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ABSTRACT

Innate immunity is an evolutionary ancient defence strategy that serves to eliminate infectious agents while maintaining host health. It involves a complex network of sensors, signaling proteins and immune effectors that detect the danger, then relay and execute the immune programme. Post-translational modifications relying on conserved ubiquitin and ubiquitin-like proteins are an integral part of the system. Studies using invertebrate models of infection, such as the nematode *Caenorhabditis elegans*, have greatly contributed to our understanding of how ubiquitin-related processes act in immune sensing, regulate immune signaling pathways, and participate to host defence responses. This review highlights the interest of working with a genetically tractable model organism and illustrates how *C. elegans* has been used to identify ubiquitin-dependent immune mechanisms, discover novel ubiquitin-based resistance strategies that mediate pathogen clearance, and unravel the role of ubiquitin-related processes in tolerance, preserving host fitness during pathogen attack. Special emphasis is placed on processes that are conserved in mammals.

KEYWORDS

Ubiquitination, SUMOylation, Host-Pathogen Interaction, Proteostasis, Unfolded Protein Response.

DECLARATION

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ABBREVIATIONS

AMP	Anti-microbial peptide
AMPK	AMP-activated protein kinase
ASK1	Apoptosis signal-regulating kinase 1
ATF	Activating transcription factor
ATFS-1	ATF associated with stress-1
ATP	Adenosine triphosphate
BATH	BTB and MATH-domain-containing
BTB	Bric-a-brac, Tramtrack and Broad complex
bZIP	basic leucine zipper
CD4	Cluster of differentiation 4
CEH	*C. elegans* homeobox
CHBP	Cycle inhibiting factor (Cif) homolog in *Burkholderia pseudomallei*
CHOP	C/EBP homologous protein
CLEC	C-type lectin
CRL	Cullin-RING ligase
DAF	Dauer formation
DAMP	Damage-associated molecular pattern
DDB1	DNA damage-binding protein 1
DCAF	DDB1 and CUL4 associated factor
DCAR-1	Dihydrocaffeic acid receptor-1

DUB	De-ubiquitinating enzyme
DVE-1	Defective proventriculus in *Drosophila*
ELT-2	Erythroid-like transcription factor family - 2
EGF	Epidermal growth factor
ER	Endoplasmic-reticulum
ERAD	ER-associated protein degradation
ErbB4	avian erythroblastic leukemia viral oncogene B homolog 4
Esp	Enhanced susceptibility to pathogen
FKH-9	Forkhead transcription factor family member 9
FOXO	Forkhead box O protein
GPCR	G protein-coupled receptor
HAMP	Homeostasis-altering molecular processes
HPLA	4-Hydroxyphenyllactic acid
HECD-1	HECT, D1 ubiquitin protein ligase homolog 1
HECT	Homologous to the E6-AP carboxyl terminus
HECW-1	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1
IGF-1	Insulin/insulin-like growth factor 1
IKK	Inhibitor of nuclear factor kappa-B kinase
INFγ	Interferon gamma
IGF-1	Insulin-like growth factor 1
IIS	Insulin/IGF-1 signaling

IPR	Intracellular pathogen response
JAMM	JAB1/MPN/Mov34 metalloenzyme domain
JNK	c-Jun N-terminal kinase
LC3	Microtubule-associated protein 1A/1B light chain 3B
LPS	Lipopolysaccharide
MAMP	Microbe-associated molecular pattern
MAPK	Mitogen-activated protein kinase
MATH	Meprin-associated Traf homology
MINDY	Motif interacting with Ub-containing novel DUB family
MJD	Machado-Josephin domain protease
MyD88	Myeloid differentiation primary response 88
NEDD	Neural precursor cell expressed developmentally down-regulated protein
NEDL1	NEDD4-like ubiquitin protein ligase 1
NF-κB	Nuclear factor-kappa B
NLP	Neuropeptide-like protein
NLR	Nucleotide-binding and oligomerization domain (NOD)-like receptor
NSY-1	Neuronal symmetry 1
NPR-1	Neuropeptide Y receptor 1
OLL	Outer labial
OTU	Ovarian tumor proteases
PALS	Protein containing ALS2CR12 signature

PAS	Proteasome alpha subunit
PBS	Proteasome beta subunit
PD	Parkinson's disease
PDK-1	Phosphoinositide-dependent protein kinase 1
PDR-1	Parkinson’s disease Related 1
PFT	Pore-forming toxin
PI3K	Phosphoinositide 3-kinase
PKB	Protein kinase B
PMK-1	p38 MAP kinase 1
PRR	Pattern recognition receptor
PTI	Pattern trigger immunity
RBR	RING-in-between-RING
RCS-1	RING protein activating with cullin and SKR protein 1
RING	Really interesting new gene
RIP	Receptor interacting serine/threonine protein kinase
RLE-1	Regulation of longevity by E3 ubiquitin-protein ligase 1
ROS	Reactive oxygen species
SARM	Sterile alpha and armadillo repeats
SATB	Special AT-rich sequence-binding protein
SEK-1	SAPK/ERK kinase-1
SENP	Sentrin-specific protease

SGK-1	Serum and glucocorticoid regulated kinase 1
SIAH	Seven in abstentia homolog
SRS	Substrate recognition subunit
STA-2	Signal transducer and activator of transcription 2
SKR	Skp1-related
SOCS-BC	Suppressor of cytokine signaling protein 1, binding to EloB-C
SUMO	Small ubiquitin-like modifier
T3SS	Type 3 secretion system
TAB	TAK-binding protein
TAK1	Transforming growth factor beta-activated kinase 1
TLR	Toll-like receptor
TIR-1	Toll/interleukin-1 receptor-1
TNFα	Tumor necrosis factor alpha
TOL-1	TOLI *Drosphila* family 1
TRAF	TNF receptor associated factor
TRIF	TIR-domain-containing adapter inducing interferon beta
UBD	Ubiquitin binding domain
UBL	Ubiquitin-like protein
UBP	Ubiquitin binding protein
UBR5	Ubiquitin-protein ligase E3 component N-recognin 5
UCH	Ubiquitin C-terminal hydrolase

ULP	Ubiquitin-like protease
UPR	Unfolded protein response
UPS	Ubiquitin proteasome system
USP	Ubiquitin-specific protease
WNT	Wingless and integration site
WWP-1	WW domain-containing E3 ubiquitin protein ligase 1
XBP-1	X-box binding protein homolog 1
ZIP-3	bZIP transcription factor family member 3
ZUFSP	Zinc finger with UFM1-specific peptidase domain protein

1. INTRODUCTION

1.1 INNATE IMMUNITY

Innate immunity is the first line of host defence against infection [1]. It is an ensemble of protective mechanisms, found in all multicellular organisms. It comes into play when pathogen avoidance, behavioural strategies that limit pathogen exposure, fail [2]. Unwanted microbial colonisation of exposed tissues, like the lungs or intestine, or microbial breaches of skin and other physical barriers, can lead to infection [3]. Host organisms are capable of sensing pathogens or the damage they cause. Notably, a large set of pattern recognition receptors (PRRs) that includes Toll-, RIG- and NOD-like receptors (TLRs, RLRs and NLRs), bind microbe-associated molecular patterns (MAMPs) such as lipopolysaccharides (LPS), peptidoglycans or viral nucleic acids, or sense damage-associated molecular patterns (DAMPs) such as extra-cellular ATP, urea or mitochondrial DNA [4]. In parallel to this pattern-triggered immunity (PTI), hosts monitor the infection-mediated perturbation of core cellular processes, aka 'homeostasis-altering molecular processes' (HAMPs) [5]. Recognition of MAMPs or DAMPs by PRRs or the sensing of HAMPs in turn trigger signaling cascades that lead to an immune response, to eliminate the pathogen and reduce the negative impact of the infection on host fitness [6]. The microbial-killing branch of this immune response is called resistance and ranges from the production of specialized inflammatory cytokines and microbicidal molecules such as antimicrobial peptides (AMP) or reactive oxygen species (ROS), to cellular processes such as phagocytosis of extracellular microbes and autophagy of intracellular pathogens [7-9]. The homeostatic branch, called tolerance, ensures host survival, by economizing energy and responding to the stress and damage caused by pathogens or by the innate immune response itself (i.e. immunopathology) [10, 11]. It can involve activation of the ubiquitin–proteasome system (UPS) or autophagy to counteract proteotoxic and organelle damage, and induction of the Unfolded Protein Response (UPR) to resolve endoplasmic reticulum (UPR^{ER}) or mitochondrial (UPR^{mt}) dysfunction [12-15].

1.2 INNATE IMMUNITY IN *C. ELEGANS*

Studies with *Drosophila melanogaster* have been instrumental to the discovery of conserved immune pathways, most notably the TLR/NF-κB pathway [16]. Similarly, work with *Caenorhabditis elegans* has contributed to our understanding of the origins and mechanisms of innate immunity [17]. This 1 mm long free-living nematode is a microbivore that is found in the wild in decaying organic matter such as rotting fruits and stems [18]. This microorganism-rich environment provides food to *C. elegans* but it also exposes the animal to potentially harmful pathogens [19]. Indeed, *C. elegans* can be naturally infected by various species of bacteria, fungi, viruses and other parasites [19], against which it has a sophisticated immune system [17]. *C. elegans* lacks circulatory immune

cells and an adaptive immune system. Its innate immune responses are mainly provided by the tissues directly exposed to pathogens such as the epidermis, or enterocytes in the intestine. Interestingly, *C. elegans* has been shown to mount an immune response when artificially infected with several human pathogens, thus opening the possibility of using *C. elegans* as a clinically-relevant infection model [20]. With its small size, numerous offspring, hermaphrodite reproductive mode and short life cycle, *C. elegans* is very easy to culture. Together with the numerous tools that have been developed since the 1960's, *C. elegans* represents a very tractable research model, including for the field of immunology [21]. One aspect of *C. elegans* biology that needs to be mentioned from the outset, however, is that much of its innate immune system has diverged substantially from other organisms [22, 23]. While the RIG-I homologue DRH-1 appears to act as a PRR that senses viral replication products to trigger anti-viral RNAi [24] as well as a transcriptional host response [25], there are no NLRs and only one TLR homologue, TOL-1, in *C. elegans* [23, 26]. TOL-1 is involved in pathogen aversion [26-28] but doesn't seem to be involved in MAMP recognition, although it has been suggested to be required for peptidoglycan-mediated tolerance against *Salmonella* infection [29]. In addition, several components of the TLR/NF-κB pathway have been lost from *C. elegans* genome, including MyD88, IKK and NF-κB itself [23]. Many of the large family of G-protein coupled receptors (GPCRs) might play a role in MAMP/DAMP recognition in *C. elegans* [30]. Currently, there is one well-documented example of a GPCR acting as a DAMP sensor to regulate AMP gene expression [31]. Other GPCRs have been implicated in immune defences, but their precise mode of action remains enigmatic [32, 33]. On the other hand, mechanisms of cellular homeostasis appear to be highly conserved in *C. elegans*, and monitoring HAMPs, referred to as 'surveillance immunity', appears to be a major infection-sensing mechanism in the nematode [34].

1.3 UB AND UBL POST-TRANSLATIONAL MODIFICATIONS

One of the processes that can be monitored is protein homeostasis. This is controlled in part by ubiquitination, a post-translational modification that regulates virtually all aspects of physiology [35]. Ubiquitin (Ub) is a highly conserved 76-amino acid polypeptide that covalently modifies a substrate on one (mono-) or several (multi-ubiquitination) lysine residue(s) via its C-terminal glycine (G76) (**Figure 1a,b**). A high diversity of homo- and heterotypic poly-Ub chains can also be formed since a distal Ub can attach to the first methionine (M1) or to one of the 7 lysine residues of a proximal Ub [36] (**Figure 1b**). Ubiquitination arises from the sequential action of 3 Ub-modifying enzymes: a Ub-activating enzyme (E1), a Ub-conjugating enzyme (E2) and a Ub-ligase enzyme (E3) (**Figure 1c, Table 1).** Ubiquitination can be reversed by de-ubiquitinating enzymes (DUBs) (**Figure 1c**, **Table 1**) [37]. Ub binding proteins (UBP), aka Ub receptors, can bind mono-, multi- and poly-Ub chains of different topology via a variety of Ub binding domains (UBD) with different affinities and avidities

[38]. These interactions with Ub receptors in turn dictate each substrate's fate, and often lead to degradation [39]. Indeed, K48 poly-ubiquitination is known to promote protein degradation by the 26S proteasome, a large multi-subunit complex of 2.5 MDa (**Figure 1c, Table 1**) [40]. In addition to the ubiquitin-proteasome system (UPS), ubiquitination is also involved in the degradation of protein aggregates and damaged organelles through selective autophagy via autophagic receptors that harbour UBDs [41]. Non-degradative functions of ubiquitination are also well documented, including roles in signal transduction [42] (**Figure 1c**).

