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Evolution of the innate immune system: the worm perspective

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Summary: Simple model organisms that are amenable to comprehensive experimental analysis can be used to elucidate the molecular genetic architecture of complex traits. They can thereby enhance our understanding of these traits in other organisms, including humans. Here, we describe the use of the nematode *Caenorhabditis elegans* as a tractable model system to study innate immunity. We detail our current understanding of the worm's immune system, which seems to be characterized by four main signaling cascades: a p38 mitogen-activated protein kinase, a transforming growth factor-β-like, a programed cell death, and an insulin-like receptor pathway. Many details, especially regarding pathogen recognition and immune effectors, are only poorly characterized and clearly warrant further investigation. We additionally speculate on the evolution of the *C. elegans* immune system, taking into special consideration the relationship between immunity, stress responses and digestion, the diversification of the different parts of the immune system in response to multiple and/or coevolving pathogens, and the trade-off between immunity and host life history traits. Using *C. elegans* to address these different facets of host–pathogen interactions provides a fresh perspective on our understanding of the structure and complexity of innate immune systems in animals and plants.

The evolutionary perspective in innate immunity

Infection by a pathogen represents one of the major threats to any living organism. Therefore, the availability of an efficient immune system, which permits recognition and subsequent elimination of a pathogen, is of high adaptive value. Not surprisingly, the immune system of almost all organisms is extremely complex. The most impressive example is found in higher vertebrates. Here, the immune defense consists of two main parts: an innate response that is immediate and an adaptive response that is delayed but highly specific and long lasting. Of these, the adaptive system has received much attention because of its ability to generate immune 'memory', a trait that was successfully exploited for vaccination programs. It was only comparatively recently that research interest was again turned to innate immune mechanisms, as it became clear that innate factors are not only responsible for the early response to an invading pathogen but also, in vertebrates,

they are involved in the initiation of the adaptive response. They seem therefore to play a fundamental role in immunity as a whole (1).

With the exception of vertebrates, all other organisms (invertebrates, plants, and fungi) rely exclusively on innate immunity. Interestingly, many features of the innate system are highly similar among these organisms, suggesting that they have a common origin and have subsequently been conserved across millions of years of evolution. Non-vertebrate model systems thus may aid our understanding of innate immunity in higher vertebrates, including humans, in two main ways:

(i) They allow us to infer the evolutionary history of immune components and thus permit the identification of conserved and variable elements. This delineation provides information about their probable functions, as conserved elements are likely to have a central, possibly regulatory role, usually under strong negative selection. The most variable factors may be involved in the direct interaction with pathogens (recognition or elimination), such that they are subject to diversifying selection in order to keep track with rapidly evolving pathogens. Alternatively, they may represent specific adaptations to the life history characteristics of the organism studied.

(ii) Most importantly, some of these model systems (e.g. *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Arabidopsis thaliana*) permit faster and more efficient genetic analysis than the typical vertebrate models (e.g. mice and zebra fish). They are also less complex, showing a much lower level of redundancy in gene regulation, which clearly facilitates delineation of regulatory pathways. Consequently, as many components of innate immunity are conserved across phyla (see below), genetic analysis in the non-vertebrate systems may be used to probe the function and interactions of previously known vertebrate proteins or even more importantly help to identify novel candidate factors of the vertebrate immune system.

The best example of studying model systems comes from research in *Drosophila*. Here, detailed characterization of the Toll pathway paved the way for the discovery of the mammalian Toll-like receptors (TLRs). These receptors are now known to 'sense' a large spectrum of microbial patterns that, in the end, activate the nuclear factor-κB (NF-κB), which can lead to the expression of different anti-microbial peptides. Similarly, analysis of anti-microbial peptides from *Drosophila* aided our understanding of the diversity and function of these molecules in the mammalian immune response (2–4).

In this review, we briefly introduce *C. elegans* as a model organism and then discuss recent findings that constitute a first view of the worm's immune system. Many aspects of the interaction of *C. elegans* with pathogens have already been described in a number of recent reviews (5–8). Here, we will specifically focus on the immune system in a strict sense, i.e. the physiological defense system, whereas other facets of defense against infection (e.g. behavioral or those involving physical barriers) are discussed briefly. Additionally, we highlight several areas that have not been the object of much previous discussion, including the evolutionary relationship between immunity, stress responses and digestion, the diversification of the immune system in response to multiple and/or coevolving pathogens, and the trade-off between immunity and host life history traits. We consider these aspects to be of capital importance for a full understanding of *C. elegans* immunity, and they may also provide a fresh perspective on the structure of the innate system in other organisms, including humans.

The nematode *Caenorhabditis elegans* as a model for the study of innate immunity

Advantages of *C. elegans*

The nematode *C. elegans* has become one of the principal model species in biological research, especially in areas such as developmental biology, neurobiology, or gerontology (9). It owes its popularity to several characteristics that greatly facilitate comprehensive molecular genetic analysis. It can be easily maintained and manipulated in the laboratory. It is transparent, such that phenotypes can often be scored using simple microscopy. It has a short generation time, thus facilitating performance of breeding experiments, and it has two gender types: hermaphrodites and males. Hermaphrodites can self-reproduce, permitting the generation of isogenic lines. Males, however, must mate hermaphrodites, and as male sperm outcompete a hermaphrodite's own sperm, male versus hermaphrodite matings result in essentially fully outbred offspring. This property facilitates enormously genetic analysis in *C. elegans*. In addition, over the last couple of years, an array of molecular genetic methods has been developed and the whole genome sequence was completed, together rendering molecular genetic applications accessible and efficient (9–11).

Pathogen models for the analysis of worm immunity

C. elegans is a soil nematode, often found in decaying material, where it is likely to feed on a diversity of microorganisms (12).

In such habitats, frequent encounters with pathogens are expected, such that the nematode should have evolved a multifaceted immune response. In consideration of its natural ecology and its advantages for genetic analysis, *C. elegans* should prove extremely valuable for deciphering the molecular genetics of immunity. Perhaps surprisingly, there was not a single publication on *C. elegans* defenses until 1999. Since then, an increasing number of research groups have become interested in the interaction of *C. elegans* with pathogens (6, 7).

In many of these studies, *C. elegans* has been employed to screen for 'universal' virulence factors of human pathogens, e.g. *Pseudomonas aeruginosa*, *Salmonella enterica* serovar *typhimurium* (for simplicity *S. enterica* in the following), *Burkholderia pseudomallei*, *Serratia marcescens*, and *Yersinia pestis* (6, 7). These studies rely on the fact that the virulence factors relevant for infection of humans are also important for full pathogenicity during the infection of *C. elegans*. However, this fact must not be expected to be true in all cases. In this context, it is worth reiterating that the impact of a certain pathogen virulence factor is not independent of the host species, but rather it is specifically determined by the presence of a particular host susceptibility factor, as documented in a diversity of organisms (13). Consequently, the *C. elegans* model will only permit identification of virulence factors with relevance for humans, if the virulence factors target host factors or cellular processes that are conserved across phyla and which are thus identical or at least similar among nematodes and primates. In turn, this also means that specific virulence factors, which have targets shared by primates and nematodes, can be employed to identify conserved host immune factors. The presence of such virulence factors is expected in pathogens which have evolved to exploit and/or hamper a diversity of host organisms, e.g. *P. aeruginosa* or *S. marcescens*. In contrast, virulence factors, which only affect the nematode model, are then likely to target a host component specific to *C. elegans* (6, 7). Thus, as discussed below, the employment of human pathogens has provided valuable insights into *C. elegans* immunity.

As there is diversity in the mechanisms that underlie pathogenesis among the existing pathogen models, one would also expect to observe differences in the immune response that they induce in *C. elegans*. These differences should reflect the disease process (e.g. associated with toxins or infection) and the site of contact (cuticle, mouth, intestine, anus, vulva, or sensory openings). For instance, a toxin-producing bacterium, which mainly infects the gut lumen (e.g. *P. aeruginosa*), should elicit a different response from a pathogen that colonizes a specific tissue in a toxin-independent fashion (e.g. *Microbacterium nematophilum* that adheres to the peri-anal surface of worms). For similar pathogenesis pathways, the host response may also vary due to differences in the interacting molecules, e.g. different toxins or different surface molecules of pathogens, which lead to an infection. Furthermore, one may also expect to see differences in the response toward natural pathogens, against which the host may have evolved a highly specific and efficient response, and 'artificial' pathogens, toward which the host may simply react with a more general response. We have attempted to classify the existing model pathogens according to these criteria and highlighted those that are being used to investigate *C. elegans* immunity (*Table 1*).

The current list includes taxa from different bacterial groups, including gram-negative and gram-positive species, and also fungi. Some of these pathogens exert their main effect with the help of a toxin (e.g. *P. aeruginosa* strain PA01), others by establishing an infection (e.g. *M. nematophilum*), and still others by both toxins and infection (e.g. *P. aeruginosa* strain PA14, *Bacillus thuringiensis*). The list includes pathogens that directly infect the host or use a specific stage for host invasion (e.g. *B. thuringiensis* spores). Most of the pathogens enter the worm via the mouth and cause their main damage in the anterior part of the intestine (e.g. *P. aeruginosa*, *S. marcescens*, and *B. thuringiensis*), while others invade via the cuticle and infest the different organs within the body cavity (e.g. *Drechmeria coniospora*). In spite of the diversity of pathogens employed, the current list still contains important gaps.

Almost all the pathogens described thus far are bacteria and the rest are fungi. The infection of *C. elegans* by viruses, protists, or multicellular parasites has not as yet been studied in detail. In fact, to date, with the exception of certain protists (P. Peyret, C. Léopold Kurz, and Jonathan J. Ewbank, unpublished results), none of these are known to be capable of interacting specifically with *C. elegans*.

Secondly, most model pathogens exert their main effect in the anterior part of the intestine. Considering that, at 20° C, an adult worm produces three pharyngeal pumps per second and with every pump takes in roughly 20 bacteria (14), *C. elegans* continuously ingests a very large number of microorganisms. Hence, the front gut may indeed be the main target for pathogen attack. At the same time, however, *C. elegans* is immersed in a 'microbial soup' in its natural habitat, such that parasite invasion via the cuticle represents a similarly probable alternative, as has been described for other nematodes (15). Therefore, the above observation may also reflect a research bias.

