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Abstract

The nematode worm *C. elegans* lends itself naturally to investigation of innate immunity, from the scale of molecules to the whole animal. Numerous studies have begun to reveal the complex interplay of signalling mechanisms that underlie host defence in *C. elegans*. We discuss here research that illustrates the connection between cell and tissue-level homeostatic mechanisms and the activation of innate immune signalling pathways. These are woven together to provide a comprehensive organismal protection against perceived threats.

Graphical abstract

Introduction

It is now generally accepted that in higher organisms, microbial infections can be recognised in one of two ways. Firstly, "microbe-associated molecular patterns" (MAMPs) can act as ligands for dedicated host receptors, setting off intracellular signal transduction cascades that lead to the production of defence molecules. The best-known examples of this type of MAMP-triggered immunity (MTI) are undoubtedly those involving members of the Toll-like receptor (TLR) family in vertebrates [1]. Alternatively, perturbations of host physiology provoked by infection can trigger an immune response. Here, three broad categories can be distinguished: (i) The host can detect "damage-associated molecular patterns" (DAMPs). These are often endogenous molecules released to an uncharacteristic location, such as nuclear proteins or mitochondrial DNA in the cytoplasm, or ATP in the extracellular milieu. (ii) Falling under the umbrella term, the "guard hypothesis", specific alterations of host proteins, or protein complexes, brought about by pathogen-delivered effectors, can be recognised and again, act as the signal for initiating defence mechanisms. (iii) Lastly, more generic alterations, such as abrupt changes in membrane potential or of translational capacity can also be triggers of host innate immune responses. These last 2 classes are examples of "effector-triggered immunity" (ETI), best characterized in plants [2], but increasingly recognised as important for animal innate immunity too [3], where the term "surveillance" is frequently applied [4].

This review is largely concerned with surveillance mechanisms. We explore the links that exist between the disruption of cellular or organismal homeostasis and innate immune defence, as revealed by recent studies with the nematode worm *Caenorhabditis elegans*. We cover subjects reflecting the diversity of known mechanisms. This choice is governed in part by the fact that in spite of more than a

decade's research, no host pathogen recognition receptors have been unambiguously defined in *C. elegans* [4], but more importantly because the surveillance mechanisms described in worms are either known to be or may be evolutionary conserved.

1. Epidermal injury.

The first DAMP important for *C. elegans* innate defences was uncovered through studies with the obligate fungal endoparasite *Drechmeria coniospora*. Infection begins with the penetration of the worm's extracellular cuticle and epidermis by specialised hyphal structures [5]. In common with physical disruption of epidermal integrity, in mutants lacking certain structural proteins (e.g. specific collagens), infection with *D. coniospora* leads to an accumulation of hydroxyphenyllactic acid (HPLA). This tyrosine-derived metabolite activates a specific G-protein coupled receptor (GPCR), DCAR-1, which acts upstream of a well-characterised p38 MAPK cascade [6], via the STAT-like transcription factor STA-2 [7], to switch on the expression of antimicrobial peptide genes [8]. DCAR-1 is also activated upon minor mechanical injury, caused by a needle wound or laser. DCAR-1 therefore appears to act as the receptor for an endogenous signal of damage, the DAMP, HPLA, as well as structurally related molecules [8]. Although there are no clear DCAR-1 orthologues in higher species, it is interesting to note that in humans, the level of HPLA increases dramatically during sepsis as a consequence of microbial degradation of tyrosine [9]. In *C. elegans*, it has not yet been established precisely how HPLA levels are controlled [8].

Injuring the *C. elegans* epidermis is associated with a separate wound-healing response [10]. This involves local production of superoxide by mitochondria (mtROS)

at the site of injury, alteration of a redox-sensitive motif in RHO GTPases and assembly of rings of F-actin, that constrict to close any open holes in the epidermal membrane [11]. mtROS are also sensed by the apoptotic pathway and can, independently of apoptosis, elicit protective mechanisms that keep the organism alive under stressful conditions [12].

Upon violent injury, the expression of antimicrobial peptide genes is induced, in a STA-2 dependent manner, but purportedly independently of p38 MAPK signalling. It was proposed that this is a consequence of the disruption of the normal physical association of STA-2 with hemidesmosomes, which attach the epidermal cells to the cuticle, although no concomitant increase in nuclear STA-2 was demonstrated. Importantly, however, it was shown that disruption of hemidesmosome structure in primary Human Epidermal Keratinocytes leads to non-canonical but STAT-dependent antimicrobial peptide gene expression [13]. There is therefore reason to believe that epithelial barriers detect danger and activate immune defences via an evolutionarily conserved mechanism more akin to mechanical than chemical signalling (see [14] for a recent review).

