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## Ubiquitin-related processes and innate immunity in *C. elegans*

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17 **ABSTRACT**

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18  
19 Innate immunity is an evolutionary ancient defence strategy that serves to eliminate infectious  
20 agents while maintaining host health. It involves a complex network of sensors, signaling proteins  
21 and immune effectors that detect the danger, then relay and execute the immune programme. Post-  
22 translational modifications relying on conserved ubiquitin and ubiquitin-like proteins are an integral  
23 part of the system. Studies using invertebrate models of infection, such as the nematode  
24 *Caenorhabditis elegans*, have greatly contributed to our understanding of how ubiquitin-related  
25 processes act in immune sensing, regulate immune signaling pathways, and participate to host  
26 defence responses. This review highlights the interest of working with a genetically tractable model  
27 organism and illustrates how *C. elegans* has been used to identify ubiquitin-dependent immune  
28 mechanisms, discover novel ubiquitin-based resistance strategies that mediate pathogen clearance,  
29 and unravel the role of ubiquitin-related processes in tolerance, preserving host fitness during  
30 pathogen attack. Special emphasis is placed on processes that are conserved in mammals.

31  
32 **KEYWORDS**

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

34  
35 **DECLARATION**

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40 contributed to the writing of the manuscript.

## 44 ABBREVIATIONS

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1		
2		
3		
4		
5		
6	45	AMP Anti-microbial peptide
7		
8		
9	46	AMPK AMP-activated protein kinase
10		
11		
12	47	ASK1 Apoptosis signal-regulating kinase 1
13		
14		
15	48	ATF Activating transcription factor
16		
17		
18	49	ATFS-1 ATF associated with stress-1
19		
20		
21	50	ATP Adenosine triphosphate
22		
23		
24	51	BATH BTB and MATH-domain-containing
25		
26		
27	52	BTB Bric-a-brac, Tramtrack and Broad complex
28		
29		
30	53	bZIP basic leucine zipper
31		
32		
33	54	CD4 Cluster of differentiation 4
34		
35		
36	55	CEH <i>C. elegans</i> homeobox
37		
38		
39	56	CHBP Cycle inhibiting factor (Cif) homolog in <i>Burkholderia pseudomallei</i>
40		
41		
42	57	CHOP C/EBP homologous protein
43		
44		
45	58	CLEC C-type lectin
46		
47		
48	59	CRL Cullin-RING ligase
49		
50		
51	60	DAF Dauer formation
52		
53		
54	61	DAMP Damage-associated molecular pattern
55		
56		
57	62	DDB1 DNA damage-binding protein 1
58		
59		
60	63	DCAF DDB1 and CUL4 associated factor
61		
62		
63	64	DCAR-1 Dihydrocaffeic acid receptor-1
64		
65		

65	DUB	De-ubiquitinating enzyme
1		
2		
3	66	DVE-1 Defective proventriculus in <i>Drosophila</i>
4		
5		
6	67	ELT-2 Erythroid-like transcription factor family - 2
7		
8		
9	68	EGF Epidermal growth factor
10		
11		
12	69	ER Endoplasmic-reticulum
13		
14		
15	70	ERAD ER-associated protein degradation
16		
17		
18	71	ErbB4 avian erythroblastic leukemia viral oncogene B homolog 4
19		
20		
21	72	Esp Enhanced susceptibility to pathogen
22		
23		
24	73	FKH-9 Forkhead transcription factor family member 9
25		
26		
27	74	FOXO Forkhead box O protein
28		
29		
30	75	GPCR G protein-coupled receptor
31		
32		
33	76	HAMP Homeostasis-altering molecular processes
34		
35		
36	77	HPLA 4-Hydroxyphenyllactic acid
37		
38		
39	78	HECD-1 HECT, D1 ubiquitin protein ligase homolog 1
40		
41		
42	79	HECT Homologous to the E6-AP carboxyl terminus
43		
44		
45	80	HECW-1 HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1
46		
47		
48		
49		
50	82	IKK Inhibitor of nuclear factor kappa-B kinase
51		
52		
53		
54		
55		
56	84	IGF-1 Insulin-like growth factor 1
57		
58		
59	85	IIS Insulin/IGF-1 signaling
60		
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65		

86	IPR	Intracellular pathogen response
1		
2		
87	JAMM	JAB1/MPN/Mov34 metalloenzyme domain
4		
5		
88	JNK	c-Jun N-terminal kinase
7		
8		
89	LC3	Microtubule-associated protein 1A/1B light chain 3B
10		
11		
90	LPS	Lipopolysaccharide
12		
13		
14		
91	MAMP	Microbe-associated molecular pattern
15		
16		
17		
92	MAPK	Mitogen-activated protein kinase
18		
19		
20		
93	MATH	Meprin-associated Traf homology
21		
22		
23		
94	MINDY	Motif interacting with Ub-containing novel DUB family
24		
25		
26		
95	MJD	Machado-Josephin domain protease
27		
28		
29		
96	MyD88	Myeloid differentiation primary response 88
30		
31		
32		
97	NEDD	Neural precursor cell expressed developmentally down-regulated protein
33		
34		
35		
98	NEDL1	NEDD4-like ubiquitin protein ligase 1
36		
37		
38		
99	NF-κB	Nuclear factor-kappa B
39		
40		
100	NLP	Neuropeptide-like protein
41		
42		
43		
101	NLR	Nucleotide-binding and oligomerization domain (NOD)-like receptor
44		
45		
46		
102	NSY-1	Neuronal symmetry 1
47		
48		
49		
103	NPR-1	Neuropeptide Y receptor 1
50		
51		
52		
104	OLL	Outer labial
53		
54		
55		
105	OTU	Ovarian tumor proteases
56		
57		
58		
106	PALS	Protein containing ALS2CR12 signature
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107	PAS	Proteasome alpha subunit
1		
2		
108	PBS	Proteasome beta subunit
4		
5		
109	PD	Parkinson's disease
7		
8		
110	PDK-1	Phosphoinositide-dependent protein kinase 1
9		
10		
11		
111	PDR-1	Parkinson's disease Related 1
12		
13		
14		
112	PFT	Pore-forming toxin
15		
16		
17		
113	PI3K	Phosphoinositide 3-kinase
18		
19		
20		
114	PKB	Protein kinase B
21		
22		
23		
115	PMK-1	p38 MAP kinase 1
24		
25		
26		
116	PRR	Pattern recognition receptor
27		
28		
29		
117	PTI	Pattern trigger immunity
30		
31		
32		
118	RBR	RING-in-between-RING
33		
34		
35		
119	RCS-1	RING protein activating with cullin and SKR protein 1
36		
37		
38		
120	RING	Really interesting new gene
39		
40		
41		
121	RIP	Receptor interacting serine/threonine protein kinase
42		
43		
44		
122	RLE-1	Regulation of longevity by E3 ubiquitin-protein ligase 1
45		
46		
47		
123	ROS	Reactive oxygen species
48		
49		
50		
124	SARM	Sterile alpha and armadillo repeats
51		
52		
125	SATB	Special AT-rich sequence-binding protein
53		
54		
55		
126	SEK-1	SAPK/ERK kinase-1
56		
57		
58		
127	SENP	Sentrin-specific protease
59		
60		
61		
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63		
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128	SGK-1	Serum and glucocorticoid regulated kinase 1
1		
2		
129	SIAH	Seven in abstentia homolog
4		
5		
130	SRS	Substrate recognition subunit
7		
8		
131	STA-2	Signal transducer and activator of transcription 2
10		
132	SKR	Skp1-related
12		
13		
143	SOCS-BC	Suppressor of cytokine signaling protein 1, binding to EloB-C
15		
16		
134	SUMO	Small ubiquitin-like modifier
18		
19		
135	T3SS	Type 3 secretion system
20		
21		
22		
136	TAB	TAK-binding protein
23		
24		
25		
137	TAK1	Transforming growth factor beta-activated kinase 1
26		
27		
28		
138	TLR	Toll-like receptor
29		
30		
31		
139	TIR-1	Toll/interleukin-1 receptor-1
32		
33		
34		
140	TNF $\alpha$	Tumor necrosis factor alpha
35		
36		
37		
141	TOL-1	TOLI <i>Drosophila</i> family 1
38		
39		
40		
142	TRAF	TNF receptor associated factor
41		
42		
143	TRIF	TIR-domain-containing adapter inducing interferon beta
44		
45		
46		
144	UBD	Ubiquitin binding domain
47		
48		
49		
145	UBL	Ubiquitin-like protein
50		
51		
146	UBP	Ubiquitin binding protein
52		
53		
54		
147	UBR5	Ubiquitin-protein ligase E3 component N-recogin 5
55		
56		
57		
148	UCH	Ubiquitin C-terminal hydrolase
58		
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149	ULP	Ubiquitin-like protease
1		
2		
150	UPR	Unfolded protein response
4		
5		
151	UPS	Ubiquitin proteasome system
7		
8		
152	USP	Ubiquitin-specific protease
10		
11		
153	WNT	Wingless and integration site
12		
13		
14		
154	WWP-1	WW domain-containing E3 ubiquitin protein ligase 1
15		
16		
17		
155	XBP-1	X-box binding protein homolog 1
18		
19		
20		
156	ZIP-3	bZIP transcription factor family member 3
21		
22		
23		
157	ZUFSP	Zinc finger with UFM1-specific peptidase domain protein
24		
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159 **1. INTRODUCTION**