Thirdly, the pathogen models may not be naturally coexisting species. The two main exceptions are *P. aeruginosa* and *P. fluorescens*, for which some strains were previously found in

Table 1. **Pathogen models of Caenorhabditis elegans**

Species	Classification	Effect‡	Target§	Natural¶	Immunity**	References
Gram negative						
Aeromonas hydrophila	γ-Proteobacteria	ND	I	–	–	(147)
Burkholderia cepacia	β-Proteobacteria	T + I?	I	P	–	(30, 148)
B. mallei	β-Proteobacteria	T + I?	I	P	–	(30, 148, 149)
B. multivorans	β-Proteobacteria	T + I?	I	P	–	(30, 148)
*B. pseudomallei**	β-Proteobacteria	T + I	I	P	+	(30, 31, 149)
B. thailandensis	β-Proteobacteria	T + I	I	P	–	(30, 149)
B. vietnamiensis	β-Proteobacteria	T + I?	I	P	–	(30, 148)
Erwinia christamthemi	γ-Proteobacteria	ND	I	–	–	(147)
Pectobacterium carotovorum	γ-Proteobacteria	ND	I	–	–	(147)
Photorhabdus luminescens	γ-Proteobacteria	ND	I	P	–	(147)
*Pseudomonas aeruginosa**	γ-Proteobacteria	T + I	I	+	+	(28, 31, 43, 72, 73, 148, 150)
P. fluorescens	γ-Proteobacteria	ND	I	+	–	(28, 73)
Salmonella enterica serovar *typhimurium**	γ-Proteobacteria	$T + I^P$	I	–	+	(29, 42, 46, 47)
*Serratia marcescens**	γ-Proteobacteria	I^P	I	P	+	(27, 44, 45, 151)
Shewanella massalia	γ-Proteobacteria	ND	I	P	–	(147)
S. oneidensis†	γ-Proteobacteria	ND	I	P	–	(147)
Xenorhabdus nematophila	γ-Proteobacteria	ND	I	P	–	(147)
Yersinia pestis	γ-Proteobacteria	B	M	–	[+]	(31, 152)
Y. pseudotuberculosis	γ-Proteobacteria	B	M	–	–	(152)
Gram positive						
Agrobacterium tumefaciens	α-Proteobacteria	ND	I	P	–	(147)
*Bacillus megaterium**	Firmicutes	ND	I	P	–	(25, 147)
*B. thuringiensis**	Firmicutes	$T + I^P$	I	P	[+]	(18–21, 71, 153–155)
*Enterococcus faecalis**	Firmicutes	I^P	I	–	+	(37, 156, 157)
Microbacterium nematophilum	Actinobacteria	I^P	A	P	[+]	(22)
Staphylococcus aureus	Firmicutes	I	I	–	+	(37, 157, 158)
Streptococcus pyogenes	Firmicutes	T	I	–	–	(157, 159)
S. pneumoniae	Firmicutes	ND	I	–	–	(157)
Streptomyces albireticuli	Actinobacteria	I	B	P	–	(160)
Fungi						
Cryptococcus neoformans	Basidiomycota	I	I	P	[+]	(161)
Drechmeria coniospora	Ascomycota	I^P	B	P	[+]	(162–164)

*Species for which several strains are under investigation and show different effects.
†The strain described as *S. frigidimarina* MR1 in (147) is in fact *S. oneidensis* MR1.
‡Main effect: B, biofilm; I, infection; I^P, persistent infection demonstrated after short exposure to pathogen; ND, not described; T, toxin.
§Main target: A, anal region; B, whole body; I, intestine; M, mouth region.
¶Natural pathogen of *C. elegans*: P, coexistence possible, because they either inhabit the soil or show a very specific relationship with the worm, suggestive of coevolution; +, coexistence; –, coexistence unlikely.
**Pathogen used for analysis of worm immunity: +, pathogen is employed; –, pathogen not employed; [+], analysis is known to be underway, but not yet published.

association with natural *C. elegans* strains (16, 17). Additional exceptions may include *S. marcescens*, *B. thuringiensis*, or the fungus *D. coniospora*, which are commonly found in the soil and which could theoretically be encountered by *C. elegans* in the wild. Of these, *B. thuringiensis* shows an additional sign of a long-term association with the worm: some strains produce toxins, which only affect nematodes (especially soil inhabitants) and which vary in their effect toward different nematode taxa, including some with high specificity toward *C. elegans* (18–20). Moreover, different natural *C. elegans* isolates also vary in their responses toward specific *B. thuringiensis* strains (21). Both factors taken together suggest the presence of specific adaptations between the two taxa, possibly as a result of coevolutionary interactions (21). Additionally, the bacterium *M. nematophilum* also shows a particular relationship with *C. elegans*, in that it is able to specifically infect the anal tissue (22). This finding may similarly be indicative of a long-term association. As for many of the worm pathogens, it is not yet known, however, whether this bacterium really coexists with the worm in nature.

Diversity of defense mechanisms

The defensive repertoire

Turning to the modes by which the worm defends itself against potential pathogens, it is worth emphasizing that *C. elegans* possesses (i) a behavioral response, (ii) physical barriers, and (iii) a physiological defense, the latter constituting its innate immune system. These responses serve either to decrease the general likelihood of pathogen encounter (behavior) or to protect possible contact zones, such as the worm's surface, the different body openings (mouth, anus, excretory

pore, vulva, and the openings of sensory neurons), and also the intestine, which could be colonized by pathogens during the process of feeding (the physical and physiological components). These lines of defense are not independent, but rather they complement each other. Most importantly, the molecules and regulatory pathways involved may overlap. For instance, the behavioral response is presumed to rely on pathogen recognition and subsequent signal processing in order to influence the activity of specific muscles. The same recognition and signal-processing pathway may be exploited to induce a physiological response of the innate immune system. Similarly, the physical barrier, e.g. the cuticle of the worm, may contain molecules with anti-microbial activity, which should thus be considered part of the physiological defense.

Behavior

The first line of defense consists of recognition of harmful microbes, followed by a coordinated behavioral reaction. The ability of *C. elegans* to perceive and respond to chemical cues is well documented, including attraction to nutritious and repulsion from noxious substances (23, 24). Worms have also been shown to be able to distinguish between different food bacteria (25, 26). Indeed, worms seem to show a preference for bacterial strains that sustain high reproductive and population growth rates (17, 26). Furthermore, *C. elegans* has been observed to exhibit two types of behavioral responses when confronted with potential pathogens: pathogen evasion and reduced food ingestion. In particular, wildtype worms placed on a bacterial lawn of pathogenic *S. marcescens* (strain Db11) were shown to increasingly avoid the bacteria (27). In a standard choice experiments, where worms were confronted with two bacterial lawns either with or without pathogenic bacteria, wildtype *C. elegans* strongly avoided pathogenic *B. thuringiensis* (21). Furthermore, reduced food ingestion rates were observed when *C. elegans* was confronted on solid agar plates with *P. aeruginosa* (28), *S. enterica* (29), *B. pseudomallei* (30), and *B. thuringiensis* (21). This response was also implicated as a reaction to pathogenic *P. aeruginosa*, *Y. pestis*, or *B. pseudomallei* in a liquid culture medium (31). However, in all these cases, it cannot be excluded that feeding inhibition is a consequence of intoxication processes instead of an active behavioral response.

These results highlight the importance of behavior as a component of worm defense. The genetic basis of this type of response is still largely unknown. The only exception refers to the observation that mutant worms homozygous for the *tol-1* (nr2033) allele, a loss-of-function allele of the only worm homolog of the *Drosophila Toll* gene (see above), lose their ability to evade the pathogenic *S. marcescens* strain Db11 (27). Although *tol-1* is expressed in some non-dopaminergic mechanosensory neurons, the observed mutant phenotype does not seem to result from a mechanosensation defect. As *tol-1* is also expressed in a subset of putative chemosensory neurons, this finding suggests that TOL-1 contributes to the recognition of a pathogen-associated compound, which subsequently leads to a change in *C. elegans* behavior (27).

Another candidate involved in pathogen-evasion behavior may be the insulin-like receptor gene *daf-2* and the associated insulin-like receptor pathway, which have been intensively studied due to their role in nematode aging (32–35). This pathway is known to respond to environmental stimuli, such as the Dauer pheromone, which induces formation of the long-lasting and highly resistant Dauer stage (33, 36). To date, it is not known whether this pathway also contributes to pathogen evasion. Nevertheless, the possibility is extremely tantalizing, as *daf-2* was recently shown to be involved in resistance against Gram-negative and especially Gram-positive bacteria (37). As discussed below, the insulin-like receptor pathway could represent a key component of worm defense and the associated trade-off with other life history traits.

Physical barriers

The most exposed body areas include the body surface and perhaps the extremities of the digestive tract (mouth, pharynx, and anus). These are protected by a multilayered cuticle, secreted by the underlying hypodermis and formed of several layers of collagens, which change in composition and structure during the worm's lifespan (38). The importance of the body surface as a protective barrier is supported by the finding that wildtype worms and mutants with altered surface antigenicity (*srf-2/-3/-5* mutants) differ in their susceptibility to *M. nematophilum* (22).

Worms also possess important internal physical barriers. The intestinal epithelium is protected by the presence of a chitinous peritrophic membrane, similar to those seen in insect guts (39). Another important structure is the grinder. This ridged tri-lobed structure made of cuticle and reinforced with chitin (Y. Zhang, personal communication) is located in the terminal bulb of the pharynx. The three lobes can be moved by simultaneous muscle contractions in a grinder-like fashion to break open bacteria, which pass back into the intestines (40). Therefore, it prevents intact bacteria from entering the gut. Genetic abrogation of its function, e.g. in the mutant *phm-2* (for pharynx morphology defective), render worms hypersensitive to *P. aeruginosa* and *S. enterica* (31, 41–43).

Similarly, as worms get older, the grinder efficiency decreases, which may in part explain why old worms are in general more sensitive to bacterial pathogens (28, 44).

