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## Interkingdom signaling between pathogenic bacteria and *Caenorhabditis elegans*

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### Abstract

Investigators have recently turned to the soil nematode *Caenorhabditis elegans* as a small animal infection model to study infectious disease. To extrapolate findings concerning bacterial pathogenesis from non-mammals to mammals, virulence factors should be conserved in function, independent of the infection model. Emerging from these studies is the observation that bacterial virulence regulatory networks function in a conserved manner across multiple hosts, including nematodes, mice, and plants. Several regulatory networks have been implicated in nematode innate immune function, and are being exploited in the *C. elegans* infection model to develop novel chemical therapies against bacterial pathogens.

### *Caenorhabditis elegans* as a model host for infectious disease

Researchers require model infection systems in order to understand microbe-host interactions, study pathogenesis, and develop anti-microbial therapies and vaccines. For many investigators, the mouse *Mus musculus* is the workhorse of our trade. However, in recent years laboratories have looked to small, invertebrate models of infection such as the wax moth *Galleria mellonella*, *Drosophila melanogaster*, the soil nematode *Caenorhabditis elegans*, and plants such as alfalfa seeds or *Arabidopsis thaliana*, to name a few. Assessing virulence in multiple hosts is often useful for comparing the utility of different model organisms, identifying and understanding host response systems that are conserved through evolution, relating observed phenotypes to disease in humans and addressing the universality of bacterial virulence factors and mechanisms.

Over the past decade researchers have demonstrated that many bacteria pathogenic to humans, other animals and plants are also harmful to *C. elegans*. A natural bacteriophage, *C. elegans* is an attractive small animal infection model because of its transparent body amenable to microscopy, short generation time (~ three days), large number of progeny from each nematode, simple and well-studied anatomy and development, genetic tractability and fully sequenced genome. Pathogenic bacteria are seeded on agar, nematodes, usually as fourth-stage (L4) larva, are placed on bacterial lawns, and then nematode viability, intestinal colony forming units (CFUs) and persistence of the bacteria are monitored over time. Two general phenotypes are observed: a slowly killing (slow kill) phenotype involving intestinal infection that occurs over several days and a fast killing (fast kill) phenotype whereby nematodes die over a few hours, usually mediated by a diffusible toxin. Additional phenotypes, and extra-intestinal infections have also been observed. For example, the nematode pathogen *Microbacterium nematophilum*, upon adherence to the post-anal cuticle, causes gross swelling of the underlying

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hypodermal tissues [1]. The bacterium *Leucobacter chromiireducens* subsp. *solipictus* enters through the vulva causing uterine infection and is correlated with reduced lifespan [2].

Approximately 35 Gram-positive, Gram-negative and fungal pathogens have been described to harm *C. elegans*, and a comprehensive listing and review of these organisms has been published elsewhere [3]. This review addresses a theme that has emerged from the growing body of literature on modeling bacterial disease in *C. elegans*. The regulatory networks of bacterial pathogens are conserved across multiple infection models and can therefore be studied effectively in *C. elegans*. Some of these bacterial regulators, particularly quorum sensing molecules, interact with innate immune function and this information is being exploited to develop novel antibacterial chemotherapies.

## Regulatory networks

### Quorum sensing

Many bacterial genes are activated by threshold quantities of small-molecule signals produced by individual cells of the same or different species. For several infections modeled in the *C. elegans*, quorum sensing is essential for virulence (Table 1). In Gram-positive bacteria, quorum sensing, or cell density signaling is mediated by small peptides. These peptides are generally secreted via ATP-binding cassette (ABC) transporters and sensing of these signal molecules occurs via two-component regulatory circuitry. The Gram-positive bacterium *Staphylococcus aureus* exhibits a slow kill phenotype over several days, and killing is attenuated in a strain deleted for the *agr* quorum sensing system [4]. *AgrB* and *AgrD* produce an octapeptide pheromone necessary for quorum sensing signaling, and *agr* mutants are attenuated in multiple animal infection models, including the mouse and a rabbit endocarditis model. *AgrA* and *AgrC* constitute a two-component response regulator that responds to the octapeptide, stimulating expression of the RNAPIII effector molecule (Figure 1). RNAPIII alters expression of a large number of virulence factors, including exoproteins, cell wall components, and regulatory loci in *S. aureus*. Similarly, the *fsr* quorum sensing system of *Enterococcus faecalis* is necessary for full virulence in the nematode [5,6]. The *fsr* system shares homology with *agr* of *S. aureus*. The *E. faecalis* proteins *FsrA* and *FsrC* make up the two-component response regulator that senses the extracellular accumulation of a peptide lactone encoded at the C terminus of the *FsrB* protein. An *fsrB* mutant of *E. faecalis* is also attenuated for virulence in a mouse model of infection [6]. These data suggest that for Gram-positive pathogens, quorum-sensing control of virulence occurs in multiple hosts, including *C. elegans*.

