017).

Pathway 1.

Tyrosine (monophenol) → [monophenoloxidase] → Dopamine (ortho-diphenol)

Dopamine → [diphenoloxidase] → Dopaqinone/Dopachrome

Dopaqinone/Dopachrome → multistep process → [phenoloxidase] → multistep process → Melanin

Pathway 2.

Dopamine (ortho-diphenol) → multistep process → [diphenoloxidase] → Dopaminequinone/Dopaminechrome

Dopaminequinone/Dopaminechrome → multistep process → [phenoloxidase] → multistep process → Melanin

Pathway 3.

Dopamine (ortho-diphenol)→ multistep process → [laccase] → multistep process → Sclerotization

Inactive promonophenoloxidase and prodiphenoloxidase and active laccase are associated with disease resistance in related taxa, while active monophenoloxidase and diphenoloxidase and prolaccase confer resistance to bleaching (Palmer et al. 2010; Palmer et al. 2012).

PO and transglutaminase activities were evaluated in the families Acroporidae, Alcyoniidae, Euphyllidae, Faviidae, Merulinidae, Mussidae, Fungiidae, Oculinidae, Pocilloporidae, Poritidae, and Sphenopidae. PO was lowest in *Acropora hyacinthus* and *A. tenuis* despite comparable assays in Pocilloporidae, Euphyllidae, and other Acroporidae, whereas the

highest was observed in Faviidae, *Galaxea* species (Oculinidae), and *Porites cylindrica* (Poritidae; Palmer et al. 2012). A coral's constituent PO activity therefore likely reflects its DT and immune investment.

"White" cells are common to all animals including corals which phagocytose debris, viruses, and pathogens in phagocytic vesicles which are later infused with reactive oxygen species (ROS; Palmer et al. 2008; Palmer et al. 2011). Phagocytes also kill and sequester pathogens and infected cells by liberating ROS and forming aggregates around foreign invaders, where some retain phenoloxidase-activating cascade and melanin deposition competencies.

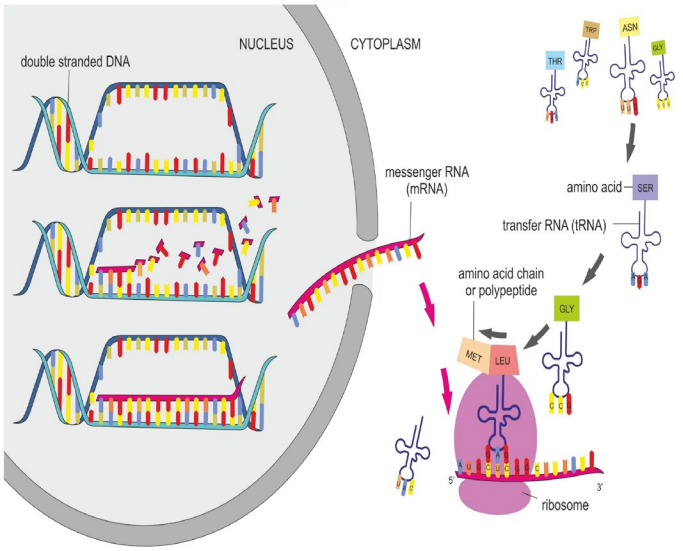

Fig 7. Polypeptide chain manufacture: transcription of nucleus-bound deoxyribonucleic acid (DNA) creates messenger ribonucleic acid (mRNA) which is destined to be translated by a ribosome (pink) after translocation to the cytoplasm. Amino acid-bound transfer ribonucleic acids (tRNAs) are concatenated according to the mRNA sequence, while their R-group (sidechain) charges and shapes determine protein folds.

Foci of pink, purple, or blue discoloured scleractinian tissue in GBR *Acropora millepora* and *Porites* species were evaluated for inflammatory biomarkers, where pigments were associated with wound healing (Fig 5.). PO activity was raised in blue tissues of *A. millepora* while mesoglea was reduced, tissues were granular, aggregates of ovoid cells were observed in the epidermis, no melanin was present, and zooxanthellae abundance was one third of that of healthy tissues. Similar structures were observed in coloured *Porites* tissue where mesoglea was reduced, zooxanthellae were halved, and the epidermis was disrupted and pervaded with four times the number of granular cells which were melanised. Although granular cells were plentiful in healthy gastrodermis (Palmer et al. 2008).