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160 **1.1 INNATE IMMUNITY**

161 Innate immunity is the first line of host defence against infection [1]. It is an ensemble of  
162 protective mechanisms, found in all multicellular organisms. It comes into play when pathogen  
163 avoidance, behavioural strategies that limit pathogen exposure, fail [2]. Unwanted microbial  
164 colonisation of exposed tissues, like the lungs or intestine, or microbial breaches of skin and other  
165 physical barriers, can lead to infection [3]. Host organisms are capable of sensing pathogens or the  
166 damage they cause. Notably, a large set of pattern recognition receptors (PRRs) that includes Toll-,  
167 RIG- and NOD-like receptors (TLRs, RLRs and NLRs), bind microbe-associated molecular patterns  
168 (MAMPs) such as lipopolysaccharides (LPS), peptidoglycans or viral nucleic acids, or sense  
169 damage-associated molecular patterns (DAMPs) such as extra-cellular ATP, urea or mitochondrial  
170 DNA [4]. In parallel to this pattern-triggered immunity (PTI), hosts monitor the infection-mediated  
171 perturbation of core cellular processes, aka ‘homeostasis-altering molecular processes’ (HAMPs)  
172 [5]. Recognition of MAMPs or DAMPs by PRRs or the sensing of HAMPs in turn trigger signaling  
173 cascades that lead to an immune response, to eliminate the pathogen and reduce the negative  
174 impact of the infection on host fitness [6]. The microbial-killing branch of this immune response is  
175 called resistance and ranges from the production of specialized inflammatory cytokines and  
176 microbicidal molecules such as antimicrobial peptides (AMP) or reactive oxygen species (ROS), to  
177 cellular processes such as phagocytosis of extracellular microbes and autophagy of intracellular  
178 pathogens [7-9]. The homeostatic branch, called tolerance, ensures host survival, by economizing  
179 energy and responding to the stress and damage caused by pathogens or by the innate immune  
180 response itself (i.e. immunopathology) [10, 11]. It can involve activation of the ubiquitin–proteasome  
181 system (UPS) or autophagy to counteract proteotoxic and organelle damage, and induction of the  
182 Unfolded Protein Response (UPR) to resolve endoplasmic reticulum (UPR<sup>ER</sup>) or mitochondrial  
183 (UPR<sup>mt</sup>) dysfunction [12-15].

185 **1.2 INNATE IMMUNITY IN *C. ELEGANS***

186 Studies with *Drosophila melanogaster* have been instrumental to the discovery of conserved  
187 immune pathways, most notably the TLR/NF-κB pathway [16]. Similarly, work with *Caenorhabditis*  
188 *elegans* has contributed to our understanding of the origins and mechanisms of innate immunity  
189 [17]. This 1 mm long free-living nematode is a microbivore that is found in the wild in decaying  
190 organic matter such as rotting fruits and stems [18]. This microorganism-rich environment provides  
191 food to *C. elegans* but it also exposes the animal to potentially harmful pathogens [19]. Indeed, *C.*  
192 *elegans* can be naturally infected by various species of bacteria, fungi, viruses and other parasites  
193 [19], against which it has a sophisticated immune system [17]. *C. elegans* lacks circulatory immune

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

### 1.3 UB AND UBL POST-TRANSLATIONAL MODIFICATIONS

219 One of the processes that can be monitored is protein homeostasis. This is controlled in part by  
220 ubiquitination, a post-translational modification that regulates virtually all aspects of physiology [35].  
221 Ubiquitin (Ub) is a highly conserved 76-amino acid polypeptide that covalently modifies a substrate  
222 on one (mono-) or several (multi-ubiquitination) lysine residue(s) via its C-terminal glycine (G76)  
223 (Figure 1a,b). A high diversity of homo- and heterotypic poly-Ub chains can also be formed since a  
224 distal Ub can attach to the first methionine (M1) or to one of the 7 lysine residues of a proximal Ub  
225 [36] (Figure 1b). Ubiquitination arises from the sequential action of 3 Ub-modifying enzymes: a Ub-  
226 activating enzyme (E1), a Ub-conjugating enzyme (E2) and a Ub-ligase enzyme (E3) (Figure 1c,  
227 Table 1). Ubiquitination can be reversed by de-ubiquitinating enzymes (DUBs) (Figure 1c, Table 1)  
228 [37]. Ub binding proteins (UBP), aka Ub receptors, can bind mono-, multi- and poly-Ub chains of  
229 different topology via a variety of Ub binding domains (UBD) with different affinities and avidities

230 [38]. These interactions with Ub receptors in turn dictate each substrate's fate, and often lead to  
231 degradation [39]. Indeed, K48 poly-ubiquitination is known to promote protein degradation by the  
232 26S proteasome, a large multi-subunit complex of 2.5 MDa (**Figure 1c, Table 1**) [40]. In addition to  
233 the ubiquitin-proteasome system (UPS), ubiquitination is also involved in the degradation of protein  
234 aggregates and damaged organelles through selective autophagy via autophagic receptors that  
235 harbour UBDs [41]. Non-degradative functions of ubiquitination are also well documented, including  
236 roles in signal transduction [42] (**Figure 1c**).

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

249 Similar to Ub, a set of evolutionary conserved Ub-like proteins (Ubls) such as Small Ubiquitin-  
250 Like Modifier (SUMO) can also covalently modify protein substrates [46]. SUMOylation also  
251 engages E1-, E2- and E3-like enzymes and Ubl-specific proteases (ULPs), conserved in *C. elegans*  
252 [47] (**Table 1**). SUMOylation is involved in a wide range of physiological processes and the majority  
253 of SUMOylated substrates are nuclear with various functions, such as transcriptional regulation and  
254 nucleo-cytoplasmic transfer [48, 49].

#### 255 **1.4 UB-RELATED PROCESSES IN C. ELEGANS IMMUNITY**

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

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

270

271

## 2. ALTERATION OF UPS, A HAMP TRIGGERING SURVEILLANCE IMMUNITY

272

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

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

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

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

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

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#### 3.1 REGULATION OF THE NEURO-IMMUNE SENSING OF PATHOGENS

326 Pathogen or parasite avoidance behaviour is an immune strategy widespread across the animal  
327 kingdom [2]. This “better safe than sorry” immune mechanism is likely to be cost-effective as it can  
328 prevent or reduce pathogen intake. *C. elegans* is a model of choice for understanding the role of the  
329 nervous system in this type of behaviour at the molecular and cellular level [65]. Nearly a third of *C.*  
330 *elegans*’ somatic cells are neurons. The anatomy of each of its 302 neurons and their synaptic  
331 connectivity are clearly defined allowing the establishment of a simple behavioural neuronal network  
332 [66-69]. *C. elegans* uses its sensory system to detect bacterial secondary metabolites,  
333 neuropeptides induced by infection, and to measure oxygen concentration. This in turn mediates



334 pathogen avoidance or aversive learning [70]. *C. elegans* avoids many environmental pathogens  
335 including *Pseudomonas aeruginosa* and *Serratia marcescens* [27, 68, 71-73]. Failure to avoid *P.*  
336 *aeruginosa* leads to infection, with the bacteria colonizing the nematode intestine, killing the worms  
337 [74]. Interestingly, Chang *et al.* demonstrated a critical role for the E3-HECT ligase HECW-1 in *C.*  
338 *elegans*' behavioural avoidance of *P. aeruginosa* [75]. Using comparative genomics of wild isolates  
339 of *C. elegans*, Chang *et al.* identified two naturally occurring polymorphism in the coding sequence  
340 of *hecw-1* resulting in reduced avoidance behaviour compared to the reference laboratory strain N2.  
341 Conversely, a deletion allele of *hecw-1* conferred increased avoidance behaviour, indicating that  
342 HECW-1 negatively regulates avoidance behaviour and that both polymorphisms most likely provide  
343 a gain of function to HECW-1. Using reporter strains, tissue specific rescue and neuronal ablation,  
344 the authors showed that HECW-1 functions specifically in the pair of outer labial (OLL) sensory  
345 neurons localized in the anterior bulb of the pharynx to inhibit avoidance behaviour (**Figure 4**).  
346 Epistasis analysis suggested that HECW-1 represses the activity of the neuropeptide receptor NPR-  
347 1. This GPCR chemoreceptor has been implicated in avoidance phenotype, aversive learning and  
348 the innate immune response against *P. aeruginosa* [73, 76, 77]. The regulation of NPR-1 by HECW-  
349 1 is likely indirect as they do not function in the same neuronal cell. Indeed, evidence suggests that  
350 HECW-1 represses the activity of NPR-1 in RMG, a pair of inter/motoneurons localized in the  
351 posterior bulb of the pharynx [75] (**Figure 4**). Altogether, these data demonstrate the critical role of  
352 an E3 ligase in extracellular pathogen sensing and the regulation of avoidance behaviour. How  
353 HECW-1 is regulated during infection, what its direct substrates are and how it affects NPR-1  
354 activity remain, however, to be determined (**Figure 4**). Several substrates of the mammalian  
355 ortholog HECW1 (aka NEDL1) have been described, including the EGF-like receptor ErbB4 and a  
356 transducer of the WNT signaling pathway, Dishevelled-1 [78, 79]. Whether the EGF or the WNT  
357 pathway are involved in avoidance behaviour in *C. elegans* merits exploration. Relatively little is  
358 known about the molecular mechanism of parasite avoidance behaviour in mammals [80], although  
359 details are starting to emerge (e.g. [81]). Interestingly, Dishevelled proteins are expressed in  
360 olfactory sensory neurons [82]. Whether NEDL1 is involved in the regulation of Dishevelled proteins  
361 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*