For Gram-negative bacteria, multiple quorum sensing systems have been described. Acylhomoserine lactones (AHL) are the best described of these and they control a wide range of phenotypes. AHLs were first elucidated in *Vibrio fischeri*, where the molecules, synthesized by *LuxI*, freely diffuse through the bacterial membrane. When the AHL concentration reaches a threshold level reflecting a high cell density they bind, in this case, to the *LuxR* regulator stimulating bioluminescence. Homologs of the *LuxI* AHL autoinducer synthase and the *LuxR* regulator have been identified in many Gram-negative bacteria. Interestingly, orphaned *LuxR*-type regulators, in the absence of a cognate *LuxI*-type synthase have also been described, and are hypothesized, in some instances, to be involved in sensing interspecies population densities [7,8].

Quorum sensing signaling is required for *Pseudomonas aeruginosa* virulence in the nematode model. This Gram-negative pathogen can exhibit either a fast kill or slow kill phenotype in the *C. elegans* infection model depending on the type of medium on which the nematodes are propagated. Both the *LasR* and *RhlR* quorum sensing regulators, which bind to the N-3-oxo-dodecanoyl-L-homoserine lactone (3O-C<sub>12</sub>-AHL) and N-butyryl-L-homoserine lactone (N-butyryl-AHL), respectively, are necessary for fast or paralytic killing, occurring over several

hours [9,10]. In a slow kill assay where death occurs at approximately three days, mutation in both the LasR and RhIR quorum sensing systems of *P. aeruginosa* were also necessary to attenuate killing [11]. Interestingly, Zaborin *et al.* describe a ‘red death’ phenotype caused by *P. aeruginosa* where the gut tube turns red during phosphate depletion, and the signaling systems involved were PhoB (Figure 2), a MvfR-PQS quorum sensing pathway and the siderophore pyoverdine [12].

Introduction of the associate iron complex in the intestine of *C. elegans* or a mouse model caused death in both organisms by epithelial cell disruption and apoptosis.

Similar to *P. aeruginosa*, *Burkholderia cepacia* complex (BCC) are a group of ubiquitous Gram-negative bacteria that cause opportunistic infections in the lungs of cystic fibrosis patients. *B. cepacia* kills nematodes over a two to three day period, and the *cep* quorum sensing system is necessary for killing [13,14]. With the goal of identifying virulence factors necessary for disease in four host organisms, Uehlinger *et al.* found that quorum sensing signaling was necessary for full virulence in all of those tested, including *C. elegans*, the wax moth, alfalfa and mice [14]. Consistent with observations concerning Gram-positive pathogens, quorum sensing is necessary for *P. aeruginosa* and BCC-mediated death observed in *C. elegans* and is conserved in this function across multiple infection systems.

### Alternative sigma factors

Alternative sigma factors confer upon the RNA polymerase holoenzyme the ability to bind to different promoters. This allows for the spatiotemporal control of specific regulons, or collections of genes controlled by a common regulatory protein. Alternative sigma factors often control large numbers of genes, and as such, have been implicated in the regulation of pathogenesis. A few alternative sigma factors are necessary for full virulence in the *C. elegans* model (Table 1). In *Salmonella enterica* serovar Typhimurium, the alternative sigma factor  $\sigma^S$ , encoded by *rpoS*, regulates gene expression in response to environmental stresses such as starvation, oxidation and low pH, signals relevant to pathogenesis, and  $\sigma^S$  also regulates the expression of *Salmonella* plasmid-encoded virulence genes [15]. *rpoS* mutants are less virulent in both *C. elegans* and in mouse models of disease [15,16]. In *P. aeruginosa*, the alternative sigma factor  $\sigma^N$ , encoded by *rpoN*, is necessary for full virulence in *C. elegans*, mice, and *Arabidopsis thaliana* [17].