The specificity of the ubiquitination reaction is ensured by the substrate-binding domain of E3s. Once bound, E3s transfer Ub to the target proteins via a HECT, RING, RBR or U-box catalytic domain [43]**.** Unlike most E3s in which the catalytic domain and the substrate recognition domain are present on a single polypeptide, a sub-category of E3-RING proteins called Cullin RING ligases (CRLs) act as multimeric complexes with a common tripartite structure: i) a cullin subunit forms the E3-CRL scaffold, ii) a catalytic RING-finger subunit bridges the cullin to the E2, iii) a substrate recognition subunit (SRS) interacts with the substrate and the cullin scaffold (**Figure 2a**). SRSs include proteins from the BTB, F-box, SOCS-BC and DCAF families. Except for SRSs from the BTB family that bind directly to the cullin scaffold, other SRSs require an adaptor subunit of the SKR, Elongin or DDB1 family to bind cullin [44] (**Figure 2b and Table 1**). All these enzymes are conserved in *C. elegans* but differ in number with human, with for example, a marked expansion of the F-box family of E3-CRLs [45] (**Table 1**).

Similar to Ub, a set of evolutionary conserved Ub-like proteins (Ubls) such as Small Ubiquitin-Like Modifier (SUMO) can also covalently modify protein substrates [46]. SUMOylation also engages E1-, E2- and E3-like enzymes and Ubl-specific proteases (ULPs), conserved in *C. elegans* [47] (**Table 1**). SUMOylation is involved in a wide range of physiological processes and the majority of SUMOylated substrates are nuclear with various functions, such as transcriptional regulation and nucleo-cytoplasmic transfer [48, 49].

1.4 UB-RELATED PROCESSES IN *C. ELEGANS* IMMUNITY

Multiple lines of evidence support a role for ubiquitination and SUMOylation in the regulation of innate immunity [35, 50]. Notably, alterations of Ub-related enzymes are associated with inflammatory and immune diseases [51, 52]. Most of our knowledge on the importance of these post-translational modifications in innate immunity, however, is related to the well-studied TLR/NF-κB pathway, while much less attention has been paid to the regulation by Ub and Ubl in other immune processes. *C. elegans* offers an opportunity to study the impact of Ub and SUMO modifications in the absence of TLR/NF-κB. We will review here the current knowledge of how innate immunity is regulated by Ub-related processes at all steps of *C. elegans* infection (**Table 2**).

We will describe Ub-related processes involved in 1) the induction of innate immune pathways, 2) the regulation of immune signaling, and 3) the host effector response. We will discuss the conservation of these regulatory mechanisms in mammals and how learning about Ub and Ubl regulation in the immune system of *C. elegans* could have an impact on the development of diagnostic tools and new therapeutic treatments for human immune and infectious diseases.

2. ALTERATION OF UPS, A HAMP TRIGGERING SURVEILLANCE IMMUNITY

Aberrant cellular physiology, if detected, can provoke an immune response [34]. An elegant study using *Nematocida parisii* has unravelled how infection-induced UPS perturbation can act as a HAMP triggering an immune reaction in *C. elegans* [53]. *N. parisii* is a fungus-related intracellular pathogen belonging to the phylum Microsporidia [54]. These eukaryotic pathogens can infect a variety of animal hosts including humans and can cause lethal diarrhoea in immunocompromised patients [55]. In *C. elegans*, *N parisii* invades intestinal cells and leads to animal death [54]. The analysis of the *N. parisii*-induced transcriptional response revealed an enrichment in ubiquitin-modifying enzymes, which led the authors to demonstrate that the UPS is required for defence against *N. parisii*. Notably, they observed that the UPS plays a greater role in controlling infection when microsporidia growth is hampered by anti-microsporidial drugs [53]. These interesting findings suggested that *N. parisii* may suppress or evade ubiquitin-mediated host defences. Using RT-qPCR and fluorescent reporters, they found that *N. parisii* infection induces a transcriptional response similar to that seen upon treatment with proteasome inhibitors. In addition, down-regulation of ubiquitin genes *ubq-1* and *ubq-2* or proteasomal subunits *pas-5* and *rpn-2* by RNAi also induced the expression of some *N. parisii* response genes [53]. These results clearly demonstrated that perturbing the UPS is sufficient to induce surveillance immunity (**Figure 3a**). They also illustrate the dynamic relationship between a host and its pathogen: *C. elegans* has evolved a mechanism to monitor UPS efficacy and to trigger an immune response if it is hampered by a pathogen. How *N. parisii* alters UPS activity and how *C. elegans* monitors UPS alteration requires further study.

Abnormal UPS activity has been proposed to contribute to the abnormal cell death that is a feature of neurodegenerative disorders such as Parkinson's disease (PD) [56, 57]. Several species of environmental bacteria from the genus *Streptomyces* produce secondary metabolites such as lactacystin with proteasome inhibitor activity [58]. Caldwell *et al.* studied the potential role of *S. venezuelae* in dopaminergic (DA) neuronal cell death, in a *C. elegans* model of PD [59]. They found that exposure of old animals to *S. venezuelae*-conditioned media triggers specific DA neuronal cell death. The nature of the secondary metabolite responsible for this phenotype has yet to be defined, but it was shown to have an inhibitory activity toward the proteasome as it heightened the fluorescence of CFP::CL-1, a proteasome-targeted fluorescent molecule, when it was expressed

within *C. elegans* DA neurons. In addition, they showed in a follow-up study that the accumulation of protein aggregates is key to neuronal cell death [60]. Indeed, *S. venezuelae* secondary metabolites induced DA neuronal cell death even in young animals when they overexpressed the aggregation-prone protein alpha-synuclein. Caldwell *et al.* further highlighted the importance of the UPS in maintaining alpha-synuclein expressing neurones alive by artificially inhibiting the UPS. Thus, they could induce alpha-synuclein-associated DA cell death by treating animals with the pharmacological proteasome inhibitor MG132 or by using RNAi to downregulate the expression of the Ub modifying enzyme E1 or of proteasomal subunits. This effect of proteasomal inhibition was epistatic to the *S. venezuelae* secondary metabolite thus confirming the capacity of the metabolite to inhibit proteasomal function [60]. Similar neurodegeneration in *C. elegans* has been observed in several other models of bacterial and fungal infection [61-63]. Interestingly, neuronal cell death observed during fungal infection was shown to be a consequence of the infection-mediated innate immune response. The antimicrobial peptide NLP-29 binds to its cognate receptor NPR-12 expressed on neurons and provokes autophagic cell death [63]. Whether the proteotoxic damage-induced neuronal cell death observed during exposure to *S. venezuelae* secondary metabolites is part of a *bona fide* but ultimately harmful immune response remains to be established.

In a recent study, it was proposed that perturbation of ubiquitin homeostasis, but not proteasomal inhibition *per se*, may act as a HAMP, conferring anti-infective capacity to mouse macrophages through the production of a robust but transient burst of reactive oxygen species (ROS) [64]. This suggests that perturbation at different steps of the UPS can trigger an immune response. It will be interesting to see whether similar ubiquitin-sensitive regulatory signals are conserved in *C. elegans*.

3. UBIQUITIN-RELATED PROCESSES IN THE REGULATION OF IMMUNE SIGNALING

3.1 REGULATION OF THE NEURO-IMMUNE SENSING OF PATHOGENS

Pathogen or parasite avoidance behaviour is an immune strategy widespread across the animal kingdom [2]. This “better safe than sorry” immune mechanism is likely to be cost-effective as it can prevent or reduce pathogen intake. *C. elegans* is a model of choice for understanding the role of the nervous system in this type of behaviour at the molecular and cellular level [65]. Nearly a third of *C. elegans’* somatic cells are neurons. The anatomy of each of its 302 neurons and their synaptic connectivity are clearly defined allowing the establishment of a simple behavioural neuronal network [66-69]. *C. elegans* uses its sensory system to detect bacterial secondary metabolites, neuropeptides induced by infection, and to measure oxygen concentration. This in turn mediates

pathogen avoidance or aversive learning [70]. *C. elegans* avoids many environmental pathogens including *Pseudomonas aeruginosa* and *Serratia marcescens* [27, 68, 71-73]. Failure to avoid *P. aeruginosa* leads to infection, with the bacteria colonizing the nematode intestine, killing the worms [74]. Interestingly, Chang *et al.* demonstrated a critical role for the E3-HECT ligase HECW-1 in *C. elegans*' behavioural avoidance of *P. aeruginosa* [75]. Using comparative genomics of wild isolates of *C. elegans*, Chang *et al.* identified two naturally occurring polymorphism in the coding sequence of *hecw-1* resulting in reduced avoidance behaviour compared to the reference laboratory strain N2. Conversely, a deletion allele of *hecw-1* conferred increased avoidance behaviour, indicating that HECW-1 negatively regulates avoidance behaviour and that both polymorphisms most likely provide a gain of function to HECW-1. Using reporter strains, tissue specific rescue and neuronal ablation, the authors showed that HECW-1 functions specifically in the pair of outer labial (OLL) sensory neurons localized in the anterior bulb of the pharynx to inhibit avoidance behaviour (**Figure 4**). Epistasis analysis suggested that HECW-1 represses the activity of the neuropeptide receptor NPR-1. This GPCR chemoreceptor has been implicated in avoidance phenotype, aversive learning and the innate immune response against *P. aeruginosa* [73, 76, 77]. The regulation of NPR-1 by HECW-1 is likely indirect as they do not function in the same neuronal cell. Indeed, evidence suggests that HECW-1 represses the activity of NPR-1 in RMG, a pair of inter/motoneurons localized in the posterior bulb of the pharynx [75] (**Figure 4**). Altogether, these data demonstrate the critical role of an E3 ligase in extracellular pathogen sensing and the regulation of avoidance behaviour. How HECW-1 is regulated during infection, what its direct substrates are and how it affects NPR-1 activity remain, however, to be determined (**Figure 4**). Several substrates of the mammalian ortholog HECW1 (aka NEDL1) have been described, including the EGF-like receptor ErbB4 and a transducer of the WNT signaling pathway, Dishevelled-1 [78, 79]. Whether the EGF or the WNT pathway are involved in avoidance behaviour in *C. elegans* merits exploration. Relatively little is known about the molecular mechanism of parasite avoidance behaviour in mammals [80], although details are starting to emerge (e.g. [81]). Interestingly, Dishevelled proteins are expressed in olfactory sensory neurons [82]. Whether NEDL1 is involved in the regulation of Dishevelled proteins in olfactory sensory neurons and regulates aversive behaviour remain to be established.

3.2 REGULATION OF INNATE IMMUNE SIGNALING PATHWAYS

3.2.1 Regulation of the p38/PMK-1 immune pathway

The PMK-1 signaling pathway, homologous to the mammalian p38 MAPK pathway, is involved in the regulation of immune responses against bacterial and fungal infection in *C. elegans*

[83, 84]. The PMK-1 “cassette” comprises NSY-1, SEK-1 and PMK-1 (homologous to mammalian ASK1, MKK3/6 and p38, respectively) and has been shown to act downstream of the TIR-domain protein TIR-1, the nematode orthologue of the human SARM1 [84-86]. We will review in this section the several lines of evidence that ubiquitination is an important regulator of the PMK-1 pathway in *C. elegans* (**Figure 5**).

The ascomycete fungus *Drechmeria coniospora* is the best-characterized causative agent of epidermal infections in *C. elegans* [87]. *D. coniospora* produces conidia that attach and then pierce *C. elegans*’ cuticle, and send hyphae throughout the animal eventually leading to its death [88]. Rapidly upon infection, *C. elegans* triggers a host response characterized by the epidermal expression of AMPs, notably a cluster of the neuropeptide-like proteins (NLPs) including NLP-29 [86, 89]. Induction of these AMPs is under the control of the TIR-1/PMK-1 pathway. In the epidermis, this pathway lies upstream of the STAT-like transcription factor STA-2 and downstream of DCAR-1, the only known DAMP-sensing GPCR alluded to above. DCAR-1 is activated by its cognate ligand, 4-hydroxyphenyllactic acid [31]. *dcar-1* was one of 360 genes found in an RNAi screen of the whole genome (ca. 20,000 genes) whose knockdown prevented *D. coniospora*-induced expression of an *nlp-29p::gfp* reporter [31]. Among the other genes, there were ten ubiquitin-related genes encoding one E2 (*let-70*), three monomeric E3s *(*E3-HECT *hecd-1,* E3-RING *rbpl-1* and the E3-U-box *prp-19),* two E3-CRL subunits (the SKR adaptor *skr-1* and the SRS *dcaf-1*), one DUB (*usp-39*) and three proteasomal subunits *(pas-3, pas-5 and pbs-2)* (**Figure 5**) [90]. The presence of an E2 enzyme and proteasomal subunits suggests that ubiquitination, and notably degradative ubiquitination, may be involved in the regulation of the epidermal p38/PMK-1 pathway. Further studies need to be performed to assess the specific role of each E3 and DUB found in this screen, and to determine whether they directly, or indirectly, regulate factors specific to *D. coniospora* infection or elements of the TIR-1/PMK-1 cassette.