2. Disruption of genome integrity

As stated in the introduction, diverse pathogens produce effector protein required for full virulence. Among them, some target host nuclei. Enteropathogenic *E. coli*, for example, secretes EspF that targets the nucleolus, depleting it of nucleolin [15]. The same effector also depletes host cell DNA mismatch repair proteins, increasing the frequency of potentially deleterious spontaneous mutations [16]. DNA damage can therefore be a surrogate signal for infection. In *C. elegans*, DNA damage has a broad

impact on gene expression. It causes the up-regulation of genes encoding lysozymes and C-type lectins [17] also induced during bacterial intestinal infection [18]. As this was not observed in mutants lacking a germline, or in an ERK MAP kinase mutant, Ermolaeva *et al*. proposed that DNA damage triggers ERK signalling in germ cells to release an unknown signal that then activates the p38 MAPK pathway in the intestine. Presumably as a secondary consequence, DNA damage also activates the ubiquitin-proteasome system (UPS) in somatic tissues, which confers enhanced proteostasis and systemic stress resistance. This was proposed to promote endurance of somatic tissues, needed if progeny production is delayed as a consequence of problems with germ cell genome integrity [17]. Fully $2/5^{th}$ of the genes induced by DNA damage are targets of the conserved FOXO transcription factor DAF-16, an important regulator of resistance to stress and infection [19]. Consistent with this, a subsequent study showed that DNA damage causes the translocation of DAF-16 into intestinal nuclei. There, it acts with a GATA factor to govern target gene expression and maintain normal cellular physiology despite sustained DNA damage [20]. In a similar manner, as in *Drosophila* [21], defence genes are expressed in somatic cells when fragmented DNA is not cleared from germ cells undergoing apoptosis [22]. There are precedents for this type of trans-tissue stress signalling in *C. elegans*. The organismal response to heat-shock is regulated cell non-autonomously, via neuronal signalling to the somatic tissues [23], by trans-cellular chaperone signalling between somatic tissues [24] as well as from somatic tissues to neurons (reviewed in [25]). Lastly, there is a global repression of stress responses, controlled by signals from germline stem cells at the onset of reproduction [26].

There are parallels between the observations of Ermolaeva *et al*. [17] and the finding by Moita and colleagues that in mice, low doses of anthracycline antibiotics, which

provoke DNA damage, confer resistance to sepsis, through the ataxia-telangiectasia mutated kinase (ATM) and Fancony Anemia pathways [27]. It is also important to note that it has recently been shown that in vertebrates, DNA damage primes the Type I Interferon system (via the cytosolic DNA sensor STING) to promote an innate immune response [28]. Further, Cossart and colleagues have shown that the toxin listeriolysin O (LLO) blocks the signalling response to DNA breaks by degrading a host sensor protein. When the normal response is compromised, bacterial replication increases, supporting the idea that detection of DNA damage is an important infection control mechanism in mice [29] as well as nematodes. It is likely to be conserved in humans too, opening promising new avenues for the management of sepsis.

3. Interference with transcription or translation

Other virulence factors target host protein synthesis. The inhibition of translation by *Pseudomonas aeruginosa* Exotoxin A, which ribosylates elongation factor 2, provokes an immune response in *C. elegans*. This requires the p38 MAPK pathway as well as *zip-2* [30,31], which encodes a transcription factor with a basic leucine zipper (bZIP) domain most similar to that of the ATF-2 family [32]. Shiga toxin that cleaves the 28S RNA of the 60S ribosomal subunit, thereby halting protein synthesis, also triggers the p38 MAPK pathway and expression of defence genes [33]. Indeed, even in the absence of any pathogen or toxin, blocking protein synthesis is enough to switch on host defences [30,31]. Subsequently, it was shown that this is an even more general phenomenon. Disruption of multiple core cellular processes can also provoke a transcriptional response in the intestine similar to that provoked by infection with bacterial gut pathogens [34]. There is thus a commonality between the consequences

of infection and of stress by ribotoxins and other microbial virulence factors. This is reflected in the existence of shared signalling mechanisms regulating the expression of cytoprotective and antimicrobial genes, including TFEB [35] and the mediator complex [36]. Both these are evolutionarily conserved and more generally, ribotoxic responses are known to activate MAPK signalling responses from yeast to mammals [37]. Toxins represent an essential part of the microbial armamentarium. Their triggering of host defence responses is likely to be evolutionary ancient (e.g. [38]). Perhaps to avoid wasteful induction of immune responses, in mammals, the mechanisms that detect changes in cellular physiology are integrated with regulators of metabolism such that a block of translation linked to amino acid starvation, for example, leads to T-cell anergy and not activation [39].