In *S. aureus*, the alternative sigma factor  $\sigma^B$ , encoded by *sigB*, positively regulates a variety of virulence related phenotypes, such as biofilm formation and hydrogen peroxide resistance, in response to environmental stress (Figure 1). Mutations in *sigB* lead to moderate attenuation of virulence in the *C. elegans* model [4] and have varying effects in mammalian hosts. For example, in mice, *sigB* mutants were less virulent in a catheter infection model, but not in an intravenous model [18]. Interestingly, although most clinical isolates of *S. aureus* are *sigB* positive, some are *sigB* deficient, indicating that although  $\sigma^B$  appears to contribute to virulence, it is not required for infection [19]. In contrast, the  $\sigma^S$  and  $\sigma^N$  alternate sigma factors of *Salmonella* and *P. aeruginosa*, respectively, are required for virulence in nematodes and mice. These regulators enable the bacterium to respond to environmental stresses such as oxidative damage, low pH and nutrient deprivation that are encountered in otherwise unrelated host organisms. The specific nature of at least some of these stresses perhaps could be best elucidated in the nematode.

### Two-component systems

Two-component systems, consisting of a sensor kinase and a response regulator, are one of the main systems bacteria use to sense and respond to their environment. The membrane-associated sensor kinase undergoes autophosphorylation in response to a chemical or physical

extracellular signal, and then transfers its phosphate group to the response regulator. Phosphorylation of the response regulator modifies the function of the protein and affects change within in the cell, often by altering gene expression or, for example, flagellar motility.

Two-component regulatory systems are necessary for virulence in many pathogenic bacteria. In *S. aureus*, two two-component regulatory systems, *saeRS* and *srrAB*, are necessary for full virulence in *C. elegans* [20]. SaeRS interacts with the *agr* quorum sensing system to integrate environmental information in the cell and regulates the transcription of many virulence factors [21]. *saeRS* mutants are less virulent in mice than wild-type *S. aureus*, indicating that this regulatory system is conserved across hosts. However, the SrrAB two-component system regulates virulence in *S. aureus* by downregulating several virulence factors in response to low oxygen conditions [22], and the overexpression of *srrAB* results in reduced virulence in a rabbit endocarditis model [23]. Thus two-component regulatory systems, sensing environmental and quorum sensing signals, play a pivotal role in the ability of *S. aureus* to cause disease in *C. elegans* and mammalian models of infection (Table 1).

In the PhoPQ system of *Salmonella*, PhoQ undergoes autophosphorylation in response to low  $Mg^{2+}$  concentration, a signal indicative of being within a host environment. PhoQ then phosphorylates PhoP, which in its activated form induces expression of multiple virulence factors [24]. The PhoPQ regulatory system has long been known to be involved in *Salmonella* virulence, and *phoP* mutants also exhibit reduced virulence in mammalian models [25]. Both a *phoP-phoQ* double knockout mutant and a mutant with a point mutation in *phoQ* exhibit reduced killing of *C. elegans* in a slow kill assay [26], indicating that it is also conserved across hosts. In addition to PhoPQ, the response regulator OmpR, which controls expression of the type III secretion system encoded within pathogenicity island 2 (SPI-2) [27] necessary for survival inside host cells and systemic infection in mice [28], is also necessary for full virulence of *Salmonella* in the *C. elegans* model [16].