The PMK-1 pathway is also required for intestinal immune responses upon infection by several bacteria such as Gram-negative bacteria *S. marcescens* and *P. aeruginosa* and the Gram-positive bacteria *Enterococcus faecalis* [83, 85, 91]. In the intestine, PMK-1 acts upstream of the transcription factor ATF-7 [92]. A small-scale RNAi screen was undertaken by Alper *et al.* who used a reporter of C-type lectin expression (*clec-85p::gfp*) to identify innate immune regulators in *C. elegans* [93]. *clec-85* is expressed in the intestine and is induced by *S. marcescens* [94]. The *clec-85p::gfp* reporter is regulated by multiple immune pathways, including PMK-1/ATF-7 following *P. aeruginosa* infection [91, 95] (**Figure 5**). Accordingly, clones targeting *nsy-1* and *tir-1* were found among the RNAi clones that altered *clec-85p::gfp* expression [93]. In addition, the E2 *let-70* as well as two E3s, the E3-RBR *ari-1.3* and the E3-RING *siah-1* were found to modulate expression of *clec-85::gfp*, reinforcing the idea that ubiquitin-related processes regulate immunity including the TIR-1/PMK-1 pathway [93] (**Figure 5**). Interestingly, several studies have suggested a potential innate

immune role for the mammalian homologues of *siah-1* [96]. In accordance with the hypothesis that SIAH-1 might control expression of *clec-85* through the regulation of the TIR-1/PMK-1 pathway, SIAH-1 was found by yeast-2-hybrid screen to interact with TIR-1 [86]. SIAH-1 might interact with and ubiquitinate TIR-1 to regulate the PMK-1 pathway following pathogen exposure (**Figure 5**). The outcome of this ubiquitination would unlikely be degradative, as SIAH-1 is a positive regulator of the TIR-1/PMK-1 signaling pathway in the intestine. On the other hand, no effect of *siah-1* RNAi was observed on *nlp-29p::gfp* expression in the epidermis following *D. coniospora* infection [90], suggesting that the putative regulation of the TIR-1/PMK-1 pathway by SIAH-1 could be tissue-specific. Interestingly, the human homologue SIAH2 has been shown to be phosphorylated by p38, thereby modulating its localization and substrate accessibility, but a reciprocal regulation of p38 by mammalian SIAH proteins has yet to be clearly established [97-99].

"Regulation of longevity by E3 ubiquitin-protein ligase" (RLE)-1, has also been shown to modulate host defence against *P. aeruginosa,* in this case through the regulation of the MAP3K NSY-1 [100]. Seeking new regulators of the NSY-1 orthologue ASK1, Maruyama *et al.* identified *roquin-2,* one of the two mammalian homologues of *rle-1,* and showed that it is required for H_2O_2-induced ASK1 degradation. In addition, Roquin-2 was found to interact with ASK1 and to induce its ubiquitination. Although ASK1 regulation by Roquin-2 was not assayed *in vitro,* these results strongly suggested that Roquin-2 is an E3 ligase regulating the degradative ubiquitination of ASK1. In *C. elegans*, expression levels of NSY-1 were increased in *rle-1* mutants compared to wild-type animals. Importantly, *rle-1* mutation or its down-regulation by RNAi was found to increase survival of animals infected with *P. aeruginosa*, in a *nsy-1* dependent manner, suggesting that increased expression of NSY-1 in RLE-1 deficient animals is sufficient to increase host defence. Consistent with such an idea, a strain over-expressing a NSY-1::GFP fusion protein displayed increased survival compared to the wild-type strain. Interestingly, mutation of *rle-1* or over-expression of NSY-1::GFP increased the level of PMK-1 phosphorylation in infected animals, suggesting that RLE-1 may regulate PMK-1 activity via NSY-1 to control host defence against *P. aeruginosa* infection [100] (**Figure 5**).

The importance of Roquin-2 mediated ASK1 ubiquitination has not been assessed in the context of innate immunity despite the fact that Roquin-2, together with Roquin-1, is known to have redundant innate, as well as adaptive immune functions [101]. These functions have, however, been attributed to the proteins' mRNA binding capacity, mediating mRNA decay of key immune genes such as Icos, IFNγ and TNFα [102] [103]. Other major innate immune genes have been found to be regulated post-transcriptionally by Roquin 1, notably the DUB A20 whose de-ubiquitinating activity represses the NF-κB pathway [104, 105]. Nonetheless, one study linked the E3 ligase activity of Roquin-1 to adaptive immunity, through the dampening of AMPK signaling

driving T-cell humoral immunity [106]. Thus, more work is required to determine if the E3 ligase activity of Roquin-1 and Roquin-2 are important for innate immune responses.

3.2.2. Regulation of the insulin/ IGF-1 signaling (IIS) pathway

The insulin/insulin-like growth factor 1 (IGF-1) signaling (IIS) pathway is highly conserved. It involves the IGFR homologue, DAF-2, a receptor tyrosine kinase. Upon ligand binding, DAF-2 triggers a kinase cascade that ultimately phosphorylates and inhibits the FOXO transcription factor DAF-16. The IIS pathway regulates multiple physiological processes including development, longevity, metabolism, as well as resistance to various environmental stresses [107]. It also plays a major role in innate immunity controlling host defence against both Gram-positive and Gram-negative pathogenic bacteria [108, 109]. The DAF-2 pathway has also been implicated in host defence against the pore-forming toxin (PFT) Cry5B from the soil bacterium *Bacillus thuringiensis* (Bt) [110]. PFTs are cytotoxic proteins that damage the plasma membrane of host cells and are required for virulence of many bacterial pathogens [111]. Chen *et al.* found that the DAF-2/PI3K/PDK-1/DAF-16 pathway is required for host defence against PFTs. They found evidence suggesting that in addition to the canonical pathway, a non-canonical DAF-2 pathway, independent of DAF-16, bifurcates at the level of PDK-1 to mediate host response to PFT (**Figure 6**). Taking advantage of publicly available datasets of protein-protein interactions, Chen *et al.* identified WWP-1 as a potential PDK-1 interactor [110]. WWP-1 is an E3-HECT of the NEDD4 subfamily [112]. They found that loss of function mutation of *wwp-1* or down-regulation of its expression using RNAi drastically reduced survival of animals exposed to Cry5B, indicating that WWP-1 is a critical positive regulator of host defence against this PFT. Interestingly, *wwp-1* RNAi was found to rescue partially the *daf-2* immune phenotype and completely rescued *daf-2;daf-16* double mutants, indicating that DAF-16 and WWP-1 must act in parallel downstream of DAF-2 to mediate full resistance to Cry5B PFT (**Figure 6**). Whether the ubiquitin-ligase activity of WWP-1 is required for host defence against PFT needs to be assessed but these results strongly suggest that ubiquitination is involved in innate immunity downstream of a non-canonical DAF-2 signaling cascade. The functional consequences of the PDK-1/WWP-1 interaction have not been explored either, but as several human E3-HECT are inhibited via phosphorylation, a model can be proposed with PDK-1 controlling WWP-1 activity through phospho-inhibition [113]. Note that Chen *et al.* also found that WWP-1 was required for host defence against *P. aeruginosa* but did not determine whether this was also dependent on the *daf-2* pathway [110]. As it will be discussed in the following section, WWP-1 might be involved in the tolerance response against *P. aeruginosa* via regulation of the UPR^{mt} [114].

WWP-1 is homologous to three mammalian E3-HECTs from the NEDD4 family: ITCH, WWP1 and WWP2 [115]. Interestingly, ITCH, so named in reference for the skin-scratching behaviour in

mice lacking this protein, has been implicated in the development of auto-immune diseases both in mice and humans [116]. This auto-immunity appears to be a consequence of multiple alterations of the adaptive immune system with aberrant CD4 T cell activation and humoral responses [116]. Of note, although in *C. elegans* WWP-1 was found to work in parallel to DAF-16, ITCH was found to mediate differentiation of CD4 follicular helper T cells by interacting and mediating UPS regulation of the DAF-16 homologue FOXO1 [117]. On the other hand, ITCH also plays a role in innate immunity and has been shown to dampen inflammation via the ubiquitin-based regulation of various kinases (RIP1, RIP2, TAK1), and UBP (TAB1) which regulate the NF-κB, p38 and JNK MAPK pathways [118-121]. WWP1 and WWP2 have also been involved in the regulation of TLR4- and TLR3- mediated inflammation through the UPS regulation of TRAF6 and TRIF, respectively [122, 123]. Hence, WWP-1's role in innate immunity is conserved among its three mammalian homologues.

3.3 . REGULATION OF IMMUNE TRANSCRIPTION

We described above how ubiquitination can be involved in the regulation of innate immune signaling pathways. We will now give several examples of how Ub- and Ubl-modifying enzymes can also affect the activity of transcription factors and their co-factors, and thereby modulate host responses to infection.

3.3.1 Regulation of the GATA transcription factor ELT-2

The erythroid-like transcription factor family (ELT)-2 is gut-specific transcription factor from the GATA family that is essential for the formation and the normal function of *C. elegans* intestine [124, 125]. In addition, ELT-2 plays an important role in the regulation of genes required for host defence, or for recovery after infection [126-128]. Recently, the proteasomal subunit RPT-6 was found to functionally interact with ELT-2. Although the ATPase activity of RPT-6 was found to be required for this interaction, the overall proteolytic capacity of the proteasome did not influence ELT-2 transcriptional activity [129]. On the other hand, a UPS-dependent regulation of ELT-2 has been revealed in the context of infection by *Burkholderia pseudomallei.* This Gram-negative bacterium is the causative agent of melioidosis and *C. elegans* animals fed with *B. pseudomallei* suffer from a lethal infection [130-132]. ELT-2 is implicated in host defence against *B. pseudomallei* as RNAi against *elt-2* reduces the survival of infected animals [133]. Further, ELT-2 has been found to be specifically targeted by *B. pseudomallei*, but not by other pathogens, as a strategy to suppress *C. elegans* immunity [133]. Whole-genome transcriptome analysis revealed that a set of ELT-2-dependent genes is progressively down-regulated over a time course of *B. pseudomallei* infection. Using a *elt-2::gfp* reporter strain, the authors could correlate this decrease with a reduction of ELT-

2::GFP protein levels. Interestingly, down-regulation of ELT-2::GFP during infection could be blocked by RNAi-treatment against the ubiquitin-encoding genes *ubq-1 and ubq-2* as well as by RNAi targeting the proteasomal subunit *rpt-2*, suggesting that ELT-2 is degraded by the UPS during *B. pseudomallei* infection. Lee *et al.* identified two host genes encoding RING-finger proteins with putative E3 ligase activity whose expression was induced during infection and required for ELT-2::GFP degradation. Direct involvement of these putative E3 ligases in ELT-2 ubiquitination was not established but these results suggested a mechanism used by *B. pseudomallei* to degrade actively ELT-2 in order to counteract the immune response of *C. elegans* [133]. Additionally, degradation of the ELT-2::GFP protein was only observed when animals were infected with wild-type bacteria but not with type III secretion system (T3SS)-deficient strains, indicating that injected bacterial effectors might be required for ELT-2 degradation. To mount an efficient infection many pathogens secrete toxins or inject effectors that can interfere with the host cell ubiquitin machinery [134]. In a mammalian cell line model, *B. pseudomallei* has been found to interfere negatively with the Ub and Ubl machinery via the T3SS-injected effector cycle inhibiting factor (Cif) homologue in *B. pseudomallei*, CHBP [135]. This effector bears a deamidase activity that targets Ub and the Ubl NEDD8, a critical regulator of E3-CRL activity. Deamidation of a conserved glutamine in position 40 of Ub and NEDD8 leads to reduced E3-ligase catalysed ubiquitin-chain synthesis and suppression of E3-CRL activity [135]. Determining whether CHBP is injected into *C. elegans* intestinal cells and is linked, via a possible compensatory mechanism, to enhanced UPS degradation of ELT-2, will require further study. The mammalian homologue of ELT-2, the transcription factor GATA4, is a central regulator of cardiac development [136] but it also acts redundantly with GATA6 to regulate intestinal epithelial differentiation during development and to maintain proper epithelial structure [137]. In addition, GATA4 is critical to the maintenance of gut barrier function and mucosal integrity following injury in mice [138], suggesting that targeting GATA4 for UPS degradation might represent an efficient strategy for bacteria invading the intestine. GATA4 UPS degradation in cardiomyocyte embryonic bodies was shown to be induced by high H_2O_2 levels, and required the JNK cascade [139], but the identity of the E3 regulating its ubiquitination is still unknown. Further studies must thus be conducted to identify Ub-modifying enzymes mediating GATA4 UPS degradation and determine whether targeting GATA4 is a conserved strategy used by pathogens to facilitate intestinal invasion.

3.3.2 Regulation of DAF-16

As a major innate immune transcription factor acting downstream of the IIS pathway, it is no surprise that DAF-16 needs to be tightly regulated. If phosphorylation appears to be the main regulatory post-translational modification controlling DAF-16 nuclear localization during infection, ubiquitination has recently emerged as another regulatory mechanism. Its ubiquitination was first

described in the context of normal longevity and was shown to involve the RLE-1 E3-RING ligase mentioned above. Mutation of *rle-1* was found to stabilize DAF-16 specifically at the protein level, and to increase *C. elegans*' longevity [140]. In contrast to the DAF-16-independent role of *rle-1* in resistance against *P. aeruginosa* infection described above [100], Heimbucher *et al.* demonstrated the importance of regulation of DAF-16 by ubiquitin in innate immunity [141]. Using an unbiased biochemical approach, Heimbucher *et al.* identified the Meprin-Associated Traf Homology (MATH) domain containing deubiquitinating enzyme, MATH-33, as a new binding partner of DAF-16a in conditions of low IIS, when DAF-16 is active in the nucleus [141]. This interaction could be confirmed by co-immunoprecipitation assays, and endogenous MATH-33, which was found to be predominantly expressed in intestinal cells, colocalized with GFP::DAF-16 in cell nuclei in a *daf-2* mutant. MATH-33 was found to display a *bona fide* deubiquitinase activity toward DAF-16 *in vitro*. Mutation of *math-33* also led to increased GFP::DAF16 ubiquitination and decreased total DAF-16 protein levels in *C. elegans*. Interestingly, this effect was only seen in a *daf-2* mutant background suggesting that IIS signaling may regulate the nuclear targeting of MATH-33 or its physical association with DAF-16. In accordance with MATH-33 positively regulating DAF-16 stability, it was found to regulate the various physiological processes controlled by the IIS pathway, including host defence against *P. aeruginosa*. Indeed, *math-33* mutation reduced the survival of *daf-2* infected mutants to the level of *daf-16;daf-2* double mutants, indicating that ubiquitination of DAF-16 during infection is an important means to control its activity and thus host defence. *rle-1* was found to act epistatically to *math-33* to regulate *daf-16*-dependent lifespan extension, metabolism and development. Its role in host defence in the context of low IIS has yet to be explored. Given the result obtained by Maruyama *et al.* [100], it is conceivable that MATH-33 counteracts DAF-16 ubiquitination mediated by an as yet unknown E3 ligase during infection to promote full transcription factor activity (**Figure 6**).