Worm immunity

General structure of the innate system

Our current understanding of the *C. elegans* immune system is derived from three approaches: (i) identification and subsequent analysis of genes that are homologous to known defense genes from other organisms such as *Drosophila* or different vertebrate taxa; (ii) molecular genetic analyses of resistance toward certain pathogen models, published data is currently available for the gram-negative bacteria *P. aeruginosa*, *S. enterica*, and *B. pseudomallei*, and the gram-positive taxa *E. faecalis*, *S. aureus*, and *B. thuringiensis*; and (iii) transcriptome studies aimed at identifying infection-induced genes.

Within the innate system, one can distinguish constitutive and inducible components. The constitutive component serves as an early and continuous physiological barrier. The current data indicate that it includes various anti-microbial or digestive peptides and proteins that are constitutively expressed in the gut, pharynx, hypodermis, or secretory cells. In contrast, the inducible component is believed to represent a highly efficient but costly defense, such that it is only activated after detection of pathogens or their detrimental effects. It was in 2002 that this component was first described in *C. elegans*, namely as a response toward the pathogenic *S. marcescens* strain Db11 (45). The analysis of high-density cDNA macroarrays revealed at least 10 genes to be reproducibly upregulated at two different time points after infection, including those encoding lysozymes (*lys*) and lectins. The expression of some of these genes (e.g. *lys-8*) is known to be under the control of a transforming growth factor-β (TGF-β)-like pathway, suggesting that activation of this pathway is also part of the inducible response (45). Three additional regulatory pathways appear to be part of the physiological defense. Genetic screens and the analysis of available mutants demonstrated that resistance toward *P. aeruginosa* and *S. enterica* is mediated by a p38 mitogen-activated protein kinase (MAPK) pathway (43, 46), that resistance toward *S. enterica* involves programed cell death (PCD) (47), and that resistance toward a diversity of pathogens, including both Gram-negative (*P. aeruginosa*) and especially Gram-positive bacteria (*E. faecalis* and *S. aureus*), is dependent on the insulin-like receptor pathway (37). Intriguingly, these pathways seem to interact, and most of them also respond to general stress conditions, suggesting that *C. elegans* utilizes an integrated stress response as part of its primary physiological defense against pathogens.

In spite of these findings, it came as a surprise that several key elements of the invertebrate immune defense, most of which are also essential in mammals, appear to be absent from *C. elegans*. In particular, this nematode seems to lack the cellular arm of innate immunity in its classic form. Although coelomocytes are present (five in males and six in hermaphrodites), these could not be shown to be involved in phagocytosis or encapsulation of bacteria as seen in diverse invertebrate species, including the nematode *Ascaris suum*. These cells, however, show high endocytotic activity, and they may still contribute to immunity by supporting detoxification processes (48). Three other important elements of the insect immune system, the immune deficiency (Imd) pathway, nitric oxide (NO), and the phenoloxidase (PO) cascade (2, 49, 50), are unlikely to be of relevance, because essential components appear to be absent from the worm genome. This absence includes homologs for NF-κB, involved in the Imd pathway, the inducible NO synthase (iNOS) required for production of NO, and phenoloxidase, the key player of the PO cascade (8). It should, however, be borne in mind that widely diverged or even non-homologous proteins might fulfill these functions in *C. elegans*. In this context, it is relevant to note that the iNOS in plants was recently demonstrated to be a variant of the P protein of the glycine decarboxylase complex, structurally unrelated to animal iNOS (51).

The Toll pathway represents another important component of immune defenses in organisms as diverse as insects, mammals, and plants (2–4). To establish its role in *C. elegans*, a reverse genetic analysis of Toll pathway homologs was performed, starting with the generation of mutants for the genes *tol-1*, *trf-1*, *pik-1*, and *ikb-1*, which correspond to the *Drosophila* genes *Toll*, *dTraf*, *pelle*, and *cactus*, respectively. However, the analysis provided no indication for a function in the *C. elegans* immune defense toward *S. marcescens*, although worms mutant for *tol-1* avoided pathogens (see above) (27). TLRs all contain a highly conserved intracellular domain, the Toll-interleukin-1 receptor (TIR) domain (52). Interestingly, the *C. elegans* genome contains another TIR-containing protein in addition to TOL-1, called TIR-1, homologous to the vertebrate protein SARM (53), a member of the TIR receptor adapter family (54). Its potential function in nematode defense is currently under investigation.

C. elegans defenses have not as yet been shown to include a complement-like system, which plays a major role in the vertebrate immune system (55) and which was recently also suggested to contribute to insect immunity (56, 57). Intriguingly, the worm genome contains a thioester-containing protein (TEP), which is related to the central component of the

complement system, the factor C3, and which shows a high degree of similarity to the TEPs implied in insect immunity (57). Furthermore, the complement system can be activated through a pathway involving lectins (55), of which there are more than 100 in *C. elegans* (58). Their role in this context will be discussed below.

Overall, the physiological response can be divided into three parts: (i) recognition of infections, e.g. via pathogen-associated molecular patterns (PAMPs); (ii) processing of the information via activation of a regulatory pathway; and (iii) expression of immune effector molecules, which either eliminate the pathogen or alleviate its effect, e.g. by the release of detoxifying enzymes to counter microbial toxins. In the following more specific description of the different immune system components, we will climb the signaling pathways, by first presenting available information on immune effectors, followed by characterization of possible regulatory pathways, and, finally, the recognition molecules.

Anti-microbial immune effectors

Two main classes of immune effectors can be discerned: anti-microbial factors and proteins involved in detoxification. For the anti-microbial factors, five groups of enzymes or peptides have been suggested to contribute to immunity: (i) lysozymes, (ii) caenopores (amoebapore-like enzymes), (iii) lipases, (iv) a new class of glycine-rich putative anti-microbial peptides, and (v) CSαβ-type anti-microbial peptides (*Table* 2).

The most convincing evidence for involvement in an inducible immune response is currently available for the lysozymes, which are well known to contribute to immunity in vertebrates. The *C. elegans* genome encodes 10 different lysozymes (lys-1 to lys-10). These do not appear to represent homologs of the well-characterized lysozyme families in vertebrates or insects (59, 60). Instead, they are most similar to those present in protists such as *Entamoeba histolytica*, where they act in synergy with amoebapores to break up bacteria (61). The macroarrays employed for characterization of the inducible immune response toward *S. marcescens* contained six of the lysozyme genes. Three of these (*lys-1/-7/-8*) were clearly induced upon infection (45). Using *in situ* hybridization, constitutive expression of these genes was mainly observed in intestinal cells. Moreover, analysis of a LYS-1 :: green fluorescence protein (GFP) fusion demonstrated a vesicular localization of the gene products with a high concentration at the apical surface of the intestinal cells (45). This localization may indicate trafficking of the lys-1 protein toward the lumen, in a similar fashion to granular exocytosis in *E. histolytica* (61) or to that seen for secretory lysosomes of cytotoxic T lymphocytes (62). Interestingly, abrogation of *lys-1* function by RNA interference (RNAi) does not have a significant impact on the survival of worms infected with *S. marcescens*, suggesting that multiple redundant factors contribute to defense toward infection. On the other hand, transgenic worms that expressed *lys-1*: *gfp* only showed an increased resistance when infected with a protease-deficient strain of *S. marcescens*, Db1140; the protective effects of the recombinant lysozyme appear to be counteracted by proteases secreted by the virulent *S. marcescens* strain Db11 (45).

The *C. elegans* genome also contains six genes for amoebapore or saposin-like proteins (*spp-1* to *spp-6*), which bear several characteristics exclusive to the worm and are thus referred to as caenopores (T. Roeder, personal communication). Their most similar homologs are the saposins from *D. discoideum* and the amoebapores from *E. histolytica*. They thus belong to the amoebapore cytolytic superfamily, which in addition to saposins (responsible for the lysosomal degradation of lipids) and amoebapores (that possess a pore-forming activity especially toward gram-positive bacteria) also include cytolytic proteins from T cells, e.g. anti-bacterial peptides such as the natural killer-lysins, and acyloxyacyl hydrolases, which are capable of cleaving lipopolysaccharide (LPS) (63). In addition to their similarity to well-known anti-bacterial factors, their involvement in *C. elegans* immunity is suggested by the following points. In *E. histolytica*, they exert their anti-bacterial effect synergistically with lysozymes, which not only are known to participate in *C. elegans* immunity but also are likely to be derived from a protist ancestor (61). Recombinant SPP-1 protein was reported to possess anti-bacterial activity *in vitro* (64). Moreover, *spp-1* transcription is under control of DAF-16, which is part of the insulin-like receptor pathway and which is known to mediate pathogen resistance (see below) (37, 65). Detailed analysis of their expression patterns and anti-bacterial characteristics is in progress and should provide more specific information about their role in immunity (T. Roeder, personal communication).

A specific lipase gene was also induced in response to *S. marcescens* (gene *ZK6.7*) (45). This enzyme is constitutively expressed in the intestines, and it is structurally similar to vertebrate gastric lipases (45). In *Drosophila*, four lipase genes were similarly upregulated after immune challenge (66, 67), supporting the involvement of *ZK6.7* in worm immunity, most likely as a direct antagonist of invading pathogens. The same study (45) identified another class of upregulated genes, which includes two glycine- and tyrosine-rich putative anti-microbial peptides. Their exact role in immunity is currently under investigation.