Multiple two-component systems are required for virulence in *P. aeruginosa* as well (Figure 2). One such system, GacAS, regulates the expression of multiple virulence factors, including the quorum-sensing molecule N-butyryl-AHL [29]. GacAS is also required for full virulence in the mouse and *Arabidopsis thaliana* models of infection [30], again highlighting the extent to which two-component systems are conserved in the virulence of multiple hosts. In addition to GacAS, two other genes encoding putative two-component regulatory systems, PA3946 and PA4380, are necessary for full virulence in *C. elegans* [31,32]. Similar to quorum sensing networks and alternative sigma factors, two component systems that have a role in mammalian infections are repeatedly identified in *C. elegans* screens of very different pathogens. These regulatory networks can be effectively investigated using the *C. elegans* infection system, which offers an unexploited advantage. The specific *in vivo* triggers for many virulence-associated two-component systems and alternate sigma factors are not known. The simplicity of the *C. elegans* intestine and the availability of complete mutant sets, interaction maps and RNA interference (RNAi) tools could make it possible to elucidate the ligands for many sensor kinases in this model.

## Innate immunity

The nematode infection model has been instrumental in deciphering host innate immunity (for reviews see Ref. [33,34]), and several examples now exist whereby gene-gene interactions between host innate immune components and bacterial regulatory circuitry have been observed. In *Salmonella*, the PhoPQ regulon and SPI-1 and SPI-2 pathogenicity islands encoding type III secretion systems are induced during infection, and are required for persistence in the nematode gut [35]. Defects in persistence due to mutation in SPI-2 and *phoP* could be rescued by RNAi-directed downregulation of the nematode gene *spp-1*. The

gene *spp-1* encodes an anti-microbial peptide that is a member of the saposin-like protein family, part of nematode innate immunity.

In another well-studied example, nematode resistance to *P. aeruginosa* killing requires the insulin-like receptor DAF-2 and transcription factor DAF-16 (Figure 2), while downregulation of DAF-16 and associated genes causes nematodes to be sensitive to *P. aeruginosa*, a phenotype requiring the two-component response regulator GacA and the LasR and RhIR quorum sensing regulators [36]. The nematode DAF-16 transcriptional factor controls multiple phenotypes including resistance to pathogens, part of innate immunity, but also aging and dauer formation [37]. These two examples in *Salmonella* and *P. aeruginosa* represent the beginnings of our understanding of interkingdom signaling between bacteria and the nematode model host.

Interestingly, although bacterial regulatory networks identified in the nematode model are important in multiple host systems and *C. elegans* has been used to identify host innate immune system components that are involved in resistance to multiple pathogens, available information suggests that the specific bacterial effectors and their nematode receptors may not be so conserved. Additionally, *C. elegans* has been used to characterize bacterial surface proteins and toxins, and it also appears well suited to identify and evaluate conserved mechanisms of global significance in both bacteria and hosts.

## Exploiting regulatory networks for therapeutic drug discovery

Some researchers might question the use of a small animal drug screening system since the molecular mechanisms of nematode killing are largely unknown, and, in most instances, disease symptoms are clearly dissimilar to disease observed in humans. For example, *E. faecalis* merely causes distention of the nematode gut lumen [5] and a specific mode of killing has not been described. Normally a harmless member of the intestinal commensal flora, *E. faecalis* causes nosocomial bacteremia, and surgical wound and urinary tract infections in humans. *Yersinia* spp. kill *C. elegans* by forming biofilms over the head of the nematode [38], ultimately starving them to death. This mechanism is allied to ‘flea-blocking’, the formation of biofilms in the gut of the flea, which blocks the flea digestive tract and stimulates them to bite new hosts [39], while in humans, the symptoms of bubonic plague are swollen lymph glands, fever, chills, headache, and exhaustion. *P. aeruginosa* is an opportunistic pathogen that infects immunocompromised individuals, e.g. affecting the lungs of cystic fibrosis patients, causing urinary tract infections and sepsis. In humans with cystic fibrosis, *P. aeruginosa* forms biofilms, producing large quantities of alginate, stimulating an immune response that ultimately leads to tissue destruction. One known mechanism of nematode death of *C. elegans* by *P. aeruginosa* is the quorum sensing dependent production of hydrogen cyanide, resulting in fast killing [31]. Hydrogen cyanide production by *P. aeruginosa* is an uncommon exception: the specific molecular mechanism of nematode death caused by bacterial pathogens, for both the fast and slow kill phenotypes, remains largely obscure and unrelated to infectious disease observed in humans.