369 [83, 84]. The PMK-1 “cassette” comprises NSY-1, SEK-1 and PMK-1 (homologous to mammalian  
370 ASK1, MKK3/6 and p38, respectively) and has been shown to act downstream of the TIR-domain  
371 protein TIR-1, the nematode orthologue of the human SARM1 [84-86]. We will review in this section  
372 the several lines of evidence that ubiquitination is an important regulator of the PMK-1 pathway in *C.*  
373 *elegans* (**Figure 5**).

374 The ascomycete fungus *Drechmeria coniospora* is the best-characterized causative agent of  
375 epidermal infections in *C. elegans* [87]. *D. coniospora* produces conidia that attach and then pierce  
376 *C. elegans*’ cuticle, and send hyphae throughout the animal eventually leading to its death [88].  
377 Rapidly upon infection, *C. elegans* triggers a host response characterized by the epidermal  
378 expression of AMPs, notably a cluster of the neuropeptide-like proteins (NLPs) including NLP-29  
379 [86, 89]. Induction of these AMPs is under the control of the TIR-1/PMK-1 pathway. In the  
380 epidermis, this pathway lies upstream of the STAT-like transcription factor STA-2 and downstream  
381 of DCAR-1, the only known DAMP-sensing GPCR alluded to above. DCAR-1 is activated by its  
382 cognate ligand, 4-hydroxyphenyllactic acid [31]. *dcar-1* was one of 360 genes found in an RNAi  
383 screen of the whole genome (ca. 20,000 genes) whose knockdown prevented *D. coniospora*-  
384 induced expression of an *nlp-29p::gfp* reporter [31]. Among the other genes, there were ten  
385 ubiquitin-related genes encoding one E2 (*let-70*), three monomeric E3s (E3-HECT *hecd-1*, E3-RING  
386 *rbpl-1* and the E3-U-box *prp-19*), two E3-CRL subunits (the SKR adaptor *skr-1* and the SRS *dcaf-1*),  
387 one DUB (*usp-39*) and three proteasomal subunits (*pas-3*, *pas-5* and *pbs-2*) (**Figure 5**) [90]. The  
388 presence of an E2 enzyme and proteasomal subunits suggests that ubiquitination, and notably  
389 degradative ubiquitination, may be involved in the regulation of the epidermal p38/PMK-1 pathway.  
390 Further studies need to be performed to assess the specific role of each E3 and DUB found in this  
391 screen, and to determine whether they directly, or indirectly, regulate factors specific to *D.*  
392 *coniospora* infection or elements of the TIR-1/PMK-1 cassette.

393 The PMK-1 pathway is also required for intestinal immune responses upon infection by  
394 several bacteria such as Gram-negative bacteria *S. marcescens* and *P. aeruginosa* and the Gram-  
395 positive bacteria *Enterococcus faecalis* [83, 85, 91]. In the intestine, PMK-1 acts upstream of the  
396 transcription factor ATF-7 [92]. A small-scale RNAi screen was undertaken by Alper *et al.* who used  
397 a reporter of C-type lectin expression (*clec-85p::gfp*) to identify innate immune regulators in *C.*  
398 *elegans* [93]. *clec-85* is expressed in the intestine and is induced by *S. marcescens* [94]. The *clec-*  
399 *85p::gfp* reporter is regulated by multiple immune pathways, including PMK-1/ATF-7 following *P.*  
400 *aeruginosa* infection [91, 95] (**Figure 5**). Accordingly, clones targeting *nsy-1* and *tir-1* were found  
401 among the RNAi clones that altered *clec-85p::gfp* expression [93]. In addition, the E2 *let-70* as well  
402 as two E3s, the E3-RBR *ari-1.3* and the E3-RING *siah-1* were found to modulate expression of *clec-*  
403 *85::gfp*, reinforcing the idea that ubiquitin-related processes regulate immunity including the TIR-  
404 1/PMK-1 pathway [93] (**Figure 5**). Interestingly, several studies have suggested a potential innate



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

416 “Regulation of longevity by E3 ubiquitin-protein ligase” (RLE)-1, has also been shown to  
417 modulate host defence against *P. aeruginosa*, in this case through the regulation of the MAP3K  
418 NSY-1 [100]. Seeking new regulators of the NSY-1 orthologue ASK1, Maruyama *et al.* identified  
419 *roquin-2*, one of the two mammalian homologues of *rle-1*, and showed that it is required for H<sub>2</sub>O<sub>2</sub>-  
420 induced ASK1 degradation. In addition, Roquin-2 was found to interact with ASK1 and to induce its  
421 ubiquitination. Although ASK1 regulation by Roquin-2 was not assayed *in vitro*, these results  
422 strongly suggested that Roquin-2 is an E3 ligase regulating the degradative ubiquitination of ASK1.  
423 In *C. elegans*, expression levels of NSY-1 were increased in *rle-1* mutants compared to wild-type  
424 animals. Importantly, *rle-1* mutation or its down-regulation by RNAi was found to increase survival of  
425 animals infected with *P. aeruginosa*, in a *nsy-1* dependent manner, suggesting that increased  
426 expression of NSY-1 in RLE-1 deficient animals is sufficient to increase host defence. Consistent  
427 with such an idea, a strain over-expressing a NSY-1::GFP fusion protein displayed increased  
428 survival compared to the wild-type strain. Interestingly, mutation of *rle-1* or over-expression of NSY-  
429 1::GFP increased the level of PMK-1 phosphorylation in infected animals, suggesting that RLE-1  
430 may regulate PMK-1 activity via NSY-1 to control host defence against *P. aeruginosa* infection [100]  
431 (**Figure 5**).

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

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

### 442 443 **3.2.2. Regulation of the insulin/ IGF-1 signaling (IIS) pathway**

444 The insulin/insulin-like growth factor 1 (IGF-1) signaling (IIS) pathway is highly conserved. It  
445 involves the IGFR homologue, DAF-2, a receptor tyrosine kinase. Upon ligand binding, DAF-2  
446 triggers a kinase cascade that ultimately phosphorylates and inhibits the FOXO transcription factor  
447 DAF-16. The IIS pathway regulates multiple physiological processes including development,  
448 longevity, metabolism, as well as resistance to various environmental stresses [107]. It also plays a  
449 major role in innate immunity controlling host defence against both Gram-positive and Gram-  
450 negative pathogenic bacteria [108, 109]. The DAF-2 pathway has also been implicated in host  
451 defence against the pore-forming toxin (PFT) Cry5B from the soil bacterium *Bacillus thuringiensis*  
452 (Bt) [110]. PFTs are cytotoxic proteins that damage the plasma membrane of host cells and are  
453 required for virulence of many bacterial pathogens [111]. Chen *et al.* found that the DAF-  
454 2/PI3K/PDK-1/DAF-16 pathway is required for host defence against PFTs. They found evidence  
455 suggesting that in addition to the canonical pathway, a non-canonical DAF-2 pathway, independent  
456 of DAF-16, bifurcates at the level of PDK-1 to mediate host response to PFT (**Figure 6**). Taking  
457 advantage of publicly available datasets of protein-protein interactions, Chen *et al.* identified WWP-  
458 1 as a potential PDK-1 interactor [110]. WWP-1 is an E3-HECT of the NEDD4 subfamily [112]. They  
459 found that loss of function mutation of *wwp-1* or down-regulation of its expression using RNAi  
460 drastically reduced survival of animals exposed to Cry5B, indicating that WWP-1 is a critical positive  
461 regulator of host defence against this PFT. Interestingly, *wwp-1* RNAi was found to rescue partially  
462 the *daf-2* immune phenotype and completely rescued *daf-2;daf-16* double mutants, indicating that  
463 DAF-16 and WWP-1 must act in parallel downstream of DAF-2 to mediate full resistance to Cry5B  
464 PFT (**Figure 6**). Whether the ubiquitin-ligase activity of WWP-1 is required for host defence against  
465 PFT needs to be assessed but these results strongly suggest that ubiquitination is involved in innate  
466 immunity downstream of a non-canonical DAF-2 signaling cascade. The functional consequences of  
467 the PDK-1/WWP-1 interaction have not been explored either, but as several human E3-HECT are  
468 inhibited via phosphorylation, a model can be proposed with PDK-1 controlling WWP-1 activity  
469 through phospho-inhibition [113]. Note that Chen *et al.* also found that WWP-1 was required for host  
470 defence against *P. aeruginosa* but did not determine whether this was also dependent on the *daf-2*  
471 pathway [110]. As it will be discussed in the following section, WWP-1 might be involved in the  
472 tolerance response against *P. aeruginosa* via regulation of the UPR<sup>mt</sup> [114].