Another group of putative immune effectors is the CSαβ-type anti-microbial peptides. Peptides of the same family were first

Table 2. Putative immune effectors in *Caenorhabditis elegans*

Genes	Induced*	*In vitro*†	*In vivo*‡	Localization§	Reference
Anti-microbial factors					
Lysozymes (*lys-1* to *lys-10*)	*lys-1/-7/-8*	?	LYS-1: *Serratia marcescens*	LYS-1/-7/-8: intestines	(45, 61, 165)
Caenopores (*spp-1* to *spp-6*)	?	SPP-1 (versus *Escherichia coli*)	?	?	(61, 64)
Lipases	ZK6.7	?	?	ZK6.7: intestines	(45)
Glycine/tyrosine-rich anti-microbial peptides	R09B5.3, *nlp-29*	?	?	?	(45)
CSαβ-type anti-microbial peptides (*abf-1* to *abf-6*)	?	ABF-2 (versus diverse microbes)	?	ABF-1/-2: pharynx	(70)
Detoxifying factors					
Catalases (*ctl-1* and *ctl-2*)	?	?	?	?	(65, 77)
Glutathione-*S*-transferase (e.g., *gst-4*)	?	?	?	GST-4: muscles and hypodermis	(82)
Metallothioneins (*mtl-1* and *mtl-2*)	?	?	?	MTL-1/-2: intestines	(65, 77, 166)
P-glycoproteins (*pgp-1* to *pgp-4*)	?	?	PGP-1/-3: *P. aeruginosa*	PGP-1/-3: intestines	(77, 79)
Phytochelatin synthase (*pcs-1*)	?	?	?	PCS-1: intestinal valves	(81)
Superoxide dismutase (e.g., *sod-3*)	?	?	?	?	(65, 77)

*Induced gene expression in response to infection with *S. marcescens*.
†*In vitro* examination of the anti-microbial or detoxifying activity of recombinant proteins.
‡*In vivo* analysis of immunity function of the genes based on mutant or transgenic worm strains and/or gene inactivation by RNAi.
§Main localization of proteins as assessed by *in situ* hybridization, immunostaining, or analysis of transgenic worms expressing a green fluorescence protein reporter construct.

isolated from the nematode *A. suum*, and they were shown to possess anti-bacterial activity against both Gram-positive and Gram-negative species (68). In *C. elegans*, the corresponding anti-bacterial factor genes are present in six copies (*abf-1* to *abf-6*). Although they share certain structural characteristics with the well-characterized insect defensins, they are most closely related to the molluscan mysticin (69, 70). The function of *abf-1* and *abf-2* has been studied in more detail. Analysis of the respective GFP fusion proteins under standard culture conditions (without pathogens) showed both of them to be most strongly expressed in the pharynx. Furthermore, the recombinant ABF-2 protein was found to be active against bacteria (both Gram-positive and Gram-negative) and also yeast (70). Their role in an inducible defense in response to pathogenic infection still remains to be investigated. Nevertheless, their constitutive expression may be part of the general defenses against infection in both the pharynx and the gut.

Detoxifying immune effectors

All above factors are supposed to target directly invading microorganisms. Some pathogens, however, such as *B. thuringiensis*, exert their main effect through the production of toxins (*Table 1*). A genetic screen permitted the isolation of a number of *C. elegans* mutants resistant to the *B. thuringiensis* toxin Cry5B. Among them, one (*bre-5*) corresponds to a β-1,3-galactosyltransferase expressed in the gut that is believed to be necessary for the post-translational modification of the cognate toxin target, as in contrast to wildtype worms, *bre-5* mutants do not take up Cry5B (71).

In other cases, detoxifying immune effectors or factors that counteract directly the detrimental effect of toxins are expected to be of importance. For example, *P. aeruginosa* PA01 produces cyanide that provokes a lethal paralysis of *C. elegans* presumably via inhibition of mitochondrial cytochrome oxidase. It has been shown that *egl-9* mutants are resistant to cyanide intoxication and thus to PA01 (72, 73). EGL-9 regulates the hypoxia inducible factor (HIF-1) by prolyl hydroxylation and thereby functions as an oxygen sensor (74). The downstream targets of HIF-1, however, are as yet unknown, and the exact basis of cyanide resistance remains to be established. As *egl-9* mutants are also more resistant to *B. pseudomallei* and *B. thailandensis* (30), this factor may contribute to a general protective mechanism; HIF-1 itself plays a central regulatory role in the stress response toward hypoxia, heavy metals, and heat (75, 76).

In the case of toxin-mediating killing by *P. aeruginosa* PA14 (fast killing provoked by phenazine toxins that are believed to act through the generation of reactive oxygen species), three genes involved in oxidative stress tolerance (*age-1*, *mev-1*, and *rad-8*) were shown to contribute to resistance (77). Whereas the exact functions of *mev-1* and *rad-8* in the stress response are as yet unclear, *age-1* is known to be part of the insulin-like receptor pathway, which regulates expression of numerous genes including detoxifying enzymes such as the catalases *ctl-1* and *ctl-2*, the superoxide dismutase *sod-3*, the metallothionein *mtl-1*, and the glutathione-*S*-transferases *gst-4* (see below) (65, 78).

The above study also provided the only direct evidence for a detoxifying immune effector. A double mutant for two P-glycoproteins (PGP-1/-3) was found to be significantly more susceptible to toxin-mediated killing (77). These proteins belong to a conserved family of adenosine triphosphate (ATP)-binding membrane transporters, and they are suggested to function as energy-dependent efflux pumps in *C. elegans*, extruding foreign compounds, thereby providing protection against exogenous toxins, including those from pathogens (77, 79).

It is clear that the majority of detoxifying immune effectors still need to be properly characterized (*Table 2*). Promising candidates are catalases, superoxide dismutases, metallothioneins, glutathione-S-transferases, and the phytochelatin synthase. These enzymes are known to contribute to detoxification (65, 78, 80–83), and most of them were shown to be controlled by one of the pathways implicated in defense (the insulin-like receptor pathway) (65, 78).

The TGF-β-like pathway

In *C. elegans*, there are at least three distinct TGF-β-like pathways. One of them that controls body size and the morphology of the male tail is referred to as either the small/male tail abnormal (Sma/Mab) or the decapentalegic/bone morphogenic protein-like-1 (DBL-1) pathway. Analysis of available mutants showed that five genes of this pathway (*dbl-1*, *sma-2/-3/-4/-6*) contribute to resistance against *P. aeruginosa* infection (84). In addition, some genes induced upon *S. marcescens* infection (see above), including *lys-8*, had previously been shown to be controlled by this pathway (85). Subsequent examination of *dbl-1* mutants showed that they exhibit a significant reduction in survival in the presence of *S. marcescens* (45). This pathway shows clear homologies to the mammalian TGF-β pathway (86), which plays an important role in immune responses (87, 88). Moreover, the *dbl-1* homolog in *Drosophila*, dpp, is upregulated upon immune challenge (66, 67). This finding suggests that one function of the TGF-β pathway in immunity has been conserved generally across evolution.

In *C. elegans*, signal processing begins with binding of the TGF-β homolog DBL-1 to the heterodimeric serine/threonine protein kinase receptor SMA-6/DAF-4, followed by phosphorylation of

the cytoplasmic signaling components SMA-3, SMA-2, and SMA-4 (*Fig. 1*). By analogy to the function of their homologs in mammals, the latter three proteins are thought to translocate to the nucleus, presumably together with a cofactor, where they regulate gene transcription (89). Currently known targets of this pathway include *mab-21*, involved in male ray pattern formation, and also *lon-1* and *lon-3*, which both regulate body size. The latter two encode a cysteine-rich secretory protein and a collagen, respectively. They are both mainly expressed in the hypodermis, where their expression is necessary and sufficient to determine proper body size formation (90, 91). Interestingly, *lon-1* bears similarities with the plant defense protein PR-1, and it is also expressed in the intestine (85, 90, 91). This finding led to speculation of a second antimicrobial role for LON-1 in the gut (8). However, recent experiments did not confirm this hypothesis (M. W. Tan, personal communication). In addition to *lys-8*, a number of other genes are also known to be controlled by the DBL-1 pathway, including three C-type lectin-like genes and several genes of unknown function (85). Most of these genes appear not to be involved in determining body size, but whether any of them play a role in innate immunity has yet to be determined.

A special role in this pathway may fall to the myocyte enhancer-binding factor-2 (*mef-2*). This transcription factor was originally identified in vertebrates as a regulator of gene expression in muscle tissue and now is known to play a wide role in controlling differentiation and apoptosis in neurons, T cells, and muscles. Its regulatory activity seems in part to depend on the interaction with Smad proteins, which are part of the mammalian TGF-β pathway and which are closely related to the *C. elegans* SMA-2 and SMA-3 proteins. Moreover, phosphorylation of this factor by the p38 MAPK seems to be a prerequisite for its interaction with Smad (92). This indicates a physical link between TGF-β and MAPK-signaling pathways, both of which are implicated in immune defense in *C. elegans* (see below). Tantalizingly, worms mutant for *mef-2* show increased susceptibility toward pathogens, and *sma-2* functions epistatically to *mef-2* (M. W. Tan, personal communication). To date, however, it is not known whether *mef-2* also interacts with *pmk-1*, the worm homolog for p38, and hence, this potential connection between TGF-β and p38 MAPK pathways currently awaits confirmation.

The upstream regulators of DBL-1 have not yet been identified. DBL-1 is expressed in the nervous system, primarily in the ventral nerve cord and in some pharyngeal neurons. This expression may indicate neuronal activation of this pathway in the course of developmental processes. At the same time, it may be induced by environmental stimuli, e.g. in response to perception of PAMPs. In this case, pathway induction might be dependent on signaling from a chemoreceptor, expressed in the chemosensory neurons in the head and pharynx (23).

The p38 MAPK pathway (PMK-1 pathway)

The p38 MAPK pathway plays an important role in cellular stress and immune responses in organisms as diverse as

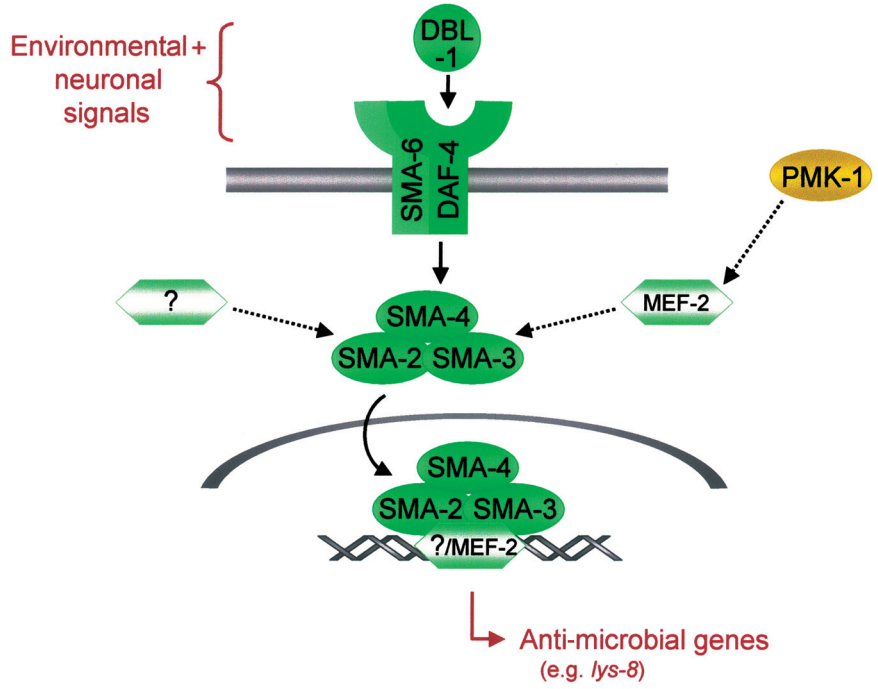

***Fig. 1.* The transforming growth factor-β-like pathway.** Elements and interactions that have been directly implicated in immunity are shown in solid color and continuous lines, respectively. Dotted arrows indicate uncertain regulatory relationships. A question mark denotes an unidentified factor.