4. Induction of mitochondrial UPR (UPR^{mt})

New cellular roles for mitochondria, in addition to their essential contribution to energy generation, continue to be uncovered (e.g. [12,40]). It is important that mitochondria function even under non-homeostatic conditions [41]. This capacity is guaranteed by a specialised unfolded protein response, the UPR^{mt} [42]. A large number of bacterial species that share the same environment as *C. elegans* induce the UPR^{mt}. Ceramide plays a key part in signalling the UPR^{mt} [43], in line with the important role of lipids in UPR generally ([44]; reviewed in [45]). This response is negatively regulated by the Jun kinase KGB-1 [46], and relies on the bZip protein ATFS-1 that can bind to promoters of genes both in the nuclear and mitochondrial genomes and coordinate mitochondria-to-nuclear communication. Thus, while ATFS-1 drives expression of mitochondrial chaperones [47], it limits the expression of genes

encoding components of the oxidative phosphorylation machinery during mitochondrial stress [48]. This mechanism is complemented by another pathway involving ROS-stimulated eIF2α kinase that leads to a reduction in protein translation [49].

As mentioned above, compromising overall translatory capacity can by itself entail the expression of defence genes, but it also prevents ROS-induced UPR^{mt} [46]. On the other hand, activation of the ATFS-1 branch of the UPR^{mt} is associated with an induction of defence gene expression, in part via *zip-2*, and contributes to protect the host against infection [50]. Many microbial products, including the antibiotics chloramphenicol and tetracycline, specifically inhibit mitochondrial protein synthesis and trigger the UPR^{mt} [51]. Equally, infection of *C. elegans* by wild-type *P. aeruginosa* causes mitochondrial dysfunction, leads to an UPR^{mt}, and up-regulation of host antimicrobial defences. Bacterial strains that do not produce siderophores, which limit available iron, essential for mitochondrial function, or cyanide, which inhibits cytochrome c oxidase, are less potent in their stimulation of the UPR^{mt} [50]. The potential importance of the mechanism is suggested by the fact that there are bacteria that block this host response [43].

In common with other stress responses in *C. elegans*, the UPR^{mt} can involve trans-tissue signalling. Thus, for example, provoking an UPR^{mt} just in neurones leads to an UPR^{mt} in the intestine (and increased longevity) [52]. Conversely, octopamine released from neurones governs mitochondrial morphology and metabolism, as well as impacting organismal ageing [53]. In this context, it should be mentioned that while worms' standard lab diet of *E. coli* (strain OP50) appears innocuous for young worms, it acts as a mild pathogen in old or immunocompromised worms [18]. In old worms, this reflects the reduction of expression of genes encoding defence proteins,

including lysozymes and C-type lectins [54,55]. Thus lifespan on live OP50 equates in part with innate immune capacity.

5. Mitophagy and autophagy

The UPR^{mt} has been described as a salvage pathway for functionally impaired mitochondria. In cases of irreparable damage, mitochondria can be removed by mitophagy (reviewed in [56]). This is a specialised form of autophagy, used by the cell generally to recycle damaged components. TFEB, which as mentioned above, controls defence gene expression also regulates autophagy [57,58]. Autophagy has been shown to play an important role in host resistance in *C. elegans* [35,59], as it does in other species. Mitophagy has a direct role in the resistance of *C. elegans* to infection, specifically against siderophore-mediated killing [60]. It is regulated via mechanisms that are interlinked with those involved in the UPR^{mt}. For example, ceramide plays an important role in both processes, across species [43,61]. Further, in *Drosophila*, mitochondrial distress in muscle causes a tissue-specific redox-dependent UPR^{mt}, and a systemic stimulation of mitophagy that involves insulin signalling [62]. Mitophagy is also regulated by the ubiquitin kinase PINK1. Under normal circumstances, PINK1 is imported into mitochondria and degraded. If this doesn't occur, PINK1 accumulates on the mitochondrial outer membrane where it phosphorylates and activates the ubiquitin ligase Parkin, which then ubiquitinates outer mitochondrial membrane proteins. This marks the mitochondrion for engulfment by autophagosomes. In *C. elegans*, the conditions that activate ATFS-1 can also cause PINK1-dependent mitophagy (reviewed in [56]).