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

475 mice lacking this protein, has been implicated in the development of auto-immune diseases both in  
476 mice and humans [116]. This auto-immunity appears to be a consequence of multiple alterations of  
477 the adaptive immune system with aberrant CD4 T cell activation and humoral responses [116]. Of  
478 note, although in *C. elegans* WWP-1 was found to work in parallel to DAF-16, ITCH was found to  
479 mediate differentiation of CD4 follicular helper T cells by interacting and mediating UPS regulation  
480 of the DAF-16 homologue FOXO1 [117]. On the other hand, ITCH also plays a role in innate  
481 immunity and has been shown to dampen inflammation via the ubiquitin-based regulation of various  
482 kinases (RIP1, RIP2, TAK1), and UBP (TAB1) which regulate the NF- $\kappa$ B, p38 and JNK MAPK  
483 pathways [118-121]. WWP1 and WWP2 have also been involved in the regulation of TLR4- and  
484 TLR3- mediated inflammation through the UPS regulation of TRAF6 and TRIF, respectively [122,  
485 123]. Hence, WWP-1's role in innate immunity is conserved among its three mammalian  
486 homologues.

### 3.3 . REGULATION OF IMMUNE TRANSCRIPTION

487  
488  
489 We described above how ubiquitination can be involved in the regulation of innate immune signaling  
490 pathways. We will now give several examples of how Ub- and Ubl-modifying enzymes can also  
491 affect the activity of transcription factors and their co-factors, and thereby modulate host responses  
492 to infection.

#### 3.3.1 Regulation of the GATA transcription factor ELT-2

493  
494  
495 The erythroid-like transcription factor family (ELT)-2 is gut-specific transcription factor from the  
496 GATA family that is essential for the formation and the normal function of *C. elegans* intestine [124,  
497 125]. In addition, ELT-2 plays an important role in the regulation of genes required for host defence,  
498 or for recovery after infection [126-128]. Recently, the proteasomal subunit RPT-6 was found to  
499 functionally interact with ELT-2. Although the ATPase activity of RPT-6 was found to be required for  
500 this interaction, the overall proteolytic capacity of the proteasome did not influence ELT-2  
501 transcriptional activity [129]. On the other hand, a UPS-dependent regulation of ELT-2 has been  
502 revealed in the context of infection by *Burkholderia pseudomallei*. This Gram-negative bacterium is  
503 the causative agent of melioidosis and *C. elegans* animals fed with *B. pseudomallei* suffer from a  
504 lethal infection [130-132]. ELT-2 is implicated in host defence against *B. pseudomallei* as RNAi  
505 against *elt-2* reduces the survival of infected animals [133]. Further, ELT-2 has been found to be  
506 specifically targeted by *B. pseudomallei*, but not by other pathogens, as a strategy to suppress *C.*  
507 *elegans* immunity [133]. Whole-genome transcriptome analysis revealed that a set of ELT-2-  
508 dependent genes is progressively down-regulated over a time course of *B. pseudomallei* infection.  
509 Using a *elt-2::gfp* reporter strain, the authors could correlate this decrease with a reduction of ELT-

510 2::GFP protein levels. Interestingly, down-regulation of ELT-2::GFP during infection could be  
511 blocked by RNAi-treatment against the ubiquitin-encoding genes *ubq-1* and *ubq-2* as well as by  
512 RNAi targeting the proteasomal subunit *rpt-2*, suggesting that ELT-2 is degraded by the UPS during  
513 *B. pseudomallei* infection. Lee *et al.* identified two host genes encoding RING-finger proteins with  
514 putative E3 ligase activity whose expression was induced during infection and required for ELT-  
515 2::GFP degradation. Direct involvement of these putative E3 ligases in ELT-2 ubiquitination was not  
516 established but these results suggested a mechanism used by *B. pseudomallei* to degrade actively  
517 ELT-2 in order to counteract the immune response of *C. elegans* [133]. Additionally, degradation of  
518 the ELT-2::GFP protein was only observed when animals were infected with wild-type bacteria but  
519 not with type III secretion system (T3SS)-deficient strains, indicating that injected bacterial effectors  
520 might be required for ELT-2 degradation. To mount an efficient infection many pathogens secrete  
521 toxins or inject effectors that can interfere with the host cell ubiquitin machinery [134]. In a  
522 mammalian cell line model, *B. pseudomallei* has been found to interfere negatively with the Ub and  
523 Ubl machinery via the T3SS-injected effector cycle inhibiting factor (Cif) homologue in *B.*  
524 *pseudomallei*, CHBP [135]. This effector bears a deamidase activity that targets Ub and the Ubl  
525 NEDD8, a critical regulator of E3-CRL activity. Deamidation of a conserved glutamine in position 40  
526 of Ub and NEDD8 leads to reduced E3-ligase catalysed ubiquitin-chain synthesis and suppression  
527 of E3-CRL activity [135]. Determining whether CHBP is injected into *C. elegans* intestinal cells and  
528 is linked, via a possible compensatory mechanism, to enhanced UPS degradation of ELT-2, will  
529 require further study. The mammalian homologue of ELT-2, the transcription factor GATA4, is a  
530 central regulator of cardiac development [136] but it also acts redundantly with GATA6 to regulate  
531 intestinal epithelial differentiation during development and to maintain proper epithelial structure  
532 [137]. In addition, GATA4 is critical to the maintenance of gut barrier function and mucosal integrity  
533 following injury in mice [138], suggesting that targeting GATA4 for UPS degradation might represent  
534 an efficient strategy for bacteria invading the intestine. GATA4 UPS degradation in cardiomyocyte  
535 embryonic bodies was shown to be induced by high H<sub>2</sub>O<sub>2</sub> levels, and required the JNK cascade  
536 [139], but the identity of the E3 regulating its ubiquitination is still unknown. Further studies must  
537 thus be conducted to identify Ub-modifying enzymes mediating GATA4 UPS degradation and  
538 determine whether targeting GATA4 is a conserved strategy used by pathogens to facilitate  
539 intestinal invasion.

### 540 541 **3.3.2 Regulation of DAF-16**

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

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

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

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### 3.3.3 Regulation of the transcriptional co-factor AKIR-1

In *Drosophila* and mammals, NF- $\kappa$ B 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- $\kappa$ B target genes [149]. In *C. elegans*, which lacks NF- $\kappa$ B, 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<sup>mt</sup> 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<sup>mt</sup> 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<sup>mt</sup> [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-

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

633 There is no evidence yet that this pathway is strictly conserved in mammals. While the  
634 mammalian UPR<sup>mt</sup> does not seem to be regulated by DVE-1's homologues, the SATB protein  
635 family, it does involve three bZIP transcription factors: ATF4, ATF5 and CHOP [13]. ATF5 has been  
636 described as the functional homologue of ATFS-1, mediating UPR<sup>mt</sup> in mammalian cells [157]. In  
637 addition, ATF5 has been found to be activated downstream the pro-inflammatory cytokine IL-1 $\beta$  via  
638 inhibition of its UPS-based degradation [158, 159] and to activate cytokine secretion in Th1 cells  
639 [160]. Interestingly, ATF5 was shown to undergo SUMO-based regulation [161]. Whether  
640 SUMOylation of ATF5 impacts its transcriptional activity and is controlled by the ULP-4 homologues  
641 SENP6 and SENP7 requires exploration. Notably, SENP6 and SENP7 are instrumental in the  
642 activation of NLRP3 [162], a key NLR activated by various stresses including mitochondrial damage  
643 [163]. Thus, SUMOylation appears to be a common feature shared between *C. elegans* and  
644 mammals involved in the regulation of immune signaling upon mitochondrial damage.

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



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

668 As mentioned above, WWP-1's homologues play key roles in mammalian innate immunity.  
669 Similarly, in addition to its role in UPR<sup>mt</sup>, the homologue of ZIP-3, ATF4, has been shown to drive  
670 expression of inflammatory cytokines in human monocytes exposed to LPS [166] and to be critical  
671 for CD4+ T cell-mediated immune responses [167]. There is, however, no evidence in the literature  
672 of WWP1, WWP2 or ITCH regulating ATF4. On the other hand, during oxidative stress, ATF4 has  
673 been found to inhibit the expression of *wwp1* as well as *nedd4.2*, which encodes another E3-HECT  
674 from the NEDD4 family, thereby stabilizing their target protein LATS1 which induces cell death  
675 [168]. Thus more work needs to be undertaken to determine in detail the link between ATF4,  
676 NEDD4 ligases and immune signaling in mammalian systems.