mammals (93), insects (94), and plants (95), suggesting that it represents one of the most ancient, evolutionarily conserved components of the metazoan defense system (8). Its involvement in *C. elegans* immunity was inferred from an elegant genetic screen (43). The F2 progeny of mutagenized worms were infected by the *P. aeruginosa* strain PA14. At a time when wildtype worms were still alive, eggs were recovered from the corpses of dead animals. As the eggs are highly resistant to infection and as *C. elegans* can reproduce by self-fertilization, hypersusceptible strains were isolated rapidly. Subsequent high-resolution gene mapping with the two most susceptible strains was employed to identify two genes, *nsy-1* and *sek-1*. These genes are members of the p38 MAPK pathway and encode a MAPK kinase kinase (MAP3K) and a MAPK kinase (MAP2K), respectively, which are required for resistance to PA14. It was further shown by RNAi-mediated gene inactivation that susceptibility depends on *pmk-1*, one of the three worm homologs for the p38 MAPK (43). The importance of this pathway in immunity was further corroborated by the finding that similar RNAi inactivation of *pmk-1* also leads to decreased resistance toward *S. enterica* (46).

These results suggest that a defense signal is transduced from NSY-1 via SEK-1 to PMK-1 (*Fig.* 2). Interestingly, the same signaling pathway mediates the nematode's stress response to arsenic and acute dehydration, whereby the arsenic response involves activation of the transcription factor skinhead-1 (SKN-1) (K. Matsumoto, personal communication). This transcription factor is related to leucine zipper proteins that regulate the major oxidative stress response in vertebrates and yeast. In *C. elegans*, stress provokes its accumulation in intestinal nuclei, where it induces expression of γ-glutamyl-cysteine synthetase-1 (GCS-1), which is required for synthesis of the major antioxidant glutathione (96). Thus, SKN-1 may also represent an interesting candidate for a downstream target of the pathogen response, where it could be involved in detoxification of pathogenesis factors such as the phenazines.

In addition to *mef-2*, another possible downstream target of the p38 pathway is the cell death abnormality-9 gene (*ced-9*), which encodes a negative regulator of PCD. RNAi inhibition of *pmk-1* leads to a reduction in both resistance toward *S. enterica* and *Salmonella*-elicited PCD. However, if *pmk-1* inactivation is combined with a loss-of-function mutation in *ced-9*, then elevated levels of PCD are observed as in the *ced-9* mutant alone (46). Hence, there could be a PMK-1-mediated connection between the p38 MAPK and the PCD pathway (see below). This connection is specific for *Salmonella* pathogenesis, because the PCD pathway is not associated with resistance toward *P. aeruginosa* PA14 (47).

Upstream regulators of the p38 pathway have not yet been identified. Pathogen resistance is independent of the Ca^{2+}/calmodulin-dependent protein kinase II UNC-43 (43), which was previously shown to act upstream of NSY-1 to control asymmetric expression of an olfactory receptor gene (97) and which is involved in activation of the p38 MAPK pathway-mediated stress response to acute dehydration (K. Matsumoto, personal communication). PMK-1-mediated resistance toward

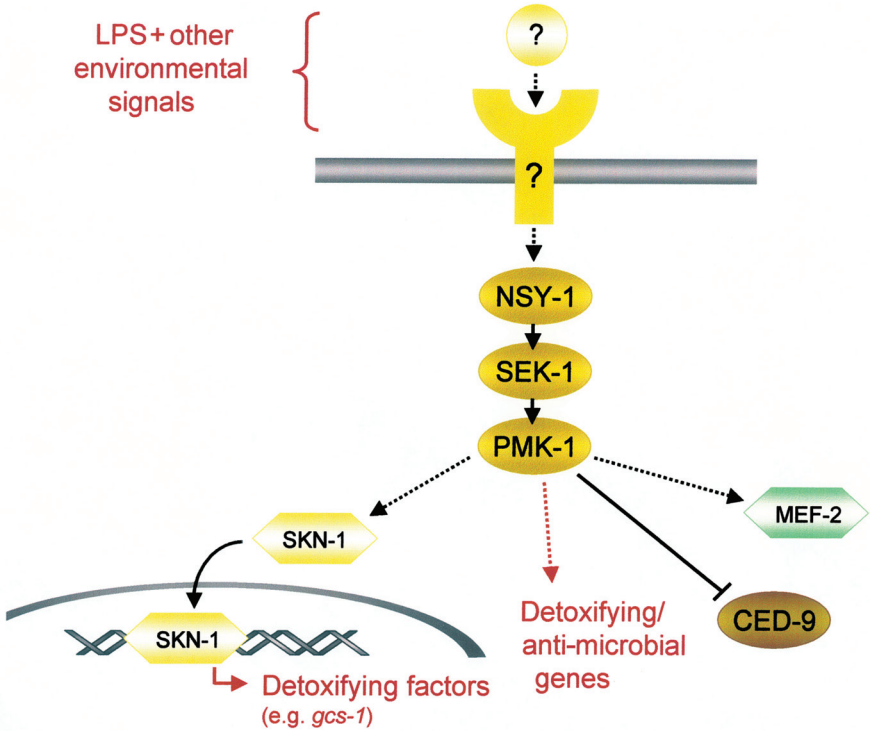

Fig. 2. The p38 mitogen-activated protein kinase pathway. For details, see comments to *Fig.* 1 and text. LPS, lipopolysaccharide.

S. enterica required intact *Salmonella* LPS, suggesting upstream involvement of a receptor for bacterial LPS. This receptor is not TOL-1, and it remains to be characterized (46).

The programed cell death pathway

In mammals, pathogen–host interactions often involve PCD of both immune and other somatic cells (98). In *C. elegans*, infection with *S. enterica* was shown to be associated with PCD in the gonads. Moreover, loss-of-function mutations in *ced*-3, *ced*-4, *egl*-1, and gain-of-function mutation in *ced*-9 lead to both inhibition of *Salmonella*-elicited PCD and reduced survival in the presence of the pathogen. These genes represent the major components of the PCD pathway in *C. elegans*, suggesting that it contributes to immunity (Fig. 3) (47). However, the exact protective mechanism remains elusive, because *S. enterica* produces an infection in the intestines (29, 42), whereas PCD is only observed in the gonads (47). PCD may thus aid in the diversion of resources from germ cell production to defense. Alternatively, the genes involved in PCD, most likely *ced*-3, may have pleiotropic effects, one of which mediates increased resistance toward the pathogen (47). Another alternative is that germ-line cell death itself elicits a signal, which activates a second regulatory pathway that generates increased resistance. This regulatory pathway could be the insulin-like receptor pathway, which is known to be inhibited in response to ablation of germ line-derived cells (99) and to mediate immunity when inactive or downregulated (37).

One upstream regulator of the PCD pathway was shown to be the p38 MAPK homolog PMK-1 (see above) (46). It is possible that others are also of relevance, e.g. some of those described for developmentally regulated PCD (100). Similarly, it still remains to be determined whether any of the previously described downstream targets of the PCD pathway also contribute to *S. enterica* resistance or whether there are other targets.

The insulin-like receptor pathway (DAF-2 pathway)

The insulin-like receptor pathway was first characterized for its role in the generation of the alternative larval dauer stage, which is formed under adverse environmental conditions, such as high worm density in combination with low food availability. Many of the genes involved were thus denoted dauer larva formation abnormal (*daf*). Subsequently, this pathway was shown to contribute to diverse traits, including longevity, thermotolerance, UV resistance, heavy metal resistance, adult motility, or brood size (32–35). Interestingly, it also seems to participate in the determination of lifespan and/or stress responses in a variety of other organisms, e.g. *Drosophila*, yeast, and mice, thus suggesting that it represents a highly conserved stress response and longevity regulation pathway (35, 101). A recent screen of available *C. elegans* mutants also revealed its importance in resistance against both Gram-negative (*P. aeruginosa*) and especially Gram-positive pathogens (*E. faecalis* and *S. aureus*) (37). In particular, worms with a deficiency in *daf*-2, the insulin-like receptor gene, show increased survival in the presence of pathogens. In contrast, mutants for *daf*-16, which encodes a forkhead transcription factor, negatively regulated by DAF-2, and *daf*-2/*daf*-16 double mutants are as susceptible to infection as wildtype worms (37).

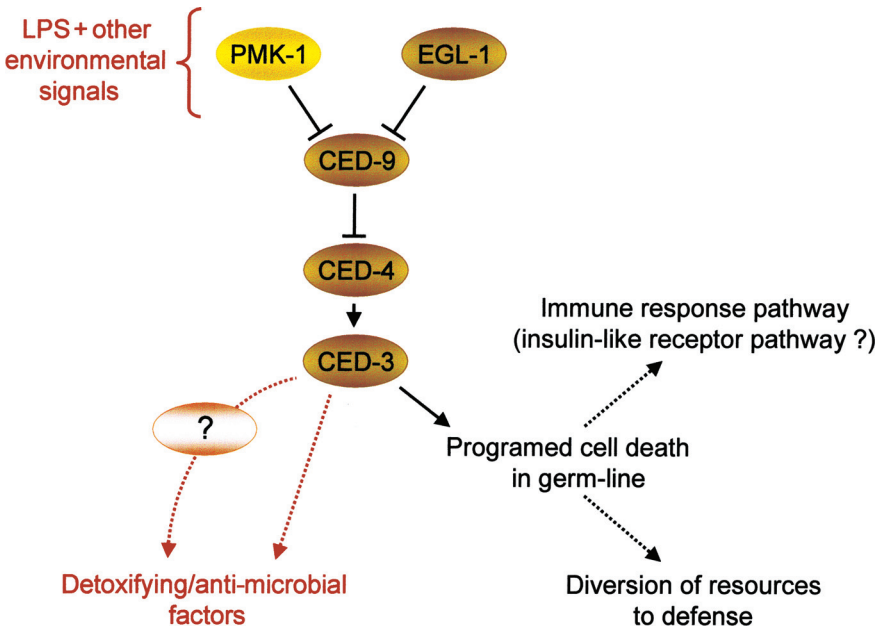

Fig. 3. The programed cell death pathway. For details, see comments to Fig. 1 and text. LPS, lipopolysaccharide.