The mammalian MATH-33 homologue, ubiquitin-specific peptidase (USP)-7, has been found to regulate FOXO proteins, the homologues of DAF-16. In this case, however, USP7 negatively regulates the transcriptional activity of FOXO proteins by promoting de-ubiquitination of a non-degradative mono-ubiquitination [142, 143]. Control of FOXO proteins by USP7 has been explored in the context of oxidative stress and serum starvation but evidence of such regulation in innate immunity in mammal is lacking. Nevertheless, USP7 has been found to play a key role in innate immunity by interacting and deubiquitinating NF-κB, which in turn increases promoter occupancy and transcriptional activity of NF-κB downstream of TLR and TNF-receptor activation [144, 145]. Thus, although the molecular function of MATH-33 seems to have substantially drifted over the course of evolution, its role in innate immunity has been conserved in mammals through the targeting of the key immune transcription factor, NF-κB.

3.3.3 Regulation of the transcriptional co-factor AKIR-1

In *Drosophila* and mammals, NF-κB forms a complex with its co-factor Akirin, which links the transcription factor to chromatin remodelers, and is required for innate immune responses [146-148]. The E3-HECT ubiquitin ligase Hyd/UBR5 ubiquitinates Akirin thereby modulating the expression of NF-κB target genes [149]. In *C. elegans*, which lacks NF-κB, the Akirin protein, AKIR-1, interacts with the POU-class transcription factor CEH-18 (**Figure 5**). Interestingly it does, however, bind the Hyd/UBR5 homologue, UBR-5. In addition, AKIR-1 also binds the DUBs MATH-33 and USP-24 that are implicated in ubiquitin-mediated protein turnover (**Figure 5**). Using fluorescent reporter strains and qPCR analysis, AKIR-1 was found to be essential for the induction of AMP genes like *nlp-29* upon fungal infection (**Figure 5**). In addition, *akir-1* RNAi substantially reduced the survival of infected animals compared to control animals [150]. Significantly, *in vitro* ubiquitination activity could be detected within AKIR-1 protein complexes immuno-purified from *C. elegans* after infection, not before [150]. Overall, these results indicate that the E3-HECT ubiquitin ligase Hyd/UBR5 has a conserved function as a nuclear selector for gene activation during the immune response, even if its target transcription factor complex in *C. elegans* is distinct from that in other species.

3.3.4 Regulation of ATFS-1, DVE-1 and ZIP-3 upon mitochondrial damage

Mitochondria are key intracellular organelles targeted by many pathogens that benefit from mitochondrial dysfunction to infect their host efficiently [151]. For instance, *P. aeruginosa* produces various virulence factors such as cyanide and phenazines that can block the mitochondrial respiratory chain. In *C. elegans*, hydrogen cyanide causes lethal paralysis, while phenazines triggers production of toxic ROS [152, 153]. Mitochondrial disruption can, however, be sensed by a host surveillance machinery, triggering a UPR^{mt} and host defence [13]. In the nematode, this transcriptional program is controlled by the bZIP transcription factor ATFS-1 and the homeobox transcription factor DVE-1 [13]. Both ATFS-1 and DVE-1 regulate mitochondrial stress response genes [13]. In addition, ATFS-1 induces expression of genes regulating mitochondrial energy metabolism, detoxification and innate immunity to promote mitochondrial recovery, restore cellular homeostasis and provide host defence against pathogens [13]. Pellegrino *et al.* described how infection of *C. elegans* with *P. aeruginosa* triggers mitochondrial damage in the intestine, which in turn promotes the UPR^{mt} and host defence [154]. Indeed, an *atfs-1* mutant was found to be highly susceptible to *P. aeruginosa* infection [154]. Interestingly, the SUMO-specific peptidase ULP-4 was shown, through a genome-wide RNAi screen, to be required for the activation of the UPR^{mt} [155]. In addition, ULP-4 expression was found to be increased upon mitochondrial stress [155, 156]. Further, ULP-4 was found to be required for host defence against *P. aeruginosa*. Indeed, down-

regulation of *ulp-4* by RNAi reduced the expression of innate immune and detoxifying genes upon *P. aeruginosa* infection, and was associated with an enhanced susceptibility to pathogen (Esp) phenotype [156]. In yeast-two-hybrid assays, ULP-4 was found to interact with both ATFS-1 and DVE-1. SUMOylation assays revealed that ATFS-1 and DVE-1 are SUMOylated on lysines K326 and K327, respectively, and that ULP-4 promotes their deSUMOylation [156] (**Figure 7**). In addition, SUMOylation regulated negatively these transcription factors by two different mechanisms. ATFS-1 SUMOylation reduced its stability and impaired its transcriptional activity, while SUMOylation of DVE-1 prevented its nuclear localization. Interestingly, the Esp phenotype of *ulp-4* RNAi treated animals could be rescued by the combined over-expression of SUMO-mutants ATFS-1 K327R and DVE-1 K326R [156], indicating that ULP-4 deSUMOylation of ATFS-1 and/or DEV-1 is required for host defence (**Figure 7**). Since ATFS-1 has been found to be required for resistance against *P. aeruginosa* [154], it is highly probable that ATFS-1 acts downstream of ULP-4 to mediate UPR^{mt}-mediated host defence. A direct assessment of a function for DVE-1 in inducing immune gene expression and promoting host defence during infection has yet to be reported. Nonetheless, this study demonstrated for the first time the importance of SUMO regulation in promoting *C. elegans* host defence against pathogenic bacteria [156].

There is no evidence yet that this pathway is strictly conserved in mammals. While the mammalian UPR^{mt} does not seem to be regulated by DVE-1's homologues, the SATB protein family, it does involve three bZIP transcription factors: ATF4, ATF5 and CHOP [13]. ATF5 has been described as the functional homologue of ATFS-1, mediating UPR^{mt} in mammalian cells [157]. In addition, ATF5 has been found to be activated downstream the pro-inflammatory cytokine IL-1β via inhibition of its UPS-based degradation [158, 159] and to activate cytokine secretion in Th1 cells [160]. Interestingly, ATF5 was shown to undergo SUMO-based regulation [161]. Whether SUMOylation of ATF5 impacts its transcriptional activity and is controlled by the ULP-4 homologues SENP6 and SENP7 requires exploration. Notably, SENP6 and SENP7 are instrumental in the activation of NLRP3 [162], a key NLR activated by various stresses including mitochondrial damage [163]. Thus, SUMOylation appears to be a common feature shared between *C. elegans* and mammals involved in the regulation of immune signaling upon mitochondrial damage.

In addition to regulation by (de)SUMOylation, ATFS-1 activity has also been found to be affected by ubiquitination, through the WWP-1-dependent UPS regulation of its co-repressor ZIP-3 (**Figure 7**). In an interesting study published recently, Deng *et al.* confirmed that phenazine-producing *P. aeruginosa* induces the UPR^{mt}, although they only observed a modest activation [114]. Surprisingly, they found *P. aeruginosa* was actually able to impair the UPR^{mt} triggered by other stimuli that cause mitochondrial dysfunction. They suggested that *P. aeruginosa*, in addition to perturbing mitochondrial function directly and thereby triggering the UPR^{mt}, has evolved a counter-mechanism to impair this host response. Mechanistically, Deng *et al.* found that the bZIP

transcription factor ZIP-3 is required for the *P. aeruginosa*-mediated inhibition of the UPR^{mt}. Indeed, bacterial clearance and survival of infected animals was increased in *zip-3* mutants. In addition, this effect of ZIP-3 was dependent on ATFS-1, indicating that ZIP-3 must inhibit ATFS-1 to repress the UPR^{mt}. ZIP-3, which was found to heterodimerize with ATFS-1 [164], alters part of the ATFS-1 transcriptional response that confers resistance to *P. aeruginosa*. How infection modulates ZIP-3 has not been explored. ZIP-3 was, however, found to be regulated by the UPS, probably through WWP-1. Indeed, while the expression of *wwp-1* is induced during mitochondrial dysfunction [165], its down-regulation causes accumulation of a ZIP-3::GFP fusion protein [114]. Similarly, a form of ZIP-3 harbouring a mutation in a canonical PY motif known to bind NEDD4 E3-HECT ligases (ZIP-3 ^{PPAX}) accumulated in the nucleus. Further, *wwp-1* RNAi, or expression of ZIP-3^{PPAX}, inhibited the UPR^{mt} triggered either by mitochondrial dysfunction or by a gain-of-function mutation of *atfs-1*. This indicates that ZIP-3 directly inhibits ATFS-1 activity to modulate the UPR^{mt}. The regulation and the impact of WWP-1 during infection was not analysed in the study. As Chen *et al.* found WWP-1 to be required for host defence against *P. aeruginosa* [110], it would be interesting to determine whether this depends on ZIP-3.

As mentioned above, WWP-1's homologues play key roles in mammalian innate immunity. Similarly, in addition to its role in UPR^{mt}, the homologue of ZIP-3, ATF4, has been shown to drive expression of inflammatory cytokines in human monocytes exposed to LPS [166] and to be critical for CD4+ T cell-mediated immune responses [167]. There is, however, no evidence in the literature of WWP1, WWP2 or ITCH regulating ATF4. On the other hand, during oxidative stress, ATF4 has been found to inhibit the expression of *wwp1* as well as *nedd4.2,* which encodes another E3-HECT from the NEDD4 family, thereby stabilizing their target protein LATS1 which induces cell death [168]. Thus more work needs to be undertaken to determine in detail the link between ATF4, NEDD4 ligases and immune signaling in mammalian systems.

4. UB-RELATED PROCESSES IN HOST DEFENCE

Besides their role in inducing and regulating innate immune signaling pathways during infection, Ub-related enzymes appear also to play a direct role as effectors of the immune response. Thus, a number of studies have reported induction of genes encoding Ub-related enzymes including E2, E3-HECT and E3-RING during infection [53, 169, 170]. We will review in this section the evidence that these enzymes are part of the host response to infection, and we will discuss the different roles they play in resistance and tolerance mechanisms, thereby contributing to host defence.

4.1. E3-CRLS IN IMMUNE RESPONSES

Comparative studies in *C. elegans* have revealed considerable similarity between the host transcriptional response to two very distinct obligate intracellular pathogens, Orsay virus and the microsporidian *N. parisii* [53, 170]. This shared response has been termed the Intracellular Pathogen Response (IPR) and appears to diverge from responses induced by extracellular pathogens [53, 170]. The IPR was found to be particularly enriched in F-box and MATH-domain encoding genes. Both of these domains are found in two categories of E3-CRL SRSs: the F-box domain is required for cullin interaction, while the MATH domain provides substrate binding when associated with a BTB cullin-binding domain. There are approximately ~520 F-box and ~50 MATH-BTB in *C. elegans,* greatly outnumbering their human homologues (**Table 1**) [171-172]. They also exhibit features of molecular evolution indicative of being under strong selective pressure, with an unusually high rate of non-synonymous codon change and a high rate of gene duplication and deletion among closely-related species [171]. These features are typical of genes involved in host-pathogen arms races, leading Thomas to propose that the 2 gene families might be involved in innate immunity, prior to any functional data supporting the hypothesis [171]. Interestingly, the positive selection in the *f-box* and *bath* genes was found to affect those regions corresponding to substrate-binding domains but not the cullin-binding regions, suggesting that protein substrates from pathogens are the evolutionary driving force. Thus, most of the F-box and MATH-BTB proteins found in *C. elegans* could be specialized in direct ubiquitination and degradation of viral proteins and/or bacterial effectors or toxins [171]. While there is still no direct evidence for pathogen proteins being targeted by the host UPS in *C. elegans*, as described below, support has been growing for the hypothesis that F-box and/or MATH-containing proteins might indeed be involved in ubiquitination of intracellular pathogens, to promote their elimination [53, 173, 174]. It should be noted, however, that some F-box and MATH-domain encoding genes are induced by several different pathogens, including extracellular bacteria. For instance, Engelmann *et al.* identified 13 F-box-containing protein that are commonly induced by three pathogenic bacteria, i.e. *Photorhabdus luminescens, E. faecalis and S. marcescens*. Among them, *fbxa-62* was also found to be induced by two species of fungi (*D. coniospora* and *Harposporium sp*) and *fbxa-182* by the Orsay virus and *N. parisii* [53, 169, 170]. Similarly, *math-15* is induced by the three bacterial species and these two intracellular pathogens [53, 169, 170]. Thus, if some F-box and MATH-domain containing proteins do act as specific intracellular PRRs detecting pathogen-specific MAMPs, others are more likely to regulate host proteins involved in the common signaling pathways triggered by different pathogens.