In spite of the differences in pathogenesis mechanisms between nematodes and mammals, there are significant advantages in using the *C. elegans* infection model to develop chemotherapeutic agents against certain bacteria. Not the least is the limitations of larger mammalian models: high throughput chemical library screens cannot be performed in mice. Targeting quorum sensing signaling and other global regulatory pathways seems particularly prudent because of the extensive list of virulence-associated phenotypes regulated via this mechanism. Biofilms are thought to be an integral part of most, if not all, bacterial diseases, and require cell-to-cell signaling, or quorum sensing for maturation. In addition to biofilms, a wide range of virulence factors and phenotypes involved in disease of humans are controlled by quorum sensing, including capsule expression, siderophores, pilus assembly, and toxin production to list a few



[40]. Because regulation of virulence determinants occurs in a manner conserved across hosts, independent of the specific mechanisms that ultimately cause damage, targeting of signaling pathways can be effectively pursued in the *C. elegans* model. The nematode is amenable to high-throughput screening, it provides a built-in check for drug toxicity to a whole eukaryotic organism, and regulatory networks controlling bacterial virulence are conserved across invertebrate and vertebrate hosts. Thus the *C. elegans* infection model is a powerful tool for drug discovery.

An emerging utility of the *C. elegans* infection model is the ability to identify and study anti-infective agents targeted at specific bacterial regulatory networks [41-43]. The current restricted pipeline for antimicrobial drugs means that new drugs are sorely needed and new screening technologies could help to identify alternate strategies for dealing with infecting pathogens. Anti-infective molecules target virulence factors or associated regulatory networks necessary for causing host damage and disease. They do not exert broad-based selective pressure like bacteriostatic and bacteriocidal drugs and therefore are less likely to select for resistance in non-target organisms. Since methods for identifying such compounds are only being developed today, there is a good chance that existing chemical libraries will contain compounds with anti-infective activity. Robotics and high throughput technologies have allowed researchers to screen libraries of compounds and plant extracts for anti-infectives in the nematode model. In one such study, 37,000 compounds and extracts were screened, giving 28 hits, including 6 new structural classes of drugs that enhance survival of *C. elegans* when infected by *E. faecalis* [42]. In addition to anti-virulence compounds, performing screens of this nature in a simple organism such as *C. elegans* makes it possible to identify hits with *in vivo* activity, which could be missed in an *in vitro* screen.

*C. elegans* can also be used to test the *in vivo* effects of specific classes of anti-infectives as with the evaluation of quorum sensing quenchers against *P. aeruginosa* [44] and *Burkholderia* [43]. Here acylases, and related enzymes, that degrade AHL molecules extend the lifespan of nematodes in the presence of these Gram-negative pathogens. The PvdQ protein degrades the 3O-C12-AHL of *P. aeruginosa* while a lactonase, AiiA, from *Bacillus thuringiensis* was used to inhibit AHL-mediated killing of *C. elegans* by *Burkholderia cepacia*. An antagonist of a LuxN-type receptor protected *C. elegans* from quorum sensing dependent killing by *Chromobacterium violaceum* [45], furthering the notion that therapeutic drug development can be achieved in this small, invertebrate infection model.

## Concluding remarks

In developing infection models to study bacterial diseases, researchers typically must choose animals that faithfully mimic related illnesses in humans. On the bacterial side, virulence factors necessary for causing clinical signs and symptoms ideally should be conserved of function within the laboratory animal host. As an example, the type III secretion systems of Gram-negative pathogens are important virulence factors in human disease and these systems inject proteins into host cells to subvert their function. For *Salmonella*, both type III secretion systems, encoded within SPI-1 and SPI-2, are required for disease in humans and for persistent infection in *C. elegans* [35]. However, unlike those of *Salmonella*, the type III secretion systems of *P. aeruginosa* and enteropathogenic *E. coli*, both thought to be necessary for virulence in humans, appear to be host specific as they are not necessary for infection of *C. elegans* [46, 47]. Thus type III secretion system function cannot be universally modeled in the nematode infection system.