The insulin-like receptor-signaling cascade is activated by binding of an insulin-like ligand to the receptor DAF-2, a transmembrane tyrosine kinase and the only worm homolog for the insulin receptor. Subsequent activation of phosphatidylinositol-3-OH kinase AGE-1 leads to conversion of phosphatidylinositol bisphosphate (PIP_2) into phosphatidylinositol trisphosphate (PIP_3). PIP_3 serves two known functions: it directly activates the complex of the Akt serine/threonine-directed kinases AKT-1/AKT-2, and it also binds to the kinase PDK-1, which additionally leads to activation of AKT-1. The AKT-1/AKT-2 complex phosphorylates the above-mentioned forkhead transcription factor DAF-16, thus preventing its translocation to the nucleus. Furthermore, the PTEN phosphatase DAF-18 is an additional regulator of the signaling cascade, which can dephosphorylate PIP_3, thus decreasing its capacity to activate the AKT-1/AKT-2 complex (*Fig.* 4) (32–35). This description is undoubtedly a simplification, and more factors are likely to be involved. For instance, detailed analysis of different *daf*-2, *age*-1, or *daf*-16 alleles suggests that there may be a second pathway from DAF-2 to DAF-16 (32, 33, 35). Moreover, the activity and specificity of this pathway also seems to depend on complex interactions with other regulatory elements, e.g. heat shock factor-1 (HSF-1) (102), or the nuclear hormone receptor DAF-12 and the cytochrome P450 DAF-9, which are also essential regulators of dauer formation (32–35).

If DAF-16 is active (i.e. it is not retained in the cytoplasm, as for example in *daf*-2 mutants), then it regulates expression of a large diversity of genes. These include several proteins with anti-microbial activity, e.g. LYS-7 and LYS-8, several saposins including SPP-1, and thaumatins, known from plants to contribute to immunity (65). Of these, *lys*-7 and *lys*-8 had previously been shown to be upregulated upon infection with *S. marcescens* (see above) (45). Furthermore, *lys*-8 seems to be under transcriptional control of both the TGF-β-like and the insulin-like receptor pathways (45, 65, 85). Other downstream targets of DAF-16 may also contribute to defense, e.g. several C-type lectins, or genes involved in detoxification (e.g. metallothioneines), resistance to oxidative stress (e.g. glutathione-*S*-transferase, catalase, and superoxide dismutase), or general stress responses (e.g. heat shock proteins) (65,78).

The insulin-like receptor pathway responds to environmental and/or neuronal signals, including exogenous Dauer pheromone

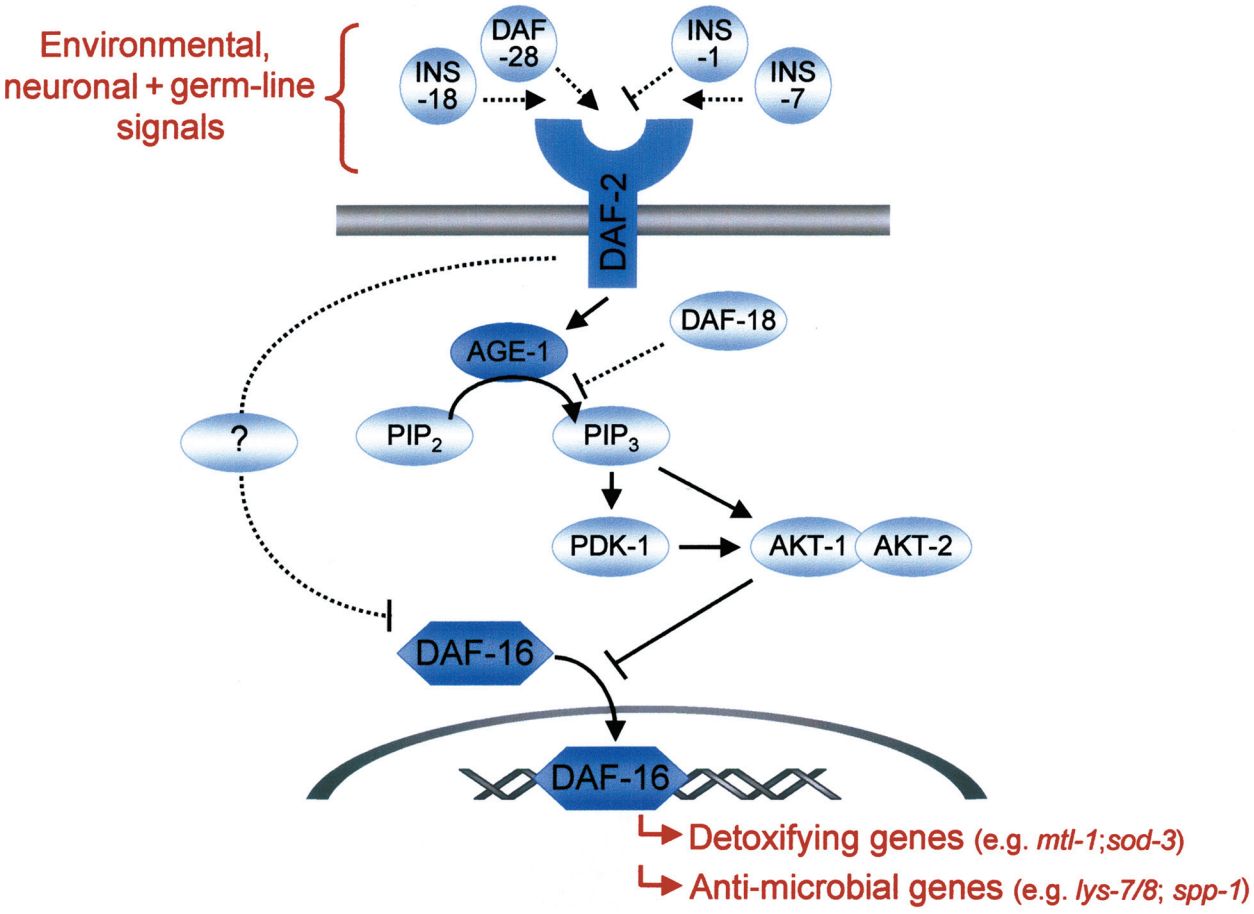

Fig. 4. The insulin-like receptor pathway. For details, see comments to *Fig.* 1 and text. PIP_2, phosphatidylinositol bisphosphate.

(33, 36, 101), and a germ-line-derived signal. In the latter case, ablation of the germ-line precursor cells was found to increase longevity through a process that depends on the activity of DAF-16 and also DAF-12 and DAF-9 (36, 99, 103). Upstream signaling seems to proceed through insulin-like hormones, which can serve as ligands for DAF-2 (34). The *C. elegans* genome contains 38 of these insulin-like peptides, and certain ones have been studied in more detail. They are mainly expressed in sensory neurons, including different amphid neurons (ASH, ASJ, and ASI). Of the peptides tested, INS-1, INS-7, INS-18, and DAF-28 were shown to interact with DAF-2, whereby INS-1 acts as an antagonist and INS-7, INS-18, and DAF-28 as agonists, leading to either slow aging and stress resistance or fast aging associated with high reproductive rate, respectively (34, 65). These insulin-like peptides are likely to mediate the 'long-distance' signal transfer from the sensory neurons to the intestinal and hypodermal cells, where the receptor DAF-2 is expressed (32–35). To date, it is unknown which if any of these upstream regulators contributes to immune defenses. Any candidate should act as an antagonist of DAF-2 in order to permit DAF-16-dependent activation of the antimicrobial and detoxifying genes. There may additionally be direct modulation of DAF-16 activity, involving a yet unknown factor.

Recognition of infection

A host can detect an infection either through direct recognition of a pathogen surface molecule or toxin or indirect perception of pathological or toxicological processes within the host tissue (cf. 'Danger model') (104). Whatever the exact mechanism, the relevant *C. elegans* receptors, which are unequivocally involved in the recognition of an infection, have not as yet been identified. Proteins with a C-type lectin-like domain (CTLD) were suggested to be of importance (45), because they are known to contribute to PAMP recognition in other organisms, including vertebrates and insects (105, 106). The *C. elegans* genome contains about 125 of the C-type lectins. Most of these show hydropathy characteristics, which are indicative of soluble secretory proteins. Only 10 appear to be membrane-associated. In addition, 19 proteins show high amino-acid conservation to the vertebrate carbohydrate recognition domain, seven of which are highly similar to the mannose-binding protein domain (58).

Interestingly, two such C-type lectin-like proteins were induced after infection with *S. marcescens* (45). They are likely to represent soluble secretory forms, and they were found to be expressed in the intestine (45). Hence, they may be involved in cell-autonomous recognition within the gut lumen or body cavity. While their constitutive expression is consistent with a sentinel role in PAMP recognition, to activate defense pathways, their induction upon infection may be indicative of the presence of a positive feedback loop. This could be advantageous via two non-exclusive mechanisms. (i) The activation of the defense pathways may be dosage-dependent, such that the abundance of PAMPs must be matched by the available PAMP-recognition molecules, in order to ensure efficient elimination of the pathogen threat. The presence of the proposed feedback-loop may then represent an economical implementation of such a dosage-dependent defense response. (ii) The C-type lectin-like proteins might not only be involved in recognition, but they may also contribute to pathogen elimination. This involvement may be achieved in a manner analogous to the complement-like system in vertebrates, such that binding of lectins to the surface of microorganisms may activate a protease complex, subsequently leading to cleavage of the putative C3 homolog, the *C. elegans* TEP, followed by activation of a membrane attack complex, which causes lysis of pathogen cells. Interestingly, *C. elegans* possesses all factors required for a complement-like system, including proteases, a TEP, and peptides, which could aid cell lysis. The possibility of their coordinated involvement in pathogen elimination clearly warrants examination.