The expansion of the E3-CRL SRS gene family is also seen in plants and could reflect a shared evolutionary immune strategy [171, 172]. In mammals, where the number of E3-CRL genes is small, SRSs nevertheless also play a role in immunity. A widely known example is the regulation of NF-κB signaling by $SCF^{\beta\text{-}TRCP}$, via ubiquitination and degradation of IkB [175]. The importance of

E3-CRL in host immunity is perhaps best revealed by the virulence factors of the Cif family, mentioned above, that interfere with E3-CRL activity through deamidation of NEDD8 [135, 176]. Cif are produced by several pathogens, including the human pathogens *Yersinia pseudotuberculosis*, and enteropathogenic and enterohemorrhagic *E. coli* (EPEC and EHEC) [177]. This underlines the importance in mammalian immunity of E3-CRL that may act in resistance or tolerance responses to pathogens.

4.2. UBIQUITIN-RELATED PROCESSES IN THE RESISTANCE RESPONSE TO INFECTION

In previous sections, we have discussed the role of Ub-modifying enzymes in the regulation of immune signaling pathways leading to the induction of defence genes such as *nlp-29* or *clec-85*. Ubiquitination of intracellular pathogens followed by their selective autophagy, aka xenophagy, represents a second important role of Ub-related genes in the response to infection. This cell-autonomous immune mechanism of pathogen elimination has been well-characterised in mammalian cells [178]. The autophagy machinery is conserved in *C. elegans* and several studies have demonstrated a role of autophagy in host defence against extracellular pathogens such as *Staphylococcus aureus*, *Salmonella* Typhimurium or *P. aeruginosa* [179-181]. Evidence of actual xenophagy of intracellular pathogens in *C. elegans* is, however, still missing. Interestingly, ubiquitination of intracellular microsporidial pathogens seems to occur *in vivo* [53, 173, 174]. Indeed, *N. parisii* has been found to be actively ubiquitinated by a Cullin-6-dependent E3-CRL ligase inside intestinal cells of *C. elegans* [53]. Further, coating *N. parisii* with ubiquitin appears to be linked with parasite clearance (**Figure 3**). Thus, RNAi targeting the IPR genes *cul-6*, *skr-3* or *skr-5*, the ubiquitin encoding gene *ubq-2*, or the proteasomal components *pas-5* or *rpn-2* resulted in a modest increase in pathogen load [53]. In addition, comparative studies using different *Nematocida* species and several C*. elegans* strains supported this hypothesis [173, 174]. Indeed, a Hawaiian *C. elegans* isolate, which can clear *N. ironsii* much more efficiently than the reference laboratory strain N2, coats the parasite with ubiquitin to a much higher level compared to N2 animals [173, 174]. In addition, ubiquitin accumulated more around *N. ironsii* compared to two other *Nematocida* species that are not cleared by the Hawaiian strain. On the other hand, even if disrupting autophagy genes led to an increased parasite load [53], this appeared to reflect a reduced colonization by the pathogen, rather than its increased elimination [174]. Specifically, the autophagy gene *lgg-2* encoding the homologue of the microtubule-associated protein 1A/1B-light chain 3 (LC3) was found to regulate the initial microsporidial colonization of intestinal cells [174]. Thus, *lgg-2*-mediated autophagy doesn't seem to be the mechanism used by the *C. elegans* Hawaiian isolate to clear infection [174]. Further studies are required to understand the molecular mechanisms underlying this effect, and whether ubiquitination contributes to elimination of microsporidia. Whether this as yet undefined mechanism of pathogen elimination could be conserved across species is of major

interest. Nonetheless, CUL-6 dependent E3-CRL might be involved in the clearance of different intracellular pathogen, as *cul-6* RNAi drastically increased loads of Orsay virus [53]. Contrary to what was observed with *N. parisii* infection, however, down-regulation of *ubq-2*, and the proteasomal subunit genes *pas-5* and *rpn-2*, dramatically reduced viral loads. This suggests that Orsay virus hijacks the host UPS to establish an infection. Many F-box and MATH-domain encoding genes are induced during infection of *C. elegans*. Some might be involved in the direct sensing of microsporidia or viruses. The future identification of a specific SRS putatively binding to these pathogens could be a key point for a better understanding of the role of ubiquitination during microsporidial and viral infection.

Emily Troemel's team took a step in that direction, studying members of an expanded family of *C. elegans* homologues of the human protein Amyotrophic Lateral Sclerosis 2 Chromosome Region Candidate 12 (ALS2CR12) [182-184]. Many of these *protein containing ALS2CR12 signature* (*pals*) genes were found highly induced by microsporidial and viral infection in *C. elegans,* as part of the IPR [53, 170]. Troemel and colleagues used *pals-5p::gfp* as a reporter of the IPR, in forward genetic screens to identify regulators of this transcriptional programme [182, 183]. Intriguingly, they identified two other *pals* genes, *pals-22* and *pals-25* that act, respectively, as negative and positive regulators of the IPR [182, 183]. Thus, a *pals-22* mutant strain displayed constitutive expression of IPR genes, which was suppressed by *pals-25* mutation [182, 183]. This signaling appeared be independent of the infection-triggered signaling pathway, as *pals-25* did not affect the IPR induced by *N. parisii* [183]. Interestingly, although *pals-22* mutants had slowed development and reduced longevity compared to wild-type animals [182], they did, however, have increased resistance against *N. parisii* and Orsay virus [183]. Indeed, loads of *N. parisii* and Orsay virus were reduced in *pals-22* mutant strains, a phenotype suppressed by *pals-25* mutations [183]. Significantly, these reduced pathogen loads were specific to natural intestinal pathogens, as loads of *P. aeruginosa* were not reduced, but rather increased, in *pals-22* mutants [183]. Additionally, deletion of the Ub-related IPR genes *cul-6*, *skr-3/5* or *skr-4/5* each had a small but significant impact on *N. parisii* pathogen load in a *pals-22* mutant background. [184]. Similarly, deletion of *rcs-1,* which encodes a RING-finger protein identified by co-immunoprecipitation as a binding partner of CUL-6, also significantly increased *N. parisii* pathogen load in the *pals-22* mutant [184]. Altogether, these results indicate that part of *pals-22* resistance phenotype relies on the activity of a CUL-6/RCS-1/SKR-3/4/5 E3-CRL [184]. F-box and/or MATH-domain proteins working within this E3-CRL might thus be involved in the direct sensing of *N. parisii*.

4.3. UBIQUITIN-RELATED PROCESSES IN THE TOLERANCE RESPONSE TO INFECTION

As previously outlined, infection, or the response to infection, can lead to proteotoxic stress or organelle damage. Hosts need to cope with this in order to survive. While very little is known in vertebrate models, *C. elegans* has considerably helped to decipher these tolerance mechanisms [14]. Evidence for pathogen-induced protein misfolding and aggregation was first shown in *C. elegans* in the context of *E. faecalis* infection, which accelerates the aggregation of poly-glutamine-containing proteins [185]. We will review below the studies that have demonstrated the importance of the UPS in this tolerance response, as part of host defence. Just like cytosolic proteins, as a consequence of infection, luminal, transmembrane or secreted proteins can also undergo misfolding during their maturation in the endoplasmic reticulum (ER) [186]. The resulting ER stress induces an UPR^{ER} that can prevent pathological ER proteotoxic damage. The UPR^{ER} encompasses notably a cellular pathway called endoplasmic-reticulum associated protein degradation (ERAD) that promotes retro-translocation and targeting of misfolded ER proteins to the cytosolic proteasome. This mechanism, also related to ubiquitin-dependent processes, will be reviewed here. Finally, as we discuss below, elimination of damaged organelles by ubiquitin-dependent autophagy is another type of tolerance response, important for maintaining homeostasis during infection.

4.3.1. Proteostasis in the tolerance response

As explained above, *pals-22* and *pals-25* have been identified, respectively, as negative and positive regulators of the IPR controlling pathogen resistance [182, 183]. In addition, with regards gene expression, the IPR partially overlaps with the response to prolonged heat stress, suggesting that part of the IPR is likely to increase proteostasis to overcome environmental stresses [182]. Accordingly, *pals-22* mutants have an increased resistance to heat stress and reduced level of stress-induced poly-glutamine aggregates, indicating that the IPR, in addition to promoting pathogen clearance, also increases cellular proteostasis [182, 183]. These results strongly suggest that such tolerance responses may also occur during infection. Interestingly, the thermotolerance phenotype of *pals-22* required the IPR E3-CRL genes [184]. Thus, for example, a *cul-6* mutation completely abrogated the increased thermotolerance of *pals-22* mutants [184]. In addition, mutations in *skr-3, skr-4* and *skr-5* also abrogated the thermotolerance of *pals-22*, suggesting that these SKP-related proteins may acts redundantly to promote proteostasis [184]. Finally, in addition to the RING-finger protein RCS-1, two F-box proteins FBXA-75 and FBXA-158 were identified by co-immunoprecipitation assay as CUL-6 binding partners, and all three CUL-6 binding partners were required for *pals-22* thermotolerance [184]. Together, these studies strongly suggest that these SKRs, F-box and RING-finger proteins are part of a cullin-6 based E3-CRL ligase that is

required to increase proteostasis, which might also contribute to more robust tolerance to infection (**Figure 3**).

4.3.2. UPS-mediated proteostasis in the tolerance response

A couple of studies have suggested that increased UPS activity upon bacterial exposure is a means to promote proteostasis and boost the host's capacity to tolerate infection [187, 188]. UPS activity can be monitored thanks to the probe Ub(G76V)-GFP. This probe contains a single, uncleavable ubiquitin N-terminally linked to GFP which mimics a mono-ubiquitinated protein that can be further polyubiquitinated and degraded by the proteasome [189, 190]. Reduced levels of Ub(G76V)-GFP thus reflect increased UPS activity.

In an interesting study analyzing the systemic stress response to genome instability, Ermolaeva *et al.* reported increased UPS activity as a tolerance response to infection and suggested that a resistance response may precede and be required to induce this tolerance response [187]. Starting from the observation that germline DNA damage also increases UPS activity and provides resistance to heat-shock, oxidative stress and *P. aeruginosa* infection, Ermolaeva *et al.* explored the underlying mechanism and found that DNA damage mediates a transcriptional response sharing similarities with innate immune responses triggered by infection with *Microbacterium nematophilum* (a Gram-positive bacterial pathogen) and *P. aeruginosa* [187]. Both DNA damage and infection-mediated responses encompass expression of putative secreted peptides that may act distantly to trigger systemic UPS activity. To explore the hypothesis that UPS activity is indeed elicited by the innate immune response rather than by the infection itself, or by the damage caused by infection, Ermolaeva *et al.* monitored UPS activity in animals fed with the immunogenic but non-pathogenic bacteria *Bacillus subtilis*. A short exposure to *B. subtillis* indeed induced an increased UPS activity. Chronic exposure to *B. subtilis*, however, decreased UPS activity. This neatly illustrates the phenomenon of immunopathology, wherein UPS dysfunction appears to be caused by a long-term immune response. Providing evidence that increased UPS activity confers tolerance to infection, the authors found that pre-exposure to *B. subtilis* extended the survival of animals infected with *P. aeruginosa*. To show that this prophylactic effect was mediated at least partially by a tolerance response and not solely by the resistance response, the authors showed that pre-exposure to *B. subtilis* increased resistance to heat-shock stress, indicating that animals exposed to *B. subtilis* acquire increased fitness allowing them to tolerate better infection [187].

In another study, Joshi *et al.* also observed that *P. aeruginosa* intestinal infection can increase UPS activity [188]. Thus, animals infected by *P. aeruginosa* exhibited reduced levels of Ub(G76V)-GFP, compared to control animals fed with the non-pathogenic *E. coli* OP50 strain.

Interestingly, increased UPS activity in infected animals required the dopamine receptor DOP-1, as *dop-1* mutants had a similar level of Ub(G76V)-GFP in infected and control animals. In addition, *dop-1* mutant were more sensitive to *P. aeruginosa* infection indicating that a signaling pathway involving dopaminergic mechanosensory neurons promotes UPS activity, helping the host to survive infection [188]. To understand the mechanism of increased UPS activity, the authors used RNAi to knockdown expression of the proteasomal subunit *pbs-5*. They saw a greater accumulation of Ub(G76V)-GFP in the *dop-1* mutant background, indicating that the proteasome is functional in *dop-1* mutants and that the accumulation of Ub(G76V)-GFP in *dop-1* animals must rather be a consequence of reduced protein ubiquitination [188].