One might hypothesize that type III secretion systems, adhesins and other factors that interact directly with host cells are host-specific virulence factors produced in response to direct *in vivo* selective pressures. However, after a little over a decade of using *C. elegans* to study

bacterial disease it has become clear that regulatory networks are highly conserved through evolution, across hosts even when downstream effectors may differ. Studying the genetic basis for quorum sensing signaling, alternative sigma factors, and two-component systems in the nematode model has been enlightening because the regulatory network<sub>[GT1]</sub> probably derives from the conserved nature of environmental stresses encountered by bacterial pathogens within a host and the requirement for regulatory networks to respond to these signals. Yet many questions concerning bacterial regulators within the nematode host remain (Box 1). Furthermore, targeting regulatory networks offers hope of developing anti-infective therapies to fight infection in the current era of increasing antibiotic resistance. Finally, the exciting discovery of bacterial regulatory networks interacting with *C. elegans* innate immunity strikes at the essence of infectious disease, communication between microbe and host.

### Box 1

#### Questions for future research

- What additional bacterial regulatory networks interact with *C. elegans* innate immune components?
- Can *in vivo* biofilms be modeled effectively in the nematode? If so, what additional regulators control their formation?
- What are the ligands for the sensor kinases of the bacterial two-component regulators within the nematode intestine?
- Do quorum sensing regulators control pathogenesis during mixed infections with multiple pathogens, or pathogens and commensal organisms?
- Are there additional regulatory networks that are amenable for targeting in developing anti-infective therapies?