Interestingly, C-type lectins and peptidoglycan receptors, which serve as the main PAMP recognition molecules in *Drosophila* (4), are also induced upon immune challenge in the fly (66, 67), suggesting the presence of a similar mechanism. Sequence analysis suggests that there might be an evolutionary relationship between these two classes of molecules (*Fig.* 5).

A second class of receptor that could be involved in defense is the large superfamily of chemoreceptors. It includes more than 1000 genes and pseudogenes of putative G-protein-coupled serpentine transmembrane receptors. They fall into four main families: the *odr-10*-like family (*ca.* 700 genes), the *sra* family (*ca.* 120 genes), the *sro* family (*ca.* 80 genes), and the *srg* family (*ca.* 40 genes). More than 500 genes are suggested to be functional, contributing to the perception of chemical stimuli by the chemosensory neurons (23, 107, 108). An involvement in PAMP recognition may be inferred from their capacity to translate environmental chemical cues into neurological signals and also from their extreme diversification, which may be the result of strong diversifying selection in response to a continuously changing range of pathogen varieties and/or coevolving pathogens (see below).

The *C. elegans* perspective

The evolutionary relationship between immunity and stress responses

The insulin-like receptor and the p38 MAPK pathways that have been shown to be important for worms' defenses against

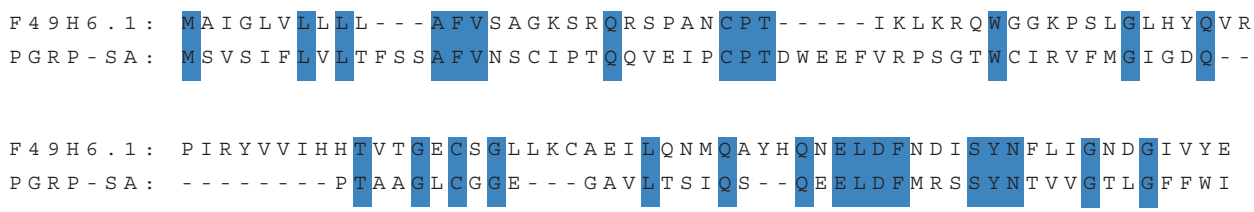

***Fig. 5.* Alignment of part of the peptidoglycan receptor PGRP-SA from *Drosophila melanogaster* and the *Caenorhabditis elegans* C-type lectin F49H6.1.** As this is the only case of obvious similarity between an insect PGRP and a nematode lectin, it may be fortuitous.

infection are also part of the nematode's stress response mechanism. There are a number of reasons why this might be the case.

(i) The pathogen strains that have been used so far to characterize the immune response do not coexist with *C. elegans* in nature or are only encountered by the worm on rare occasions. In this case, the worm is unlikely to have evolved a more sophisticated immune response toward these particular pathogens and only reacts to the incurred damage with a general stress response. This possibility could be tested by an analysis of the response of *C. elegans* to other soil microorganisms, in particular, those that are likely to be associated with *C. elegans* under natural conditions, e.g. additional strains of *P. aeruginosa* or *M. nematophilum* and *B. thuringiensis*.

(ii) Until now, all published research on the composition of the immune response has used the standard *C. elegans* strain N2. Since its isolation at least 40 years ago, this strain has been maintained under laboratory conditions, which clearly differ from its natural environment in the absence of pathogens and in the presence of an abundance of a slow-growing strain of *Escherichia coli* (the uracil auxotroph OP50). Thus, it has likely undergone extensive adaptation toward this specific environment. One could imagine that, in nature, worms possess a more developed immune system, but that this development would be energetically costly and the rate of reproduction would be suboptimal. In the laboratory, not only would there be no positive selection for the retention of such an immune system but also there would be strong negative selection, as worm strains that invest all available resources into development and reproduction (and thus reproduce more quickly) will clearly outcompete conspecific strains that allocate resources to an expensive but unnecessary defense system (109). Under these conditions, pathogen-specific immune responses may have been lost. The validity of this alternative may be tested by analysis of natural strains, which have not been maintained in the laboratory for many generations. Interestingly, such natural strains show significant variation in resistance toward both *B. thuringiensis* (21) and *S. marcescens* (Hinrich Schulenburg and Jonathan J. Ewbank, unpublished data).

(iii) The third possibility is simply that immunity in *C. elegans* does rely to a large extent on a general stress response. This possibility provides an interesting perspective on the dynamics of the evolution of innate immune systems. According to the current consensus on the metazoan phylogeny, nematodes and insects form a monophyletic group, the Ecdysozoa, whereas vertebrates are found on a different evolutionary branch (110, 111). As many parts of the more complex innate systems of insects and vertebrates show enormous similarities (e.g. components of cellular defenses or the Toll and imd pathways), these are likely to have a single origin. Therefore, they must have evolved before the separation of the Ecdysozoa and the vertebrate lineages. In this case, these parts were lost in nematodes or at least in the nematode lineage leading to *C. elegans*, because other nematodes such as *A. suum* seem to posess a more complex defense, possibly including a cellular component (i.e. phagocytically active coelomocytes) (112). The hypothesis of a lineage-specific loss of innate immune mechanisms in *C. elegans* is also supported by the fact that although its genome encodes a number of homologs of Toll pathway components, these genes appear not to contribute directly to immunity (see above) (27, 46).

This type of reduction could be explained by the fact that *C. elegans* represents a classic r-strategist with a high reproductive rate, a comparatively simple organization, and a short generation time, where investment of resources may be more efficiently directed toward egg production rather than a complex defense system (109). This conclusion is consistent with previous theories on the optimal investment in immunity, which suggest that life history requirements ultimately determine the structure and complexity of immune defenses, which in turn may be subject to rapid evolutionary changes (113–116). This conclusion then predicts that the stress response is indeed energetically less expensive

than alternative responses, possibly because it simultaneously provides protection against a whole range of harmful agents (different pathogens and toxins). It also predicts that a more complex immune response is retained in closely related taxa with different life history strategies (e.g. *K*-strategists with low reproductive rate and high investment in individual offspring).

Regardless of the underlying cause, the current findings clearly demonstrate that the general stress response plays an important role in *C. elegans* innate immunity. In this context, it is interesting to note that, in *C. elegans*, this response encompasses additional MAPK regulatory pathways. These are known to mediate resistance against oxidative stress and also various toxins. Therefore, it is conceivable that they also contribute to immunity especially against toxin-producing pathogens (8).

The evolutionary relationship between immunity and digestion

A number of digestive enzymes have been identified as being among the putative immune effectors. This finding suggests that a close relationship exists between immunity and digestion in *C. elegans*. Some digestive enzymes may be constitutively expressed along any of the possible contact zones (body surface, body openings, and digestive tract), where they serve as physiological barriers to pathogen invasion, either independent of (body surface, openings of sensory neurons, excretory pore, or the vulva) or in association with digestive processes (throughout the digestive system). For example, judged by a GFP reporter construct, in addition to its intestinal expression, the lysozyme gene *lys-1* is expressed in the six interleukin-1 (IL-1) and six IL-2 neurons as well as in certain neurons in the head ganglia (45). The use of digestive enzymes as a defense mechanism has the added advantage that it can result in the conversion of a potential threat into a source of nutrition. Interestingly, the employment of digestive enzymes as immune effectors seems to be finely regulated in response to an infection. In particular, the expression of *lys-8* that is upregulated by infection with the *S. marcescens* strain Db11 has been shown to be controlled both by the TGF-β-like and the insulin-like receptor pathways (*Figs 1 and 4*) (45, 65, 85).

In the context of an understanding of the evolutionary origin of the innate immune system, it is very significant that the digestive enzymes found to be associated with immunity in *C. elegans* are all more closely related to enzymes found in protists than in vertebrates (see above). As their original role was likely to be the degradation of nutrition (61), their function in defense should be derived. Moreover, by analogy to the above argument on the origin of the immune-signaling cascades in *C. elegans*, this observation indicates that the worm has retained an ancestral form of immune defense after separation of the nematode and insect lineages within the Ecdysozoa or after the separation of the lineage leading to *C. elegans* from the remaining nematodes. Furthermore, if both the stress response and employment of digestive enzymes represent the ancestral immune defense, then it is tempting to speculate that they are part of the same signaling cascades. Future identification of the targets of the stress response pathways should provide an answer to this hypothesis. In this context, it is notable that SKN-1, which contributes to defense against oxidative stress (see above), is also responsible for the initiation of the development of the digestive system (96).

Diversification of immune components due to multiple and/or coevolving pathogens

As highlighted above, immune defenses do not constitute a static system, but rather they continuously change in response to the evolution of other host life history characteristics and also the pathogen threats encountered. This latter factor is expected to be particularly important, if the diversity of pathogens continuously changes over time and space, and especially, if the host is engaged in a coevolutionary arms race with specific pathogens. In both cases, there is very strong selection on the host to adapt continuously to the new pathogen varieties (117, 118). As *C. elegans* seems to live primarily in decaying material, it is expected to encounter a continuously changing range of pathogens (12). Moreover, coevolving pathogens are thought to be widespread (117, 118), such that they are also likely to play a role in *C. elegans* biology. Although truly coevolving pathogens have not yet been reported, two currently used taxa, *M. nematophilum* and especially *B. thuringiensis*, are likely candidates because of their specific relationship with *C. elegans* (see above).

The occurrence of a continuously changing range of pathogens and/or coevolving antagonists has important consequences for the evolution of the different components of the immune system. In general, pathogens may escape eradication in two main ways. (i) They are not detected by the immune system. This scenario is the most probable one, if pathogens are recognized by non-essential PAMPs, which may then simply be altered. (ii) In contrast, if the pathogens are recognized by essential PAMPs, then they may escape elimination by counteracting the immune effectors. Consequently, there is strong selection on the host's pathogen recognition system to detect essential PAMPs. As PAMPs may not be essential in an absolute sense (there may not be a single surface molecule motif that cannot be altered, if selection is high, a given PAMP may only be essential in one pathogen, but not others), this selection is likely

to be an ongoing process, resulting in continuous diversifying selection on PAMP recognition molecules. Furthermore, one also expects diversifying selection to act on the host's immune effector molecules, although possibly to a lesser extent. Therefore, the PAMP recognition molecules should be most diverse, followed by the immune effectors, whereas the regulatory pathways are likely to be most conserved. The importance of such selective constraints on the different parts of the immune system is supported by a number of studies in vertebrates, insects and plants. Here, regulatory pathways are highly conserved, e.g. the p38 MAPK pathway (8) or the Toll pathway (2). Moreover, PAMP receptors and immune effectors are often found to be subject to strong diversifying selection (119–121).