677

#### 678 4. UB-RELATED PROCESSES IN HOST DEFENCE

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

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#### 686 4.1. E3-CRLS IN IMMUNE RESPONSES

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687 Comparative studies in *C. elegans* have revealed considerable similarity between the host  
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688 transcriptional response to two very distinct obligate intracellular pathogens, Orsay virus and the  
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689 microsporidian *N. parisii* [53, 170]. This shared response has been termed the Intracellular  
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690 Pathogen Response (IPR) and appears to diverge from responses induced by extracellular  
8  
691 pathogens [53, 170]. The IPR was found to be particularly enriched in F-box and MATH-domain  
10  
692 encoding genes. Both of these domains are found in two categories of E3-CRL SRSs: the F-box  
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693 domain is required for cullin interaction, while the MATH domain provides substrate binding when  
13  
694 associated with a BTB cullin-binding domain. There are approximately ~520 F-box and ~50 MATH-  
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695 BTB in *C. elegans*, greatly outnumbering their human homologues (**Table 1**) [171-172]. They also  
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696 exhibit features of molecular evolution indicative of being under strong selective pressure, with an  
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697 unusually high rate of non-synonymous codon change and a high rate of gene duplication and  
20  
698 deletion among closely-related species [171]. These features are typical of genes involved in host-  
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699 pathogen arms races, leading Thomas to propose that the 2 gene families might be involved in  
23  
700 innate immunity, prior to any functional data supporting the hypothesis [171]. Interestingly, the  
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701 positive selection in the *f-box* and *bath* genes was found to affect those regions corresponding to  
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702 substrate-binding domains but not the cullin-binding regions, suggesting that protein substrates from  
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703 pathogens are the evolutionary driving force. Thus, most of the F-box and MATH-BTB proteins  
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704 found in *C. elegans* could be specialized in direct ubiquitination and degradation of viral proteins  
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705 and/or bacterial effectors or toxins [171]. While there is still no direct evidence for pathogen proteins  
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706 being targeted by the host UPS in *C. elegans*, as described below, support has been growing for  
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707 the hypothesis that F-box and/or MATH-containing proteins might indeed be involved in  
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708 ubiquitination of intracellular pathogens, to promote their elimination [53, 173, 174]. It should be  
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709 noted, however, that some F-box and MATH-domain encoding genes are induced by several  
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710 different pathogens, including extracellular bacteria. For instance, Engelmann *et al.* identified 13 F-  
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711 box-containing protein that are commonly induced by three pathogenic bacteria, i.e. *Photobacterium*  
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712 *luminescens*, *E. faecalis* and *S. marcescens*. Among them, *fbxa-62* was also found to be induced by  
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713 two species of fungi (*D. coniospora* and *Harposporium sp*) and *fbxa-182* by the Orsay virus and *N.*  
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714 *parisii* [53, 169, 170]. Similarly, *math-15* is induced by the three bacterial species and these two  
48  
715 intracellular pathogens [53, 169, 170]. Thus, if some F-box and MATH-domain containing proteins  
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716 do act as specific intracellular PRRs detecting pathogen-specific MAMPs, others are more likely to  
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717 regulate host proteins involved in the common signaling pathways triggered by different pathogens.  
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718 The expansion of the E3-CRL SRS gene family is also seen in plants and could reflect a  
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719 shared evolutionary immune strategy [171, 172]. In mammals, where the number of E3-CRL genes  
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720 is small, SRSs nevertheless also play a role in immunity. A widely known example is the regulation  
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721 of NF- $\kappa$ B signaling by SCF $^{\beta}$ -TRCP, via ubiquitination and degradation of I $\kappa$ B [175]. The importance of  
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722 E3-CRL in host immunity is perhaps best revealed by the virulence factors of the Cif family,  
723 mentioned above, that interfere with E3-CRL activity through deamidation of NEDD8 [135, 176]. Cif  
724 are produced by several pathogens, including the human pathogens *Yersinia pseudotuberculosis*,  
725 and enteropathogenic and enterohemorrhagic *E. coli* (EPEC and EHEC) [177]. This underlines the  
726 importance in mammalian immunity of E3-CRL that may act in resistance or tolerance responses to  
727 pathogens.

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

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

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

767 Emily Troemel's team took a step in that direction, studying members of an expanded family  
768 of *C. elegans* homologues of the human protein Amyotrophic Lateral Sclerosis 2 Chromosome  
769 Region Candidate 12 (ALS2CR12) [182-184]. Many of these *protein containing ALS2CR12*  
770 *signature (pals)* genes were found highly induced by microsporidial and viral infection in *C. elegans*,  
771 as part of the IPR [53, 170]. Troemel and colleagues used *pals-5p::gfp* as a reporter of the IPR, in  
772 forward genetic screens to identify regulators of this transcriptional programme [182, 183].  
773 Intriguingly, they identified two other *pals* genes, *pals-22* and *pals-25* that act, respectively, as  
774 negative and positive regulators of the IPR [182, 183]. Thus, a *pals-22* mutant strain displayed  
775 constitutive expression of IPR genes, which was suppressed by *pals-25* mutation [182, 183]. This  
776 signaling appeared be independent of the infection-triggered signaling pathway, as *pals-25* did not  
777 affect the IPR induced by *N. parisii* [183]. Interestingly, although *pals-22* mutants had slowed  
778 development and reduced longevity compared to wild-type animals [182], they did, however, have  
779 increased resistance against *N. parisii* and Orsay virus [183]. Indeed, loads of *N. parisii* and Orsay  
780 virus were reduced in *pals-22* mutant strains, a phenotype suppressed by *pals-25* mutations [183].  
781 Significantly, these reduced pathogen loads were specific to natural intestinal pathogens, as loads  
782 of *P. aeruginosa* were not reduced, but rather increased, in *pals-22* mutants [183]. Additionally,  
783 deletion of the Ub-related IPR genes *cul-6*, *skr-3/5* or *skr-4/5* each had a small but significant impact  
784 on *N. parisii* pathogen load in a *pals-22* mutant background. [184]. Similarly, deletion of *rsc-1*, which  
785 encodes a RING-finger protein identified by co-immunoprecipitation as a binding partner of CUL-6,  
786 also significantly increased *N. parisii* pathogen load in the *pals-22* mutant [184]. Altogether, these  
787 results indicate that part of *pals-22* resistance phenotype relies on the activity of a CUL-6/RCS-  
788 1/SKR-3/4/5 E3-CRL [184]. F-box and/or MATH-domain proteins working within this E3-CRL might  
789 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<sup>ER</sup> that can prevent pathological ER proteotoxic damage. The UPR<sup>ER</sup> 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 act 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

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

### 827 828 **4.3.2. UPS-mediated proteostasis in the tolerance response**

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

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

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

860 Interestingly, increased UPS activity in infected animals required the dopamine receptor DOP-1, as  
861 *dop-1* mutants had a similar level of Ub(G76V)-GFP in infected and control animals. In addition,  
862 *dop-1* mutant were more sensitive to *P. aeruginosa* infection indicating that a signaling pathway  
863 involving dopaminergic mechanosensory neurons promotes UPS activity, helping the host to survive  
864 infection [188]. To understand the mechanism of increased UPS activity, the authors used RNAi to  
865 knockdown expression of the proteasomal subunit *pbs-5*. They saw a greater accumulation of  
866 Ub(G76V)-GFP in the *dop-1* mutant background, indicating that the proteasome is functional in *dop-*  
867 *1* mutants and that the accumulation of Ub(G76V)-GFP in *dop-1* animals must rather be a  
868 consequence of reduced protein ubiquitination [188].