Cytotoxic intermediates are formed in the melanin cascade which are critical components of defence while abnormally pigmented tissues are likely integral to an inflammatory response. Nevertheless nil immune cell infiltration and aggregation suggests that *Acropora* and *Porites* species lack "white" cells compared to octocorallian sea fans. Phenoloxidase does not always cause melanin deposition which was observed in *A. millepora* which suggests an alternative role, while the granular cells observed in abnormally coloured tissues of *Porites* are consistent with the inflammatory response in *P. compressa*, where tissues were infiltrated with chromophore amoebocytes (Palmer et al. 2008).

Zooxanthellae appear to be expelled immediately after injury yet multiply over the following 48 hours to within 75 percent of their previous maximum. The melanin of healthy epidermis is twice that of healthy gastrodermis which was diminished by 50 percent 6 hours postinjury which suggests trauma-associated melanin release, where levels were restored to 80 percent at 24 and fully replenished by 48 hours. Wounded epidermis becomes densely packed with melanised granular cells, while *Porites cylindrica* cnidae were large and dispersed throughout the epidermis but were also found in mesenterial filaments. Melanin was associated with enlarged nuclei, while the protein concentration of infiltrating granular eosinophilic amoebocytes increased over the first 6 hours which aggregated at the advancing epithelial front (Fig 6.).

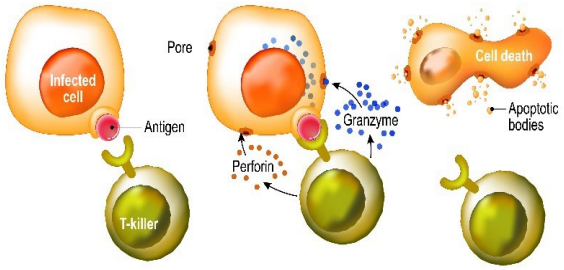

Fig 8. The receptor of a fish CD8 killer T-lymphocyte binds the foreign peptide on the major histocompatibility complex (MHC) class I of an infected fish cell, where CD8 activation releases apoptosis-inducing perforin and granzyme. These mechanisms are integral to the more advanced adaptive immunity of fish, however primitive "white" cells like amoebocytes perform analogous cytotoxic roles in corals using pattern recognition receptors (PRRs).

A coagulation plug was formed from cellular debris, invading organisms like ciliates, cnidae, pink coloured zooxanthellae, and possibly melanin-liberating granular cells and eosinophilic amoebocytes. The latter commenced to reconstruct epithelium on the lesion edge while melanised granular cells formed along the restorative boundary. Pink non-granular fibroblasts with pseudopodia interwove the plug and deposited a collogen-like substance which was interspersed with low numbers of non-granular putative hyalinocytes. Perpendicularly aligned aggregates of granular amoebocytes were present behind the nascent epithelial boundary some of which had begun to differentiate into gastrodermis, with endodermal zooxanthellae distal to the advancing front at 24 hours. Granular amoebocytes had diminished while melanised granular cells remained densely aggregated and cell types were separated by a thin layer of mesoglea 48 hours postinjury. The front appeared to be reconstructed by eosinophilic granular amoebocytes containing golden brown granules of melanin which suggest these cells may transform into melanised granular cells, albeit several contained basophilic clumps typical of apoptosis. Such preprogrammed cell death is consistent with healing where potentially aberrant cells are killed after clot consolidation and initiation of epithelial remodelling (Palmer et al. 2011).

Part I is foundational to the remainder of this series which clarifies how coral holobionts awash with biocides and unstable intermediates can nurture healthy communities of microbes to their advantage whilst excluding others in a precise manner. Next time we have a comparatively sedate journey in part II where we can recharge our batteries in preparation for the fact-packed journey of part III.

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