6. Neurodegeneration

Mitophagy has been implicated in the pathogenesis of several neurodegenerative disorders (reviewed in [63]). A link between such conditions and innate immunity was suggested from recent investigation of a *C. elegans* model of amyotrophic lateral sclerosis (ALS). Neuronal expression of ALS-causing mutant proteins, but not polyglutamine toxicity, induces expression of the antimicrobial peptide gene *nlp-29* in other tissues. This response, and the underlying neurodegeneration, required neurosecretion [64], in contrast to the up-regulation of *nlp-29* seen upon fungal infection [6]. These results complement prior work linking immune and nervous systems in *C. elegans* [65-68], and suggest that the worm may help understand the molecular basis of these connections under normal and pathological situations.

Conclusions

The *C. elegans* immune system is comparatively simple, since the worm has no specialized immune cells, nor any motile macrophage-like cells. Unbiased functional approaches in *C. elegans*, via genetic or genome-wide RNAi screens, have contributed to an understanding of the molecular underpinnings of its innate immune system and revealed hitherto unsuspected connections between different fundamental cellular processes. Several overarching themes have emerged. First, antimicrobial defences are intertwined with those that help protect the animal from abiotic stress. Second, disruption of any number of basic cellular functions, including translation or energy generation, can act as a trigger for switching on immune defences. Third, there is an extensive, as yet relatively poorly characterised cross-talk between the different tissues, involving the germline and the somatic tissues, with a prominent role for the

nervous system. A number of the mechanisms described for the first time in *C. elegans* appear to be present in higher animals. Work with this powerful model system will undoubtedly continue to provide insights into conserved aspects of innate immunity.

Figure 1. Model for diverse triggers of defense gene expression in response to infection. *C. elegans* uses effector-triggered immunity, as well as DAMP-triggered immunity, to upregulate defense gene expression in response to infection. MAMP triggered immunity is well-described for other hosts, but has not yet been described for *C. elegans* (see Box 1). In addition to cell-autonomous mechanisms, innate immune defences can be activated by perturbations in distant tissues. Figure adapted, with permission, from [4].

Box 1: PAMP receptors in *C. elegans*?

C. elegans lacks many of the families of proteins involved in PAMP recognition in other species [69]. There are a number of potential scavenger receptors (SR), including the SCARF ortholog CED-1, and 6 SCAV proteins of the SR-B family. Loss of function *scav-1* mutants are highly susceptible to infection by *Candida albicans* and *Cryptococcus neoformans* [70]. *ced-1* mutants also display a decreased resistance to these 2 intestinal fungal pathogens. But whether these *C. elegans* proteins actually recognize yeast cell wall beta-glucans and thereby trigger downstream effector gene expression has not been formally demonstrated. Knocking down *scav-4* increases susceptibility to the nematocidal toxin Cry5B [71], suggesting they may play an indirect role in host defense.

The most prominent candidate PAMP receptor, TOL-1 (the unique nematode TLR), was hypothesized to play a direct role in pathogen recognition since *tol-1* mutants are defective in their avoidance of pathogenic bacteria [72]. This hypothesis was subsequently revised when it was found that *tol-1* mutants do not have a problem in recognizing pathogens, but rather a defect in sensory integration [73]. A recent study has provided the explanation for these observations. It turns out that *tol-1* is required for the terminal differentiation and function of the BAG neurons [74]. These chemosensory neurons are activated by CO_2. Microbial respiration alters local CO_2 concentrations [75], and this is one signal that guides worms' behaviour [76]. In common with other BAG-defective worms, *tol-1* mutants are defective in CO_2 sensing; this alters their comportment in the presence of highly metabolically active microbes [74]. These results, together with others [77,78], put to rest the idea that TOL-1 functions as a PAMP receptor in *C. elegans*.