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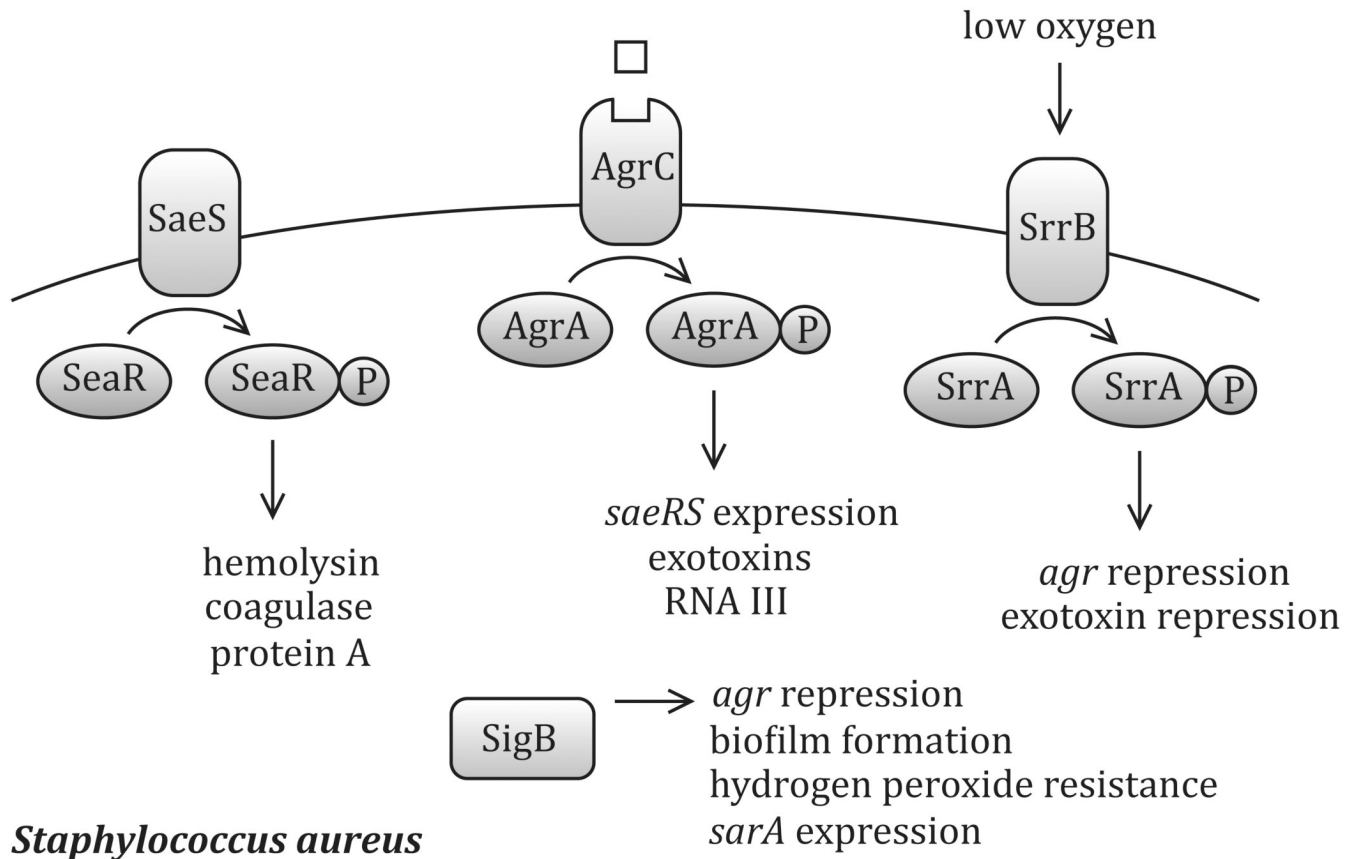
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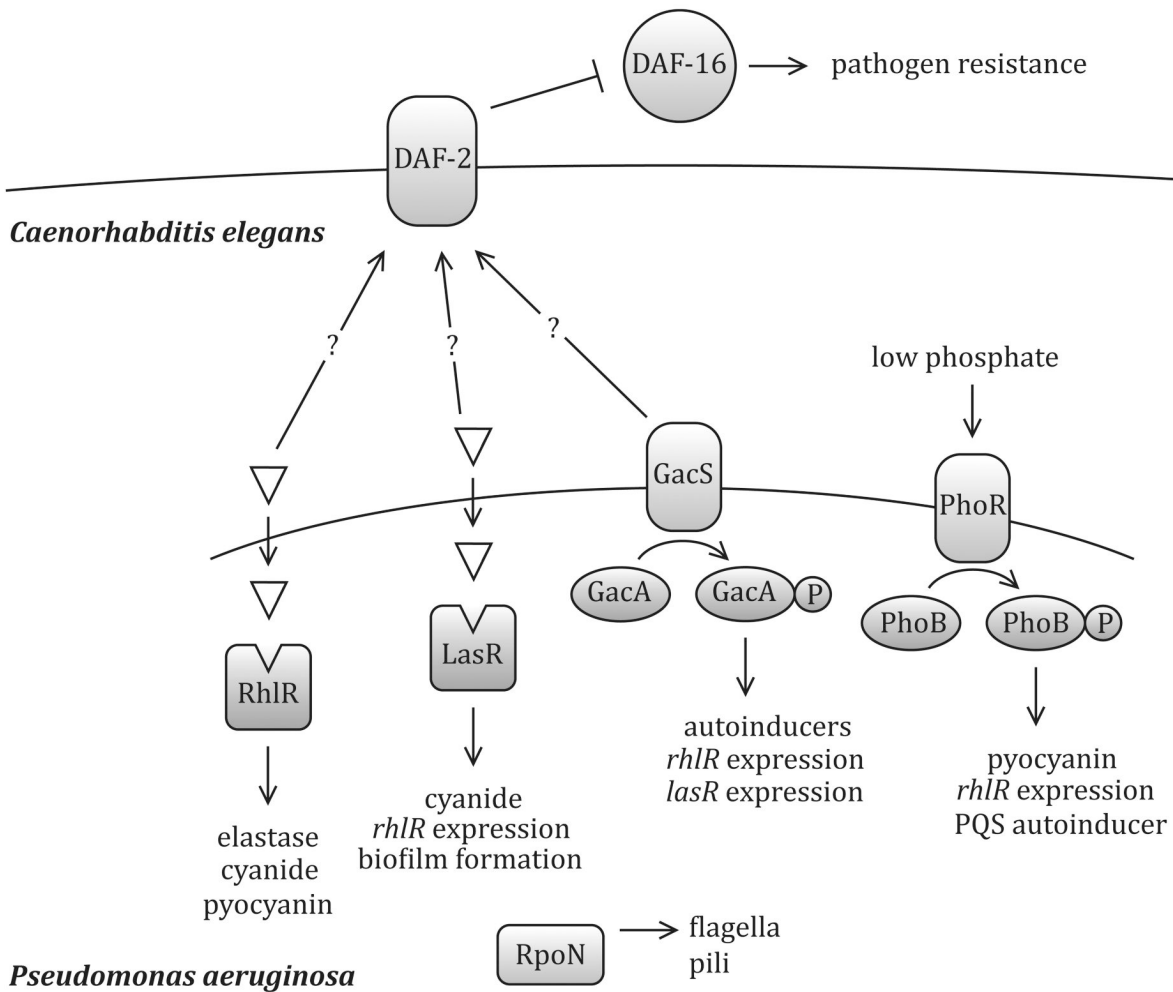
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**Figure 1.**