The appreciation of such processes should help to explain the structure and diversity of the *C. elegans* immune system. In this context, it is worth noting that diversification in PAMP recognition and immune effectors may be attained by highly variable single loci, which then bear many alleles in the population, or by few alleles at many related loci, generated by gene duplication (122, 123). These two alternatives are also of importance for our general understanding of the evolutionary dynamics of parasite–host interactions, which are usually modelled with the help of either the matching-alleles or the gene-for-gene hypothesis (124, 125). Of these, the latter is more compatible with a system where defense is mediated by many related loci with few alleles, whereas the former is consistent with either of the two mechanisms. In *C. elegans*, the presence of diverse genes with similar function (e.g. PAMP recognition) within a single genome should be favored, because this nematode seems to reproduce primarily via selfing in nature (12), leading to genetically uniform populations with only few different alleles at single loci (M. Haber and Hinrich Schulenburg, unpublished data). A similar situation has previously been reported for the plant *A. thaliana*, which also shows comparatively low levels of intrapopulational diversity due to inbreeding (126) and which possesses large families of PAMP recognition genes spread across the genome (127, 128). In addition, strong selection pressure imposed by pathogens may also lead to a specific genomic distribution of duplicated immunogenes. Diversification of duplicated genes is increased, if they are relocated into different chromosomal regions, because this enhances escape from gene homogenization through intergenic exchange, which primarily acts across short distances along the same chromosome (129, 130). At the same time, selection should favor clustering of genes with complementary effects, i.e. they mediate recognition of diverse pathogens and subsequently induce the same regulatory defense pathway (122). Furthermore, such duplicated genes are expected to be subject to strong diversifying selection. Hence, non-synonymous substitutions should be significantly more frequent than synonymous changes in orthologous genes between individuals (allele diversity per locus within populations) and/or among the duplicated genes within single individuals (allele diversification across genomes). Such signatures should be particularly common in those parts of the genes that are directly involved in the interaction with pathogens (121, 131). These three patterns have been observed in the above example *A. thaliana*. Here, PAMP recognition genes are found in clusters in close proximity and then control recognition of diverse pathogens (128). They occur in dispersed and highly diversified clusters across the genome (127), and they also show signatures of strong diversifying selection (132).

Intriguingly, the *C. elegans* genome contains two large groups of genes, which meet at least some of the expectations. They include the CTLD-containing proteins and the large superfamily of chemoreceptors, which may both be involved in PAMP recognition. Importantly, both are likely to have diversified via duplication events and include more than 100 or even 1000 members, respectively. In addition, they are found in groups in close proximity and also in highly diverse clusters dispersed across the genome (58, 107, 108). This finding especially applies to the large superfamily of chemoreceptors (107, 108). In addition, some of the implicated immune effector genes also occur in clustered groups in different parts of the genome, e.g. the genes for the lysozyme family, the caenopores, or the ABFs. However, these gene families are comparatively small and not extremely diverse. Unfortunately, the presence of diversifying selection has not as yet been examined for any of these genes.

The trade-off between immunity and host life history traits

Immunity comes at a price. The maintenance and utilization of an immune system requires resources, which are usually limited. Furthermore, the immune response may rely on compounds that are toxic to pathogens but that may also be detrimental to the host's own cells. These costs of immunity imply a trade-off with other fitness-related traits, e.g. reproductive effort, competitive ability, or longevity. In turn, this trade-off selects for an optimized investment in host defense, where optimization may be achieved along two 'dimensions' (cf. the defense component model) (133). The first dimension concerns the use of the defense strategy where the two extremes are constitutive and induced expression of defense factors. Here, a constitutive defense permits a rapid response and is therefore advantageous toward parasites with a high

damage potential. In contrast, the inducible defense system only provides a delayed response but may be more economic, as it does not require permanent expression of defense factors. The second dimension refers to the degree of specificity of the defense. A general non-specific response may be advantageous in the face of a diverse, unpredictable set of parasites, even though such a defense may not be entirely efficient. In contrast, high specificity is directed against a restricted set of parasites and assures their complete eradication. It comes at the cost, however, of providing no protection against numerous other parasites. The evolutionary optimal defense strategy may then be manifested as a fixed (i.e. purely genetic) and/or a conditional (i.e. phenotypically plastic) response, such that its exact form in individuals of the species is either independent or dependent on the general environmental conditions, respectively (133, 134).

This trade-off is one of the key determinants of the evolutionary dynamics of pathogen–host interactions (124, 135). The underlying mechanisms and the evolutionary consequences have thus become a major topic in the analysis of infectious diseases. They are addressed using both theoretical approaches (113–115) and empiric studies (136–138). For instance, lines of *D. melanogaster* were selected for resistance against its hymenopteran parasitoid *Asobara tabida* over several generations. The resulting resistant lines possessed about twice as many hemocytes (phagocytosing cells of the cellular immune defense) as susceptible lines. At the same time, however, they showed significantly reduced ability to acquire food as larvae when in competition with conspecifics (137, 138). The molecular genetic basis for this trade-off is largely unknown. In insects, the juvenile hormone and its main antagonist, ecdysone, are possibly involved, because they not only regulate development but also decrease (juvenile hormone) or increase (ecdysone) activity of different parts of the immune function (139, 140).

The trade-off is to a large extent responsible for the structure and complexity of the immune system, highlighting again that immunity is not a static system but rather the product of multifaceted interactions with the parasite threat encountered, general environmental conditions, and also other life history traits. In *C. elegans*, early reproduction (i.e. fast larval development) and generation of large offspring numbers during early adulthood are expected to be selectively advantageous, because they ensure optimal usage of available nutrition and also displacement of possible competitors in a generally short-lived or unstable environment (decaying material in the soil). Under laboratory conditions, it was indeed possible to show that a mutant with delayed onset of egg production was less successful than a wildtype competitor in populating an *E. coli* lawn (109). In turn, an activated immune response should be costly until the early reproductive period, when the available resources are better directed toward development and egg production. During this phase, a low level of constitutively expressed immune factors is expected and defense should mainly rely on an inducible system. Here, protection is more important for the larva than the young adult, because once egg laying has started, it will only lead to a minor advantage in reproductive success. Moreover, the immune function is predicted to be affected by environmental conditions that correlate with the likelihood of pathogen encounters, e.g. high temperatures, which often enhance microbial proliferation. In contrast, the life history stages, which specifically allow the worm to persist under unfavorable environmental conditions, should be highly protected against pathogen attack. These include the Dauer stage and possibly eggs.

Some of the available data is consistent with the above expectations. For instance, resistance against pathogenic *S. marcescens* is highest in eggs and the early larval stages, followed by the late larval stages, young adults, and it rapidly decreases with age in adult worms (44) (unpublished data). Similar observations were made for slow killing of *P. aeruginosa* strain PA14, where young adults were more susceptible than L4 larvae. However, exactly the opposite pattern was recorded for fast killing of this *P. aeruginosa* strain (L4 larvae more susceptible than young adults) (28, 77). It is evident that more detailed information is required for a full understanding of the immune function of different life history stages in relation to pathogen attack and general environmental conditions.

Intriguingly, one of the currently implicated signaling pathways shows all hallmarks of a regulatory switch for the trade-off between immunity and other life history traits. Such a switch must mediate investment in development and reproduction on one hand and in immunity on the other hand. Furthermore, it is required to respond to developmental and environmental signals, in order to ascertain optimality of the trade-off in consideration of the developmental stage, availability of resources, and risk of pathogen attack. These requirements seem to be fulfillled by the insulin-like receptor pathway. When activated, resources are invested in metabolism (as required for fast development) and reproduction, whereas expression of the stress and pathogen resistance genes is suppressed. In contrast, inhibition or downregulation of the pathway leads to decreased metabolic and reproductive rates but increased resistance toward pathogens (32, 33, 35, 37). Most importantly, it is known to respond to environmental cues (inactivation by the dauer pheromone and heat and activation by presence of food) and perhaps

stage-dependent neuronal signals (32–35, 101), which should contain all the relevant information for the life history trade-off.

Consequently, the insulin-like receptor pathway may integrate diverse physiological and environmental inputs to optimize allocation of resources to reproduction, metabolism, and resistance (33, 141–143). As such, it may represent one of the key determinants of *C. elegans* life history. The diversity of functions would then also explain its high complexity. The exact processes involved in signal perception and integration remain exciting challenges for future research.

Future prospects

C. elegans has enormous advantages as an experimental system, including a comparatively simple organization and accessibility to comprehensive genetic analysis. Therefore, it will continue to provide a very valuable invertebrate model to dissect the genetics and molecular processes that underlie innate immunity. Its use may lead to the identification of regulatory pathways and molecules that are involved in immunity in other organisms including humans where functional studies are often hampered by extensive genetic redundancy.

In addition, this nematode is an ideal model to address certain aspects that are central to an understanding of the complexity of immune systems but which are usually neglected in molecular genetic studies. These relate to the diversification of different components of the immune system in response to pathogen threats and the genetic integration of immune functions within other fitness-related traits. Furthermore, detailed knowledge of the diversity of PAMP receptors, immune effectors, and the corresponding signaling pathways should help to determine how invertebrates achieve high levels of specificity in pathogen resistance and long-lasting inducible protection reminiscent of an immune memory. Both factors are well known from vertebrates where specificity and immune memory are mainly mediated by the adaptive system. As the adaptive system is absent in invertebrates, it is yet unclear how the recently reported highly similar phenotypic patterns are determined genetically in these organisms, e.g. high levels of genotype-specific resistance in *Daphnia* (144) and *C. elegans* (Hinrich Schulenburg and Jonathan J. Ewbank, unpublished results) or highly specific and long-lasting inducible immunity in *Daphnia* (145) and copepods (146).

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