4.3.3. ERAD-mediated proteostasis in the tolerance response

In a recent study, Tillman *et al*. also showed how proteasomal degradation of misfolded ER proteins by the ERAD pathway may contribute to survival during infection [191]. The importance of the UPR^{ER} during infection had been nicely illustrated by the work of Richardson *et al.* who found that the high levels of secreted antimicrobial peptides and proteins induced by *P. aeruginosa* infection generate an ER stress, beneficial for host defence. Consequently, *C. elegans* larvae lacking XBP-1, a major UPR^{ER} mediator, fail to develop on *P. aeruginosa* [186]. Following this study, Tillman *et al.* conducted a genetic screen to identify regulators of ER homeostasis that could compensate for XBP-1 deficiency during infection. They identified a mutation in the transcription factor FKH-9 that rescued the developmental failure of *xbp-1* mutation provoked by *P. aeruginosa*, without altering the immune response [191]. In accordance with a role for FKH-9 compensating ER stress, *fkh-9* mutant was also found to promote resistance to the ER stress-inducer tunicamycin. Digging into the underlying mechanism, mutation of *fkh-9* was found to increase ERAD pathway activity, which might account for the increased survival of *xbp-1;fkh-9* larvae during infection. Using the Ub(G76V)-GFP probe, however, *fkh-9* mutant had a mild negative impact on cytosolic proteasomal degradation, highlighting a possible balance between ERAD and cytosolic protein degradation. As mentioned previously, interference with the UPS can be perceived as a HAMP [53]. It will be interesting to explore whether their mild UPS alteration also contributes to triggering a host response and the rescue of larval lethality seen in *xbp-1;fkh-9* mutant.

4.3.4. Organelle autophagy in the tolerance response

In addition to proteotoxic damage, organelles can also be altered over the course of infection. We previously evoked the example of mitochondria that are targeted by several pathogens, triggering a UPR^{mt} [13, 151]. An alternative host response to mitochondrial damage consists of eliminating the damaged organelle by selective autophagy, a process referred to as mitophagy [192]. Upon loss of

mitochondrial membrane potential, the E3-RBR ubiquitin ligase Parkin is activated by PINK-dependent phosphorylation and promotes ubiquitination of mitochondrial outer membrane proteins, ultimately leading to mitochondrial degradation by mitophagy [193]. Interestingly, Parkin and mitophagy have recently been shown to be involved in *C. elegans* host defence in a model of *P. aeruginosa* liquid pathogenesis [194]. *P. aeruginosa* induces diverse pathologies in *C. elegans*, depending on the growth conditions [195]. On solid agar media, *P. aeruginosa* virulence involves principally bacterial colonization, phenazines or cyanide production [195]. Pathogenesis in liquid culture is dominated instead by the siderophore pyoverdine, which induces a lethal hypoxic crisis [196, 197]. The importance of this iron-chelator agent in *P. aeruginosa* virulence was demonstrated by the fact that bacterial mutants deficient in pyoverdine biosynthesis exhibit reduced killing [197]. In part, this reflects the fact that upon iron chelation, ferripyoverdine can function as a signaling molecule, regulating the production of several secreted toxins [198]. Pyoverdine can also enter the cells of *C. elegans* and, alone provoke animal death [194]. During exposure to *P. aeruginosa* in liquid culture, pyoverdine as well as other chelators were found to damage mitochondria, leading to their removal. In accordance with mitochondrial clearance being mediated by mitophagy, autophagy and mitophagy reporters were induced by *P. aeruginosa* or by an iron-chelator alone. Most importantly, RNAi or mutation of autophagy and mitophagy genes, including a mutant of the E3-RBR Parkin encoding gene *pdr-1,* increased animal death upon exposure to *P. aeruginosa* or an iron-chelator. These results suggest that Parkin likely mediates ubiquitination of damaged mitochondria and subsequent mitophagy, and that this process is required to maintain *C. elegans* fitness during *P. aeruginosa* infection (**Figure 7**). The protective mechanism of ubiquitin-dependent mitophagy remains, however, to be determined. Interestingly, iron-chelation was also shown to promote mitochondrial clearance in mammalian HEK293T cells. As Parkin-mediated ubiquitin-dependent mitophagy plays a conserved role in mitochondrial clearance, it is highly plausible that this E3 ubiquitin ligase might also be required in iron-chelation-mediated mitophagy in mammalian cells. A recent study from the same research group demonstrated the importance of pyoverdine in the virulence of *P. aeruginosa* isolates from patients with cystic fibrosis. Using *C. elegans* as well as an acute murine pneumonia model, the authors could correlate the severity of *P. aeruginosa* infection with levels of pyoverdine, and rescue the pyoverdine-mediated host pathology with pyoverdine inhibitors [199]. Enhancing the Parkin-dependent ubiquitin-mediated mitophagy pathway might also represent a potential therapeutic approach to help patients with cystic fibrosis to recover from *P. aeruginosa* infection.

5. CONCLUSION

Since its adoption as a model system by Sydney Brenner in the 1960's, *C. elegans* has played a pivotal role in fundamental research, allowing the discovery of universal cellular and molecular core processes, like apoptosis or RNAi, findings which have been rewarded with Nobel Prizes. With 40% of its genes conserved in humans [115], this nematode has helped deciphering essential conserved physiological pathways involved for instance in aging and metabolism [200, 201]. The field of immunology is no exception and continues to benefit from *C. elegans* research. Here, we reviewed how it is helping to reveal the importance of ubiquitin-related processes in innate immunity. Ub-modifying enzymes act at all steps of host defence. It has become clear that Ub-related processes also play key roles in maintaining host health during infection. In addition to regulating canonical immune signaling pathways, when they malfunction, this itself can act as a danger signal, triggering immunity. We highlighted a potential cooperation between different Ub-modifying enzymes, the deSUMOylating enzyme ULP-4 and the E3 ligase WWP-1. Their action, downstream of mitochondrial damage, increases the activity of the key transcription factor ATFS-1. We suggest that different branches of the Ub and Ub-related network may work in an integrated manner to induce a coordinated immune response. This is clearly an area that merits further investigation. Studying WWP-1 has also helped uncover another interesting feature of Ub-related enzymes in innate immunity. WWP-1 was found to be involved not only in mitochondrially-triggered immune responses, but also in the regulation of the insulin/DAF-2 pathway upon PFT exposure. This is probably not a peculiarity of WWP-1, and other Ub-related enzymes such as MATH-33 might also be expected to play multiple roles in innate immunity. At the same time, given the large repertoire of F-box and MATH encoding genes, we predict that some enzymes must act in a more specific manner. Examining the expression patterns of Ub-related enzymes, determining what stimuli trigger their expression, and whether they are expressed in a single tissue or in the whole organism, will help identify those that have a specific role in immunity.

How many of the known enzymes are able to modulate immune responses remains unclear. A critical point to understand better the role of these enzymes will be to identify their substrate(s). Although *C. elegans* is a well-adapted model for immune research, such enzyme/substrate identification remains technically complex. Several non-biased approaches have been developed, such as affinity purification proteomics, or Ub ligase trapping and proximity labelling [202]. Used in *C. elegans*, these tools will help us understand the molecular mechanism behind Ub-related immune processes. This will help answer key questions in the field, such as how Ub-related enzymes modulate tissue cross-talk in neuro-immune signaling, how they mediate cell-autonomous clearance of intracellular pathogens, and how tolerance and resistance cooperate to provide efficient host defence.

In addition to its role in fundamental research, *C.elegans* has also made a real contribution to biomedical research, serving as a model of human diseases, as well as a powerful manageable tool

for high-throughput drug discovery [203, 204]. *C. elegans* serves as a model of infectious diseases and also offers the possibility to screen very large chemical libraries for anti-infective molecules with antimicrobial, immunomodulatory or anti-virulence properties [203, 204]. Targeting Ub-related processes has potential as a therapeutic approach to fight infections. While proteasome inhibitors such as Bortezomib, aka Velcade™, have already proven to be very effective cancer therapies in the clinic, their intrinsic toxicity compromises the health of patients health of patients [205]. Fundamental research is needed to understand more fully the mechanism involved in the UPS and to define a mode of action for each Ub-related enzyme. Given the hundreds of enzymes involved in the regulation of Ub and Ubl pathways, the use of a genetically tractable model offers a practical way to decipher key conserved factors of innate immune responses. Further investigation of *C. elegans* Ub and Ubl pathways during host–pathogen interactions promises to pave the way for the identification of novel tolerance-enhancing molecules and/or anti-infective drugs.

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FIGURE LEGEND

Figure 1: Regulation and functional consequences of the ubiquitin reaction.

a. Amino-acid sequence of human ubiquitin. Red: the N-terminal methionine and the seven lysine residues are acceptor sites that can be modified by the addition of another ubiquitin molecule. Blue: glycine 76 allows covalent linking to a substrate or another ubiquitin molecule. **b. Ubiquitination diversity and recognition.** Ubiquitinated substrates can bind with a variety of ubiquitin binding proteins (UBPs) that possess ubiquitin-binding domains recognizing the different ubiquitination types. UBPs binding mixed or branched chains remain to be identified. A single ubiquitin molecule can attach to a substrate via one (mono-Ub) or several (multi-Ub) lysine residues. In addition, the first methionine and/or one of the seven lysine residues of ubiquitin can serve as ubiquitin acceptor sites, forming poly-Ub chains with different topologies. Homotypic poly-Ub chains, engaging the same linkage within the polymer, can display a compact (K48) or linear (K63 and M1) conformation. Heterotypic poly-Ub chains include chains with mixed linkages or branched chains, with two distal ubiquitin molecules attached to at least two acceptor sites of a single proximal ubiquitin moiety. **c. Ubiquitin reaction and outcome**. E1 activates Ub in an ATP-dependent manner, then transfers Ub to an E2 Ub-conjugating enzyme. The E2 active site cysteine forms a thioester bond (represented as ~) with the C-terminal carboxyl group of Ub. Finally, E3 Ub-ligases mediate the transfer of Ub from the E2 to a lysine residue of the substrate. De-ubiquitinating enzymes (DUBs) can reverse the reaction, restoring the pool of free ubiquitin. K48 and K11 chains, which display a compact conformation, are known to direct proteasomal degradation, while K63 and M1 linear chains are involved in autophagy and intracellular signaling, respectively.

Figure 2: Tripartite structure of E3-CRL

a. Schematic representation of Cullin-RING E3 ligase (E3-CRL) multimeric complexes. A catalytic RING-containing enzyme binds to the E2~Ub intermediate and the cullin subunit, which acts as a scaffold of the complex. Interaction with the substrate is mediated by a substrate recognition subunit (SRS) that interacts with the cullin subunit either directly or indirectly via an adaptor protein. E3-CRLs mediate the direct transfer of Ub from the E2 to the substrate (dashed arrow). **b. SRS and adaptor families**. BTB (Broad-complex, Tramtrack and Bric-à-brac) containing SRSs bind directly to the cullin subunit. F-box, SOCS-BC (Suppressor Of Cytokine Signaling protein 1, binding to EloB-C) and DCAF (DDB1-Cul4A-Associated Factor)-containing SRSs interact with cullin via SKR (SKp-1 Related), EloB-C (Elongin B-C complex) and DDB1 (DNA damage-binding protein 1), respectively.

Figure 3: Model of Ub-dependent mechanisms in surveillance immunity against microsporidia.

Intracellular microsporidia cells interfere with proteasome function through an unknown mechanism. This triggers surveillance immunity and induction of “intracellular pathogen response” genes such as *cul-6*, *skr-3*, *skr-4* and *skr-5*. CUL-6 dependent E3-CRL then promotes proteostasis on one hand, and ubiquitination and subsequent elimination of microsporidian cells on the other. Together, CUL-6 mediated proteostasis and pathogen ubiquitination contributes to host defence.

Figure 4: Model of neuronal regulation of NPR-1 by the E3-HECT ligase HECW-1

a. Respective position of OLL (yellow) and RMG (red) neurons in the anterior and posterior bulb of the pharynx (green). Adapted from WormAtlas (https://www.wormatlas.org/) **b.** Schematic intercellular signaling pathway of *P. aeruginosa* sensing and subsequent induction of avoidance behaviour. During infection, HECW-1, which is expressed in OLL sensory neurons, must induce ubiquitination of a yet to identify substrate. This regulation ultimately inhibits activity of the NPR-1 receptor localized in RMG neurons, impairing avoidance behaviour.

Figure 5: Model of PMK-1 pathway regulation by Ub-related genes during *P. aeruginosa* and *D. coniospora* infection.

D. coniospora activates the PMK-1 pathway following binding of HPLA to the GPCR DCAR-1. This triggers expression of genes such as *nlp-29* encoding antimicrobial peptides, via the transcription factor STA-2. Seven Ub-modifying enzymes and three proteasomal subunits potentially positively regulate the DCAR-1/PMK-1/STA-2 pathway in the epidermis (green box #1). The transcription co-factor AKIR-1, which binds to the POU transcription factor CEH-18 and regulates expression of *nlp-29* in the epidermis upon fungal infection, also interacts with and might be regulated by the Ub-related enzymes UBR-5, MATH-33 and USP-24. In intestinal cells, through a uncharacterised mechanism, *P. aeruginosa* also activates the PMK-1 pathway, and triggers expression of genes such as *clec-85* that encode antimicrobial proteins, through the transcription factor ATF-7. Three Ub-modifying enzymes are potential positive regulators of this pathway (green box #2), including SIAH-1 which may directly regulate TIR-1. RLE-1 is a negative regulator of the pathway, specifically targeting NSY-1 to the UPS (red box #3).