### 869 870 **4.3.3. ERAD-mediated proteostasis in the tolerance response**

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

### 889 890 **4.3.4. Organelle autophagy in the tolerance response**

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



895 mitochondrial membrane potential, the E3-RBR ubiquitin ligase Parkin is activated by PINK-  
896 dependent phosphorylation and promotes ubiquitination of mitochondrial outer membrane proteins,  
897 ultimately leading to mitochondrial degradation by mitophagy [193]. Interestingly, Parkin and  
898 mitophagy have recently been shown to be involved in *C. elegans* host defence in a model of *P.*  
899 *aeruginosa* liquid pathogenesis [194]. *P. aeruginosa* induces diverse pathologies in *C. elegans*,  
900 depending on the growth conditions [195]. On solid agar media, *P. aeruginosa* virulence involves  
901 principally bacterial colonization, phenazines or cyanide production [195]. Pathogenesis in liquid  
902 culture is dominated instead by the siderophore pyoverdine, which induces a lethal hypoxic crisis  
903 [196, 197]. The importance of this iron-chelator agent in *P. aeruginosa* virulence was demonstrated  
904 by the fact that bacterial mutants deficient in pyoverdine biosynthesis exhibit reduced killing [197]. In  
905 part, this reflects the fact that upon iron chelation, ferripyoverdine can function as a signaling  
906 molecule, regulating the production of several secreted toxins [198]. Pyoverdine can also enter the  
907 cells of *C. elegans* and, alone provoke animal death [194]. During exposure to *P. aeruginosa* in  
908 liquid culture, pyoverdine as well as other chelators were found to damage mitochondria, leading to  
909 their removal. In accordance with mitochondrial clearance being mediated by mitophagy, autophagy  
910 and mitophagy reporters were induced by *P. aeruginosa* or by an iron-chelator alone. Most  
911 importantly, RNAi or mutation of autophagy and mitophagy genes, including a mutant of the E3-  
912 RBR Parkin encoding gene *pdr-1*, increased animal death upon exposure to *P. aeruginosa* or an  
913 iron-chelator. These results suggest that Parkin likely mediates ubiquitination of damaged  
914 mitochondria and subsequent mitophagy, and that this process is required to maintain *C. elegans*  
915 fitness during *P. aeruginosa* infection (**Figure 7**). The protective mechanism of ubiquitin-dependent  
916 mitophagy remains, however, to be determined. Interestingly, iron-chelation was also shown to  
917 promote mitochondrial clearance in mammalian HEK293T cells. As Parkin-mediated ubiquitin-  
918 dependent mitophagy plays a conserved role in mitochondrial clearance, it is highly plausible that  
919 this E3 ubiquitin ligase might also be required in iron-chelation-mediated mitophagy in mammalian  
920 cells. A recent study from the same research group demonstrated the importance of pyoverdine in  
921 the virulence of *P. aeruginosa* isolates from patients with cystic fibrosis. Using *C. elegans* as well as  
922 an acute murine pneumonia model, the authors could correlate the severity of *P. aeruginosa*  
923 infection with levels of pyoverdine, and rescue the pyoverdine-mediated host pathology with  
924 pyoverdine inhibitors [199]. Enhancing the Parkin-dependent ubiquitin-mediated mitophagy pathway  
925 might also represent a potential therapeutic approach to help patients with cystic fibrosis to recover  
926 from *P. aeruginosa* infection.

## 5. CONCLUSION

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930 Since its adoption as a model system by Sydney Brenner in the 1960's, *C. elegans* has played  
931 a pivotal role in fundamental research, allowing the discovery of universal cellular and molecular  
932 core processes, like apoptosis or RNAi, findings which have been rewarded with Nobel Prizes. With  
933 40% of its genes conserved in humans [115], this nematode has helped deciphering essential  
934 conserved physiological pathways involved for instance in aging and metabolism [200, 201]. The  
935 field of immunology is no exception and continues to benefit from *C. elegans* research. Here, we  
936 reviewed how it is helping to reveal the importance of ubiquitin-related processes in innate  
937 immunity. Ub-modifying enzymes act at all steps of host defence. It has become clear that Ub-  
938 related processes also play key roles in maintaining host health during infection. In addition to  
939 regulating canonical immune signaling pathways, when they malfunction, this itself can act as a  
940 danger signal, triggering immunity. We highlighted a potential cooperation between different Ub-  
941 modifying enzymes, the deSUMOylating enzyme ULP-4 and the E3 ligase WWP-1. Their action,  
942 downstream of mitochondrial damage, increases the activity of the key transcription factor ATFS-1.  
943 We suggest that different branches of the Ub and Ub-related network may work in an integrated  
944 manner to induce a coordinated immune response. This is clearly an area that merits further  
945 investigation. Studying WWP-1 has also helped uncover another interesting feature of Ub-related  
946 enzymes in innate immunity. WWP-1 was found to be involved not only in mitochondrially-triggered  
947 immune responses, but also in the regulation of the insulin/DAF-2 pathway upon PFT exposure.  
948 This is probably not a peculiarity of WWP-1, and other Ub-related enzymes such as MATH-33 might  
949 also be expected to play multiple roles in innate immunity. At the same time, given the large  
950 repertoire of F-box and MATH encoding genes, we predict that some enzymes must act in a more  
951 specific manner. Examining the expression patterns of Ub-related enzymes, determining what  
952 stimuli trigger their expression, and whether they are expressed in a single tissue or in the whole  
953 organism, will help identify those that have a specific role in immunity.

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

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



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

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## FIGURE LEGEND

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### 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.

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**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

1604 14-3-3 (upper panels). In condition of low IIS, unphosphorylated DAF-16 is targeted to the nucleus  
1605 and enhances host defence against *P. aeruginosa* and Cry5B pore-forming toxin (PFT) (lower  
1606 panels). The DUB MATH-33 is also targeted to the nucleus of intestinal cells, and interacts,  
1607 deubiquitinates and stabilizes DAF-16, boosting host defence against *P. aeruginosa* (left panel). It is  
1608 not known what E3 ligase ubiquitinates DAF-16 nor whether this occurs in the cytoplasm or in the  
1609 nucleus. In parallel, the E3-HECT ligase WWP-1 acts downstream of PDK-1 and enhances host  
1610 defence against PFT, probably through the Ub-dependent regulation of one or several substrates.  
1611 PDK-1 may regulate WWP-1 through an inhibitory phosphorylation.

1612  
1613 **Figure 7: Model of Ub and Ubl-dependent regulation of mitochondrial damage responses**  
1614 **during *P. aeruginosa* infection.**

1615 Depending on the culture conditions, *P. aeruginosa* virulence involves pyoverdine (left) or cyanide  
1616 and phenazines (right), virulence factors that induce mitochondrial damage. Following pyoverdine  
1617 exposure, damaged mitochondria are cleared by mitophagy in a PDR-1 dependent manner, which  
1618 provides protection against *P. aeruginosa* infection. By analogy with mammalian autophagy, PDR-1  
1619 might induce ubiquitination of a mitochondrial outer protein, which might recruit UBP bound to the  
1620 isolation membrane to promote mitochondrial engulfment into autophagosome. Fusion of  
1621 autophagosomes with lysosomes induces degradation of damaged mitochondria in  
1622 autophagolysosome structures in a process known as mitophagy. In solid culture, mitochondrial  
1623 damage leads to increased expression of the deSUMOylase ULP-4, which removes the SUMO  
1624 homologue SMO-1 from ATFS-1 and DVE-1. DeSUMOylated ATFS-1 displays increased stability  
1625 and transcriptional activity, while deSUMOylation of DVE-1 increases its nuclear translocation. The  
1626 increased expression of innate immune genes and/or UPR<sup>mt</sup> ultimately promotes host defence. *P.*  
1627 *aeruginosa* production of phenazines represses UPR<sup>mt</sup> in a ZIP-3 dependent manner. ZIP-3  
1628 negatively regulates ATFS-1-mediated host defence. WWP-1, induced upon mitochondrial stress,  
1629 appears to be the E3 ligase responsible for ZIP-3 degradation by UPS. Data suggest a model where  
1630 *P. aeruginosa* prevents ZIP-3 UPS degradation to counteract ATFS-1 mediated host defence.  
1631 Although shown here in the cytoplasm, the site of ZIP-3 UPS regulation is not known.

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

Ubiquitin-related genes				
Type	Family	<i>C. elegans</i>	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]
DUB	BTB	>100	169	[206, 210]
	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	<i>C. elegans</i>	Human	Ref.
SUMO		1	4	[215]
E1		2*	2*	[215]
E2		1	1	[215]
E3		? <sup>§</sup>	? <sup>§</sup>	[215, 216]
Peptidase	SENP	4	6	[215, 216]
	DeSI	1	2	[215, 217]
Proteasome-encoding genes				
Type		<i>C. elegans</i>	Human	Ref.
20S		14	14	[45]
19S		17	17	[45]

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\*: 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.