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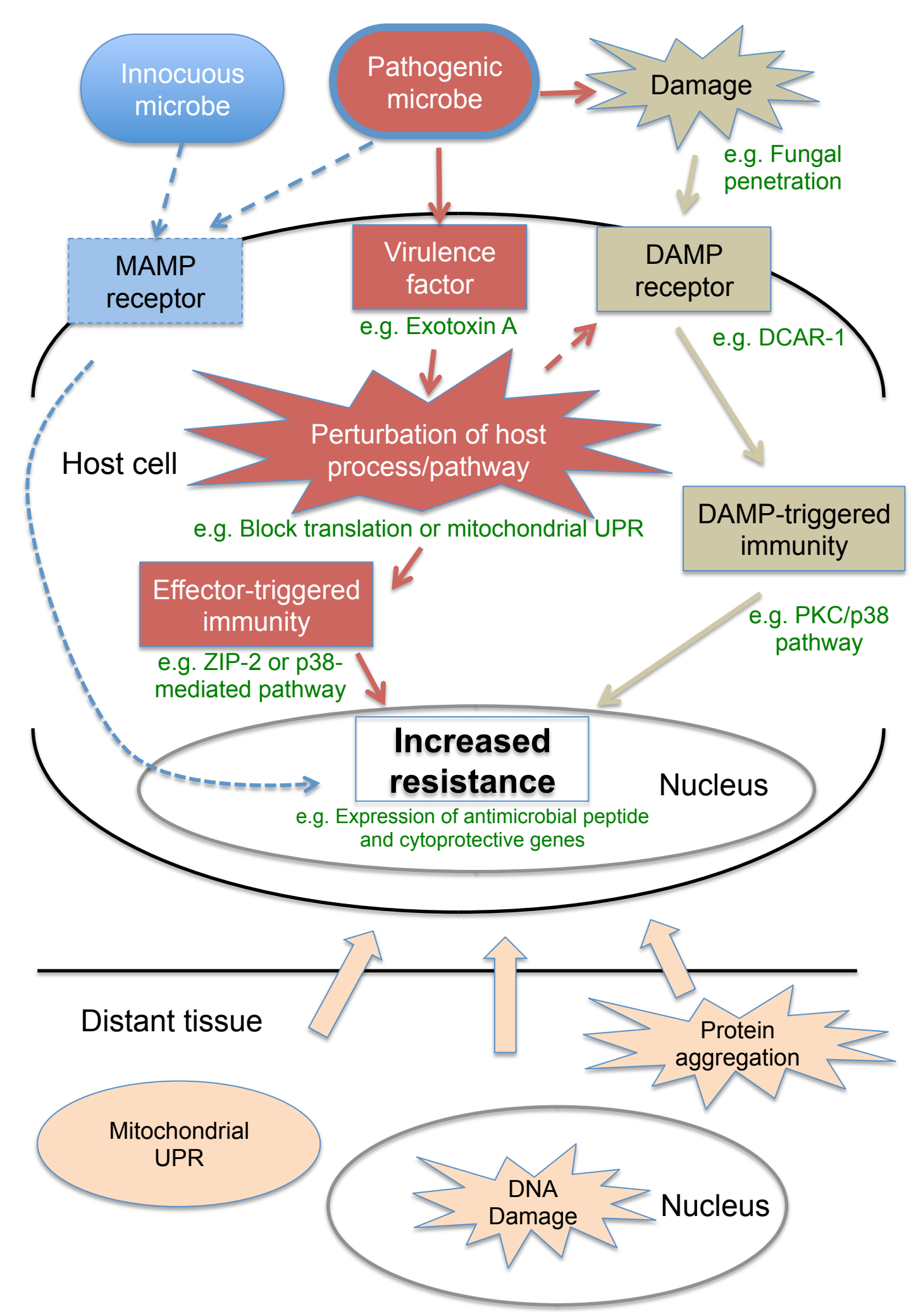
Innocuous microbe
Pathogenic microbe
Damage
e.g. Fungal penetration
MAMP receptor
Virulence factor
e.g. Exotoxin A
DAMP receptor
e.g. DCAR-1
Host cell
Perturbation of host process/pathway
e.g. Block translation or mitochondrial UPR
DAMP-triggered immunity
Effector-triggered immunity
e.g. ZIP-2 or p38-mediated pathway
e.g. PKC/p38 pathway
Increased resistance
Nucleus
e.g. Expression of antimicrobial peptide and cytoprotective genes
Distant tissue
Protein aggregation
Mitochondrial UPR
DNA Damage
Nucleus