Figure 6: Model of Ub-dependent regulation of the IIS signaling pathway

Under conditions where IIS is activated, the receptor DAF-2 auto-phosphorylates and activates downstream kinases, leading to the phosphorylation of DAF-16 and its retention in the cytosol by

14-3-3 (upper panels). In condition of low IIS, unphosphorylated DAF-16 is targeted to the nucleus and enhances host defence against *P. aeruginosa* and Cry5B pore-forming toxin (PFT) (lower panels). The DUB MATH-33 is also targeted to the nucleus of intestinal cells, and interacts, deubiquitinates and stabilizes DAF-16, boosting host defence against *P. aeruginosa* (left panel). It is not known what E3 ligase ubiquitinates DAF-16 nor whether this occurs in the cytoplasm or in the nucleus. In parallel, the E3-HECT ligase WWP-1 acts downstream of PDK-1 and enhances host defence against PFT, probably through the Ub-dependent regulation of one or several substrates. PDK-1 may regulate WWP-1 through an inhibitory phosphorylation.

Figure 7: Model of Ub and Ubl-dependent regulation of mitochondrial damage responses during *P. aeruginosa* infection.

Depending on the culture conditions, *P. aeruginosa* virulence involves pyoverdine (left) or cyanide and phenazines (right), virulence factors that induce mitochondrial damage. Following pyoverdine exposure, damaged mitochondria are cleared by mitophagy in a PDR-1 dependent manner, which provides protection against *P. aeruginosa* infection. By analogy with mammalian autophagy, PDR-1 might induce ubiquitination of a mitochondrial outer protein, which might recruit UBP bound to the isolation membrane to promote mitochondrial engulfment into autophagosome. Fusion of autophagosomes with lysosomes induces degradation of damaged mitochondria in autophagolysosome structures in a process known as mitophagy. In solid culture, mitochondrial damage leads to increased expression of the deSUMOylase ULP-4, which removes the SUMO homologue SMO-1 from ATFS-1 and DVE-1. DeSUMOylated ATFS-1 displays increased stability and transcriptional activity, while deSUMOylation of DVE-1 increases its nuclear translocation. The increased expression of innate immune genes and/or UPR^{mt} ultimately promotes host defence. *P. aeruginosa* production of phenazines represses UPR^{mt} in a ZIP-3 dependent manner. ZIP-3 negatively regulates ATFS-1-mediated host defence. WWP-1, induced upon mitochondrial stress, appears to be the E3 ligase responsible for ZIP-3 degradation by UPS. Data suggest a model where *P. aeruginosa* prevents ZIP-3 UPS degradation to counteract ATFS-1 mediated host defence. Although shown here in the cytoplasm, the site of ZIP-3 UPS regulation is not known.

Table 1. Ub-, SUMO- and proteasome-related genes in *C. elegans* and human

Ubiquitin-related genes				
Type	Family	*C. elegans*	Human	Ref.
ubiquitin		2	4	[45, 206]
E1		1	1	[45, 206]
E2	UBC	22	37	[45, 206]
	UEV	3	2	[45, 206]
E3	HECT	9	28	[45, 206]
	RBR	11	14	[207, 208]
	U-Box	4	9	[45, 206]
	RING	152	~300	[45, 206]
E3-CRL accessory protein	Cullin scaffold	6	8	[206, 209]
	RING subunit	3°	2	[45], [184]
	SKR adaptor	21	1	[45]
	DDB1 adaptor	1	3	[210]
	Elongin B/C adaptor	2*	2*	[45]
	F-box SRS	~520	61	[171, 209-211]
	DCAF SRS	36	60	[209, 212, 213]
	SOCS-BC	11[#]	37	[209, 210, 214]
	BTB	>100	169	[206, 210]
DUB	USP	27	56	[37, 45]
	OTU	5	17	[37, 45]
	MJD	2	4	[37, 45]
	UCH	4	4	[37, 45]
	MINDY	1	5	[37]
	JAMM	8	12	[37, 45]
	ZUFSP	0	1	[37]
SUMO-related genes				
Type	Family	*C. elegans*	Human	Ref.
SUMO		1	4	[215]
E1		2*	2*	[215]
E2		1	1	[215]
E3		?[$]	?[$]	[215, 216]
Peptidase	SENP	4	6	[215, 216]
	DeSI	1	2	[215, 217]
Proteasome-encoding genes				
Type		*C. elegans*	Human	Ref.
20S		14	14	[45]
19S		17	17	[45]

*: indicates that the 2 proteins act as dimers
[#]: based on SOCS box #IPR001496 domain search (https://www.ebi.ac.uk/InterPro)
[$]: the exact number of E3 SUMO ligases is not known. Only a few E3 SUMO ligases have been identified and correspond to the PIAS family, RanBP2 and RNF451.
°: The RING-finger proteins RBX1 and RBX2 are conserved across evolution. The RCS-1 has been identified in *C. elegans* as an functional interactor of CUL-6 and appears as an additional CRL RING finger protein in this nematode.

Table 2. Summary of Ub-related processes in innate immunity of *C. elegans*

Pathogen/Toxin	Enzymes / Proteasome	Targets	Function	Ref.
		Regulation of immune surveillance		
N. parisii	Proteasome	N/A	Pathogen-mediated proteasome alteration inducing surveillance immunity	[53]
S. venezuelae	Proteasome	N/A	Proteasomal inhibition induced neuronal cell death	[59, 60]
		Regulation of immune signaling		
P. aeruginosa	HECW-1	?	Regulation of pathogen sensing	[75]
P. aeruginosa	ARI-1.3, SIAH-1, LET-70	?	Regulation of clec-85 intestinal expression	[93]
D. coniospora	LET-70, HECD-1, RBPL-1, PRP-19, SKR-1, DCAF-1, USP-39, PAS-3, PAS-5, PBS-2	?	Regulation of nlp-29 epidermal expression	[90]
P. aeruginosa	RLE-1	NSY-1	Regulation of PMK-1 immune signaling	[100]
B. thuringiensis/ Cry5B	WWP-1	?	Regulation of DAF-2/PDK-1 non-canonical immune signaling	[110]
P. aeruginosa	RPT-6	ELT-2	Non proteolytic regulation of ELT-2 mediated innate immune response	[129]
B. pseudomallei	F54B11.5?, ZK637.14? Bacterial E3?	ELT-2	Regulation of ELT-2-mediated innate immune response	[133]
P. aeruginosa	MATH-33	DAF-16	Regulation of DAF-16-mediated innate immune response	[141]
D. coniospora	UBR-5, MATH-33, USP-24	AKIR-1	Potential regulation of AKIR-1 by UPS	[150]
P. aeruginosa	ULP-4	ATFS-1, DVE-1	Regulation of UPR^{mt} and innate immune response	[156]
P. aeruginosa	WWP-1	ZIP-3	Regulation of ATFS-1-mediated UPR^{mt} and innate immune response	[114]
		Regulation of host response		
N. parisii *Orsay Virus*	F-box / MATH proteins	?	Induced by infection	[53, 169, 170]
Orsay Virus	CUL-6, PAS-5, RPN-2		Regulation of viral infection	[53]
N. parisii	CUL-6, RCS-1, SKR-3, SKR-4, SKR-5, UBQ-2, PAS-5, RPN-2	?	Regulation of pathogen load	[53, 173, 174, 184]
N. parisii	CUL-6, SKR-3, SKR-4, SKR-5, RCS-1, FBXA-75, FBXA-158	?	Regulation of proteostasis	[182-184]
P. aeruginosa	Proteasome	N/A	Systemic UPS activity-mediating tolerance to infection downstream of the resistance response	[187]
P. aeruginosa	?	?	Tolerance response conferred by increased protein ubiquitination and UPS downstream dopamine signaling	[188]
P. aeruginosa	N/A	N/A	Tolerance response mediated by FKH-9-regulated ERAD during ER-stress	[191]
P. aeruginosa	PDR-1	unknown	Tolerance response mediated by mitophagy	[194]

Figure 1, Garcia-Sanchez

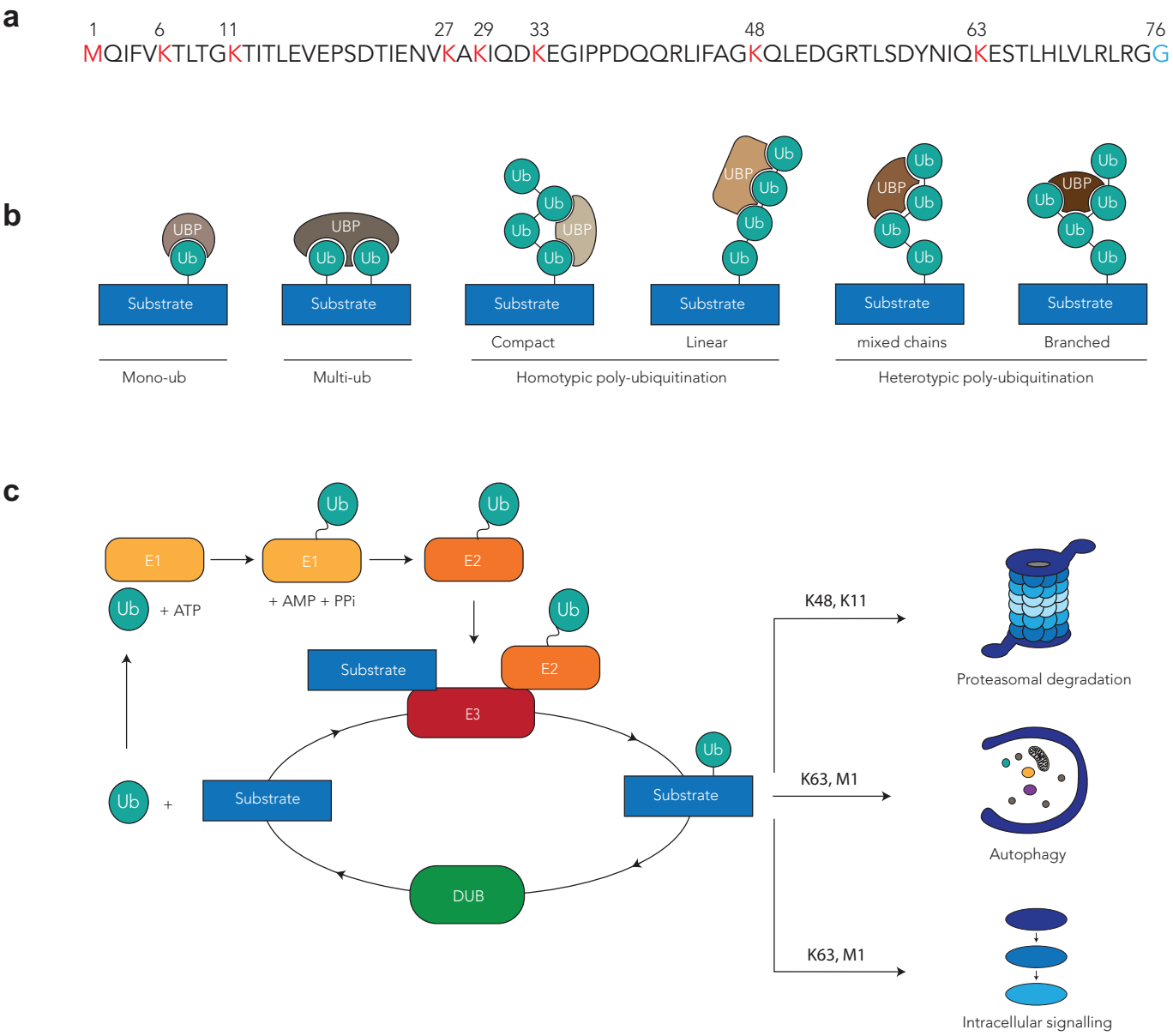
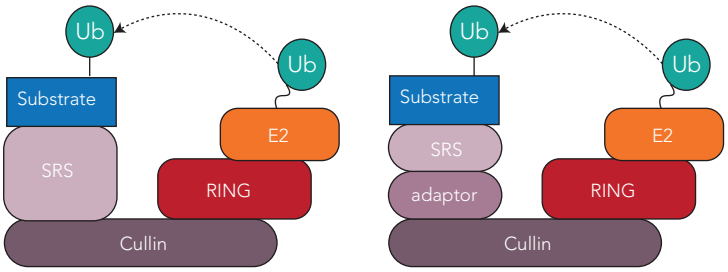