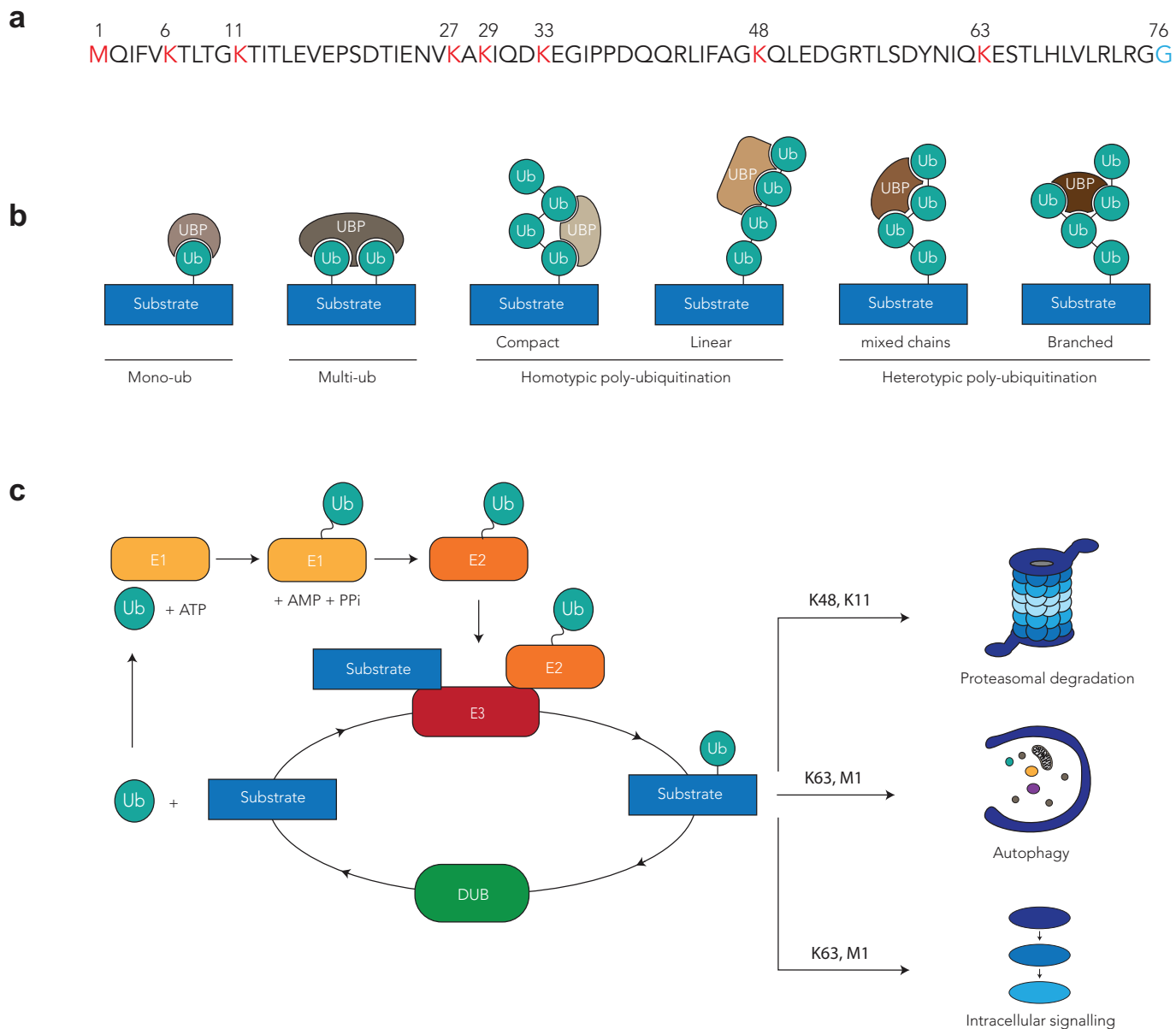
°: The RING-finger proteins RBX1 and RBX2 are conserved across evolution. The RCS-1 has been identified in *C. elegans* as a functional interactor of CUL-6 and appears as an additional CRL RING finger protein in this nematode.

1644  
1645**Table 2. Summary of Ub-related processes in innate immunity of *C. elegans***

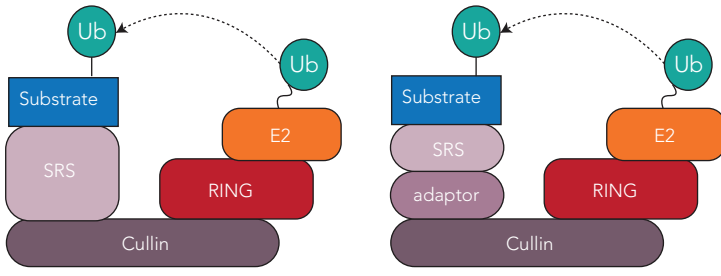
Pathogen/Toxin	Enzymes / Proteasome	Targets	Function	Ref.
<i>Regulation of immune surveillance</i>				
<i>N. parisii</i>	Proteasome	N/A	Pathogen-mediated proteasome alteration inducing surveillance immunity	[53]
<i>S. venezuelae</i>	Proteasome	N/A	Proteasomal inhibition induced neuronal cell death	[59,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

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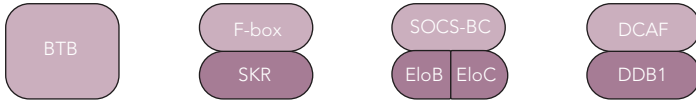
**Figure 1, Garcia-Sanchez**

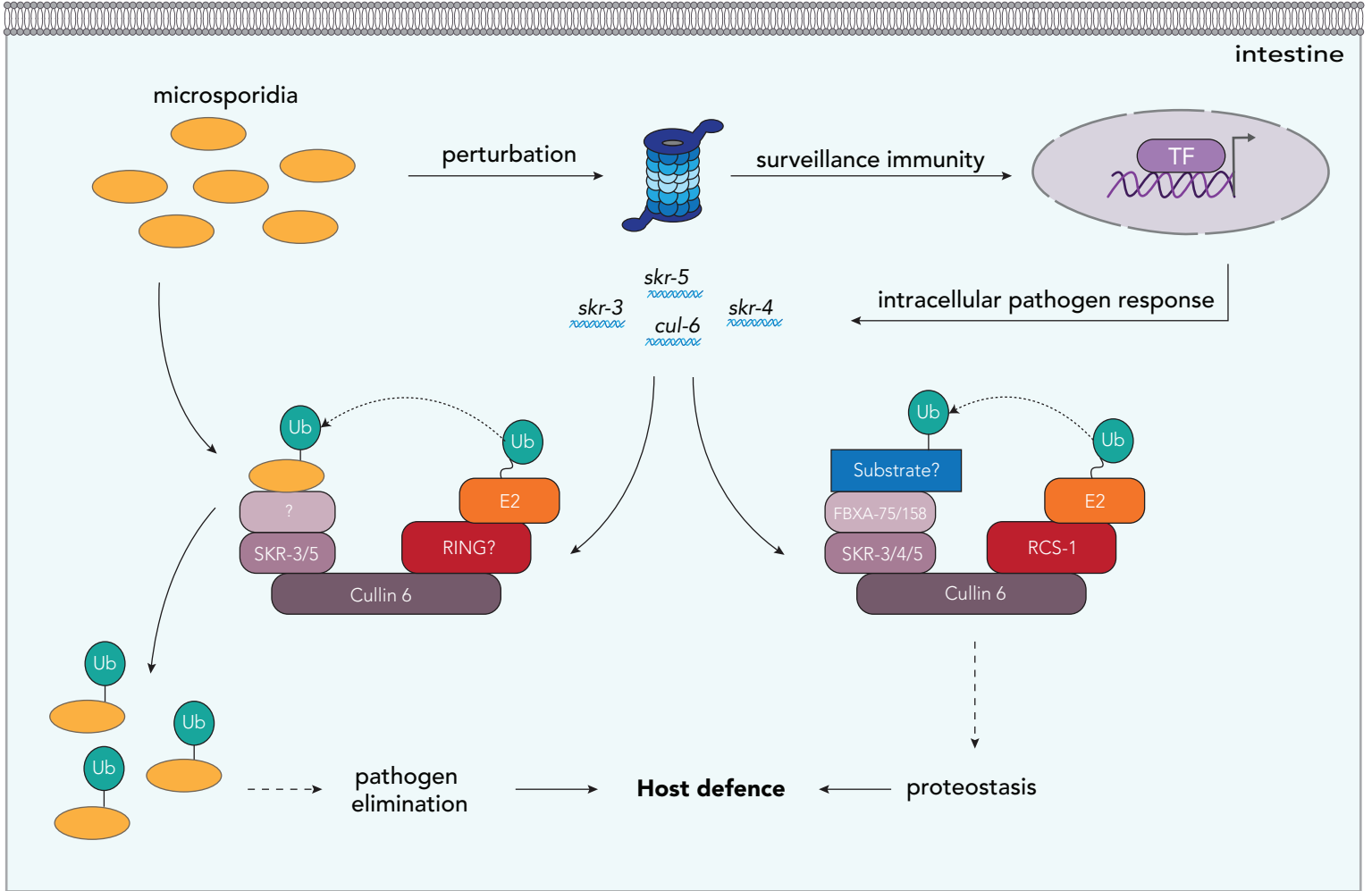


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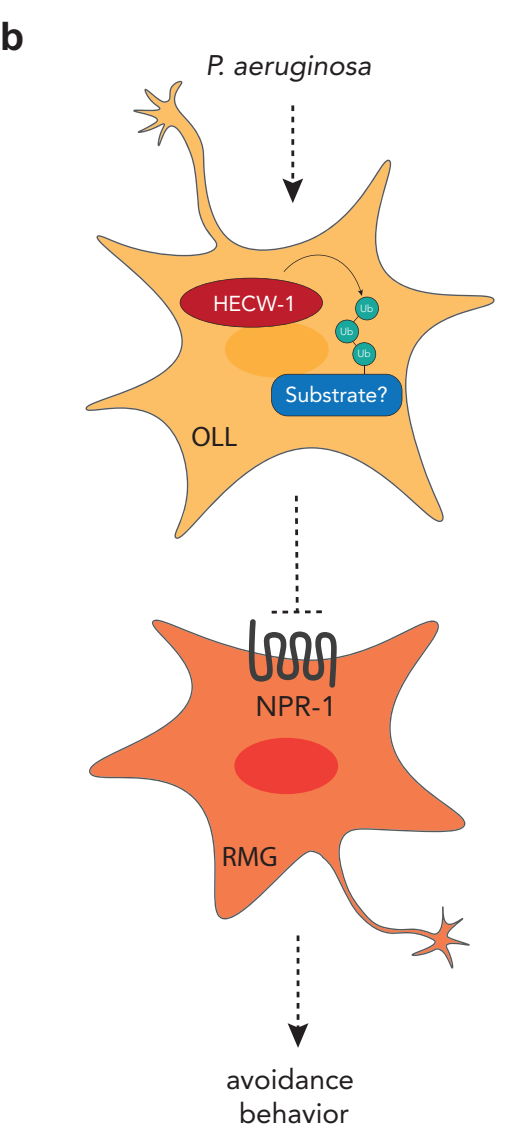
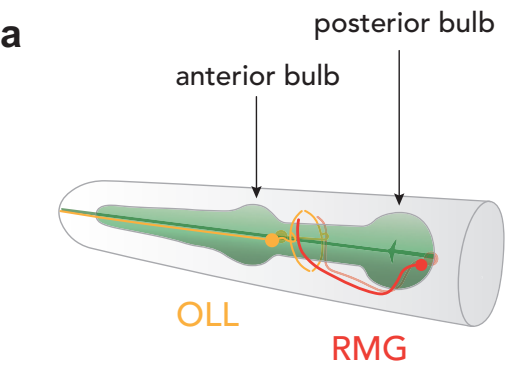


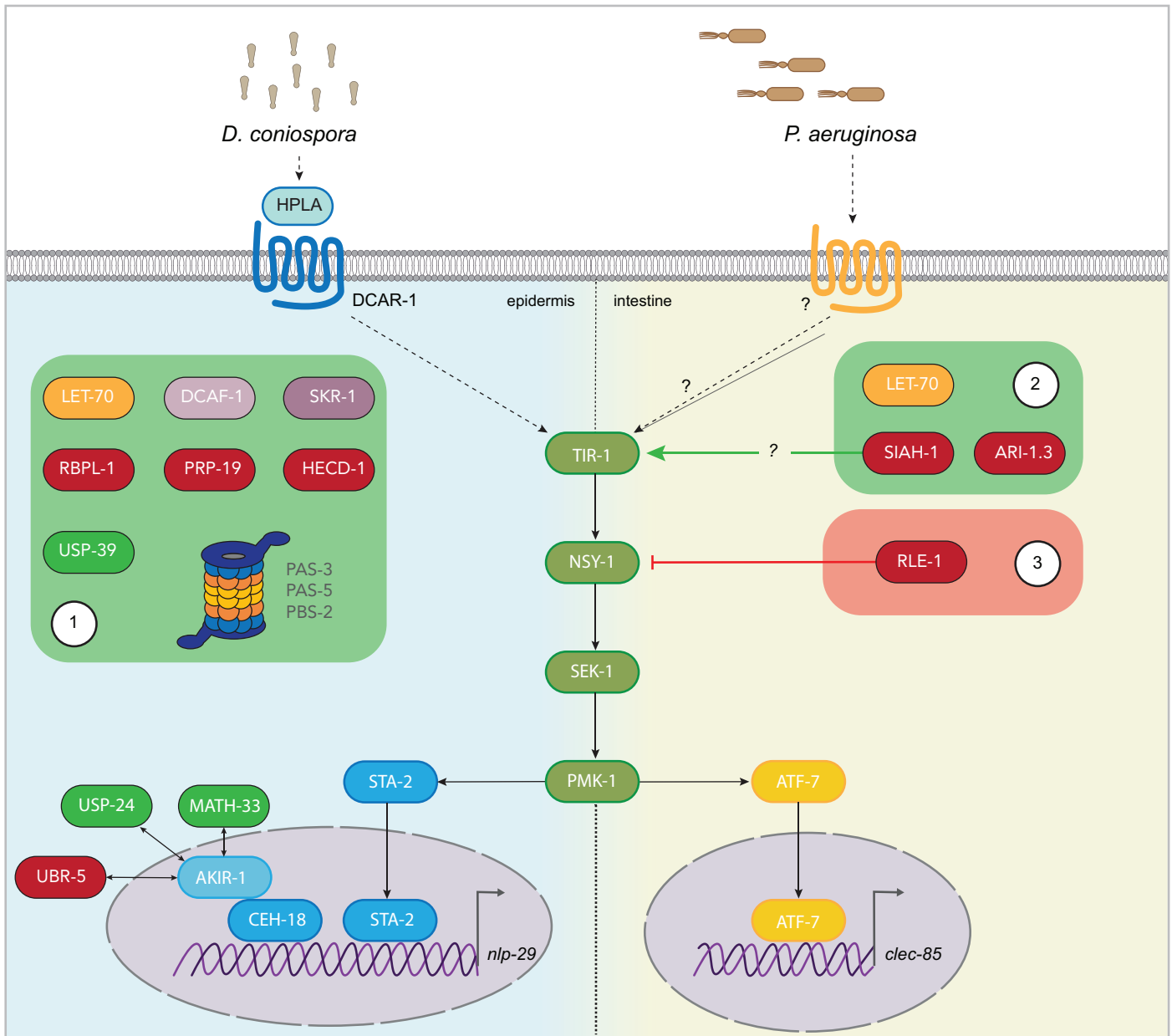
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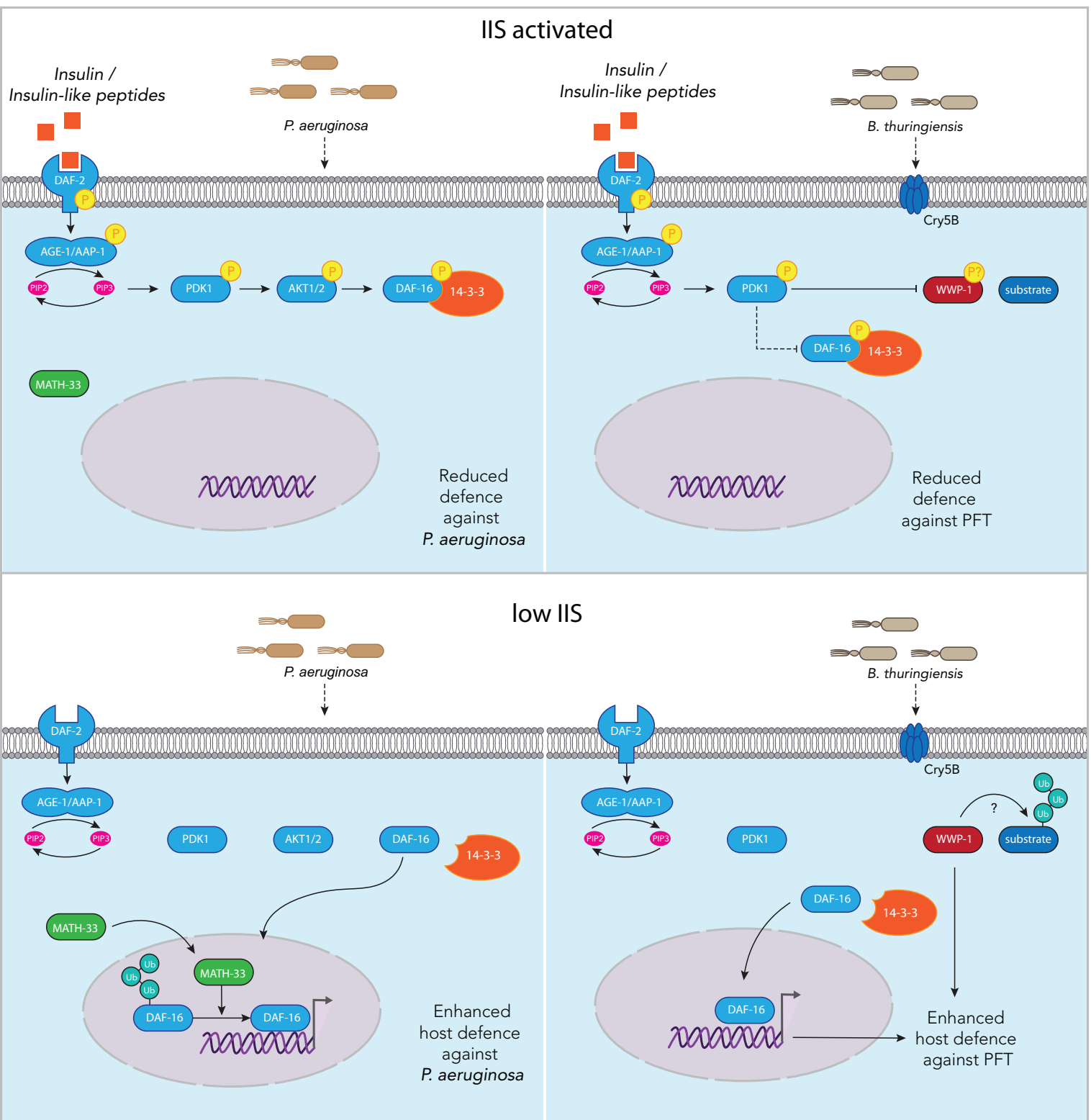












Figure

Figure 7, Garcia Sanchez