Figure 2, Garcia-Sanchez

a



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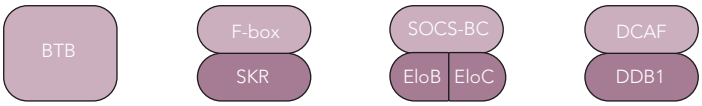
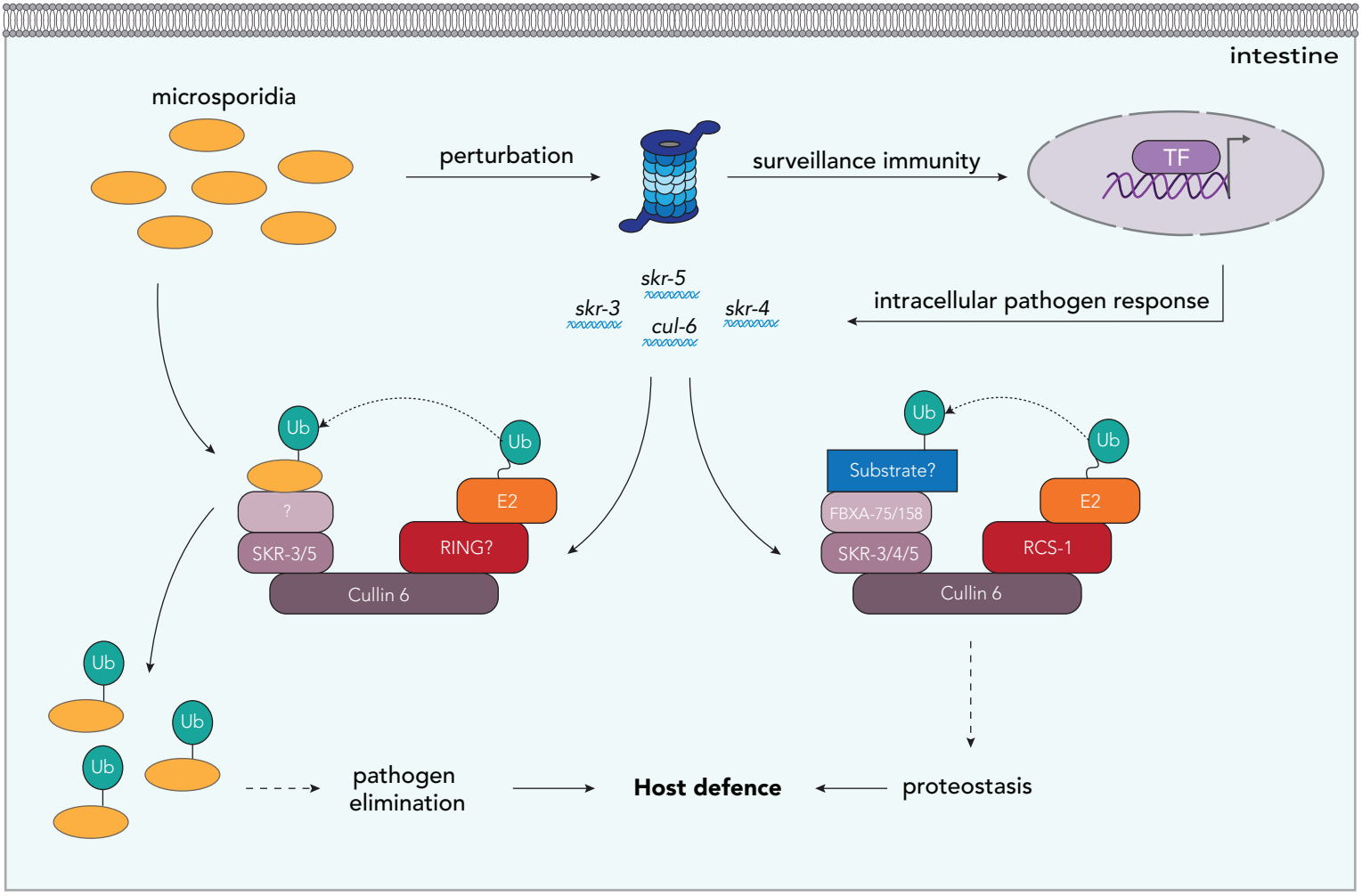


Figure 3, Garcia-Sanchez



Figure

Figure 4, Garcia-Sanchez

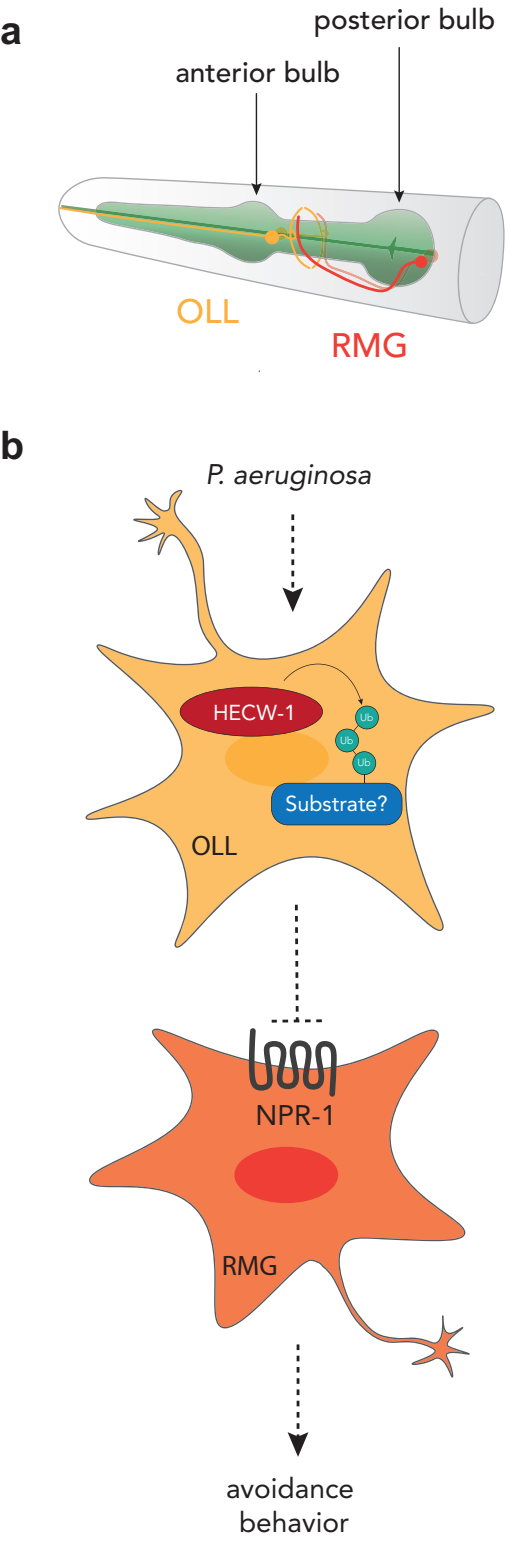
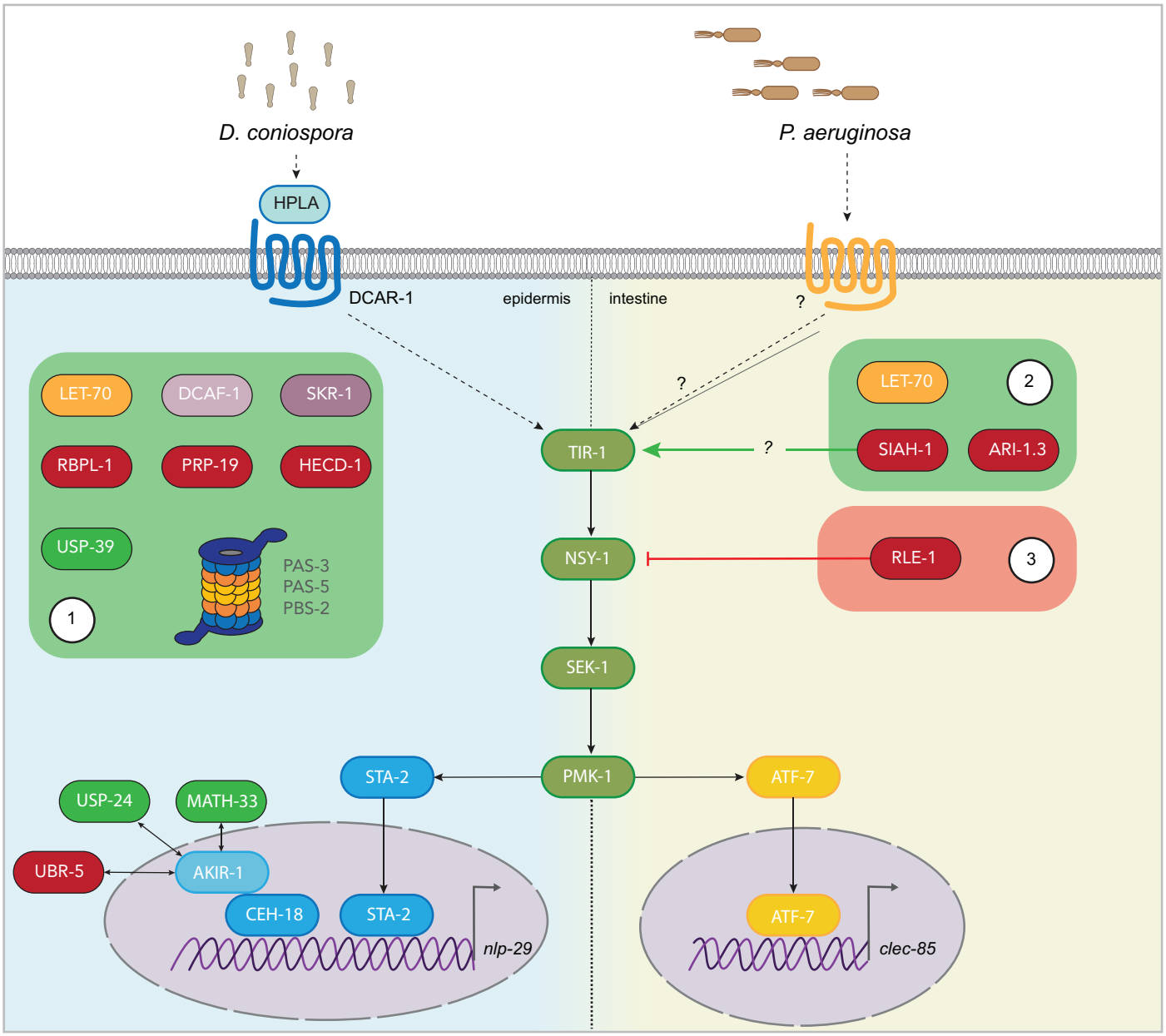
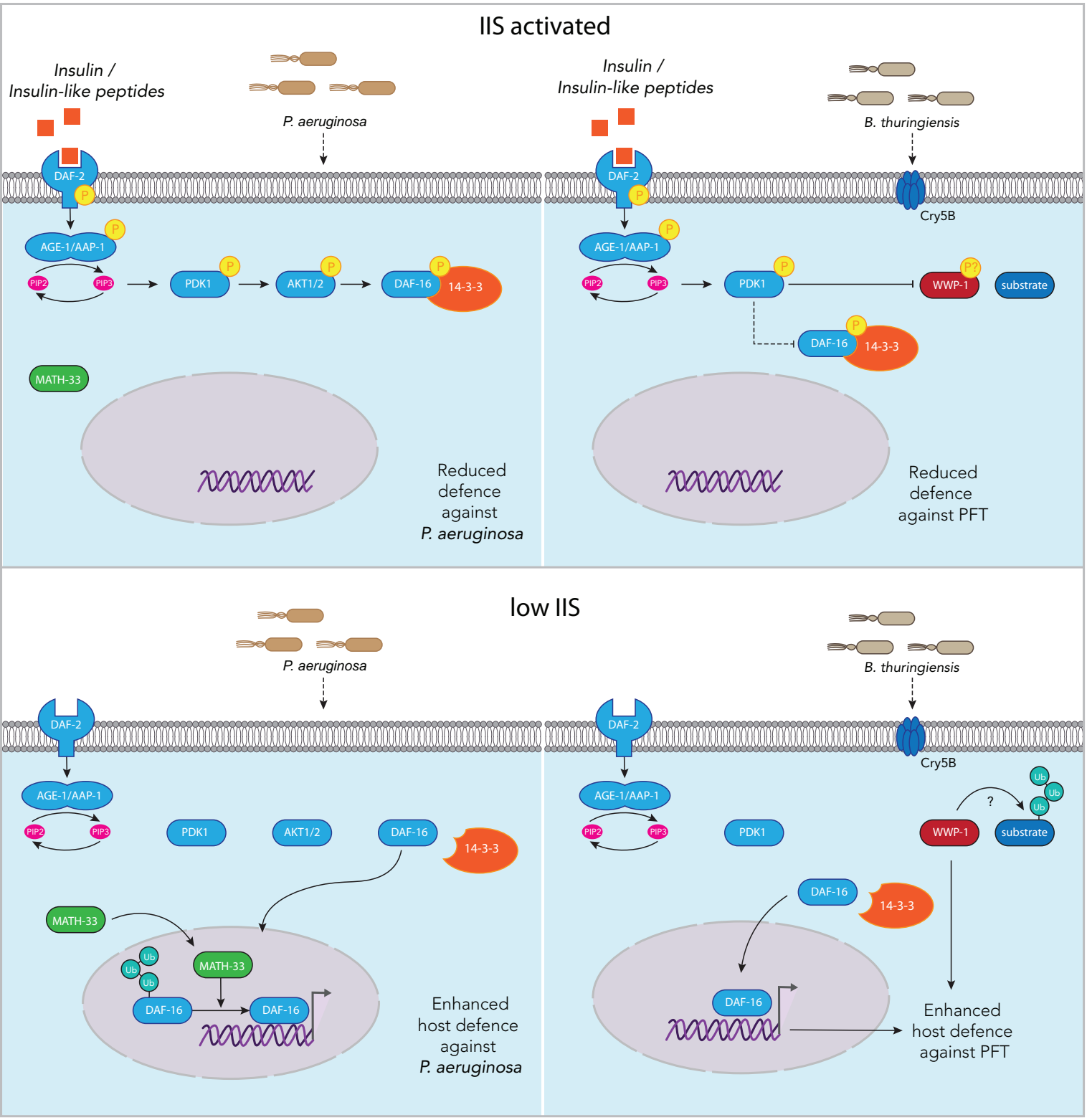


Figure 5, Garcia-Sanchez



Figure

Figure 6, Garcia-Sanchez



Figure

Figure 7, Garcia Sanchez