Regulatory networks required for infection of *C. elegans* by *Staphylococcus aureus*. The two-component system SaeRS regulates hemolysin, coagulase and protein A production [49], while SrrAB represses *agr* and exotoxin expression in response to low oxygen levels [22]. The AgrAC quorum sensing system controls *saeRS* and exotoxin expression [50,51] in response to an octapeptide pheromone (light green square). The alternative sigma factor SigB is involved in regulation of *agr* [52], biofilm formation [53], hydrogen peroxide resistance [54] and *sarA* [55].

**Figure 2.**

Regulatory networks required for infection of *C. elegans* by *Pseudomonas aeruginosa*. GacAS regulates *rhlR*, *lasR*, and AHL autoinducers [29]. PhoRB also regulates the quorum sensing components *rhlR* and the PQS autoinducer, as well pyocyanin production [56]. The quorum sensing regulator LasR binds to the autoinducer produced by LasI, and regulates biofilm formation [57]. LasR is also necessary for cyanide production [58] as well as expression of another quorum sensing system *rhlR* [59]. RhlR regulates production of cyanide [58], elastase and pyocyanin [60]. The alternative sigma factor RpoN is involved in regulation of flagella and pili [61]. Light green triangles represent AHLs used in quorum sensing. *P. aeruginosa* overcomes *C. elegans* host immune defenses by downregulating (blunt arrow) the DAF-16 transcription factor [36]. This phenotype requires the nematode insulin-like receptor DAF-2, and the *P. aeruginosa* GacA protein and quorum-sensing regulators LasR and RhlR.

**Table 1**Bacterial regulatory genes necessary for virulence in *C. elegans*

Type of Regulator	Gene(s)	Organism	Non-Nematode Models <sup>a</sup>	Refs
<b>Quorum Sensing</b>	<i>agr</i>	<i>S. aureus</i>	Mouse, Rabbit	[4]
	<i>fsr</i>	<i>E. faecalis</i>	Mouse	[5,6]
	<i>lasR</i>	<i>P. aeruginosa</i>	Mouse, <i>Arabidopsis</i>	[9,10]
	<i>rhIR</i>	<i>P. aeruginosa</i>		[9,11]
	<i>phoB</i>	<i>P. aeruginosa</i>		[12]
	<i>cep</i>	<i>Burkholderia</i> sp.	Mouse, Wax Moth, Alfalfa	[13,14]
<b>Alternate Sigma Factors</b>	<i>rpoS</i>	<i>S. enterica</i> <sup>b</sup>	Mouse	[15,16]
	<i>rpoN</i>	<i>P. aeruginosa</i>	Mouse, <i>Arabidopsis</i>	[17]
	<i>sigB</i>	<i>S. aureus</i>	Mouse <sup>c</sup>	[4,18]
<b>Two-Component Systems</b>	<i>saeRS</i>	<i>S. aureus</i>	Mouse	[20,48]
	<i>srrAB</i>	<i>S. aureus</i>	Rabbit <sup>d</sup>	[20,23]
	<i>ompR/envZ</i>	<i>S. enterica</i> <sup>b</sup>		[16]
	<i>phoPQ</i>	<i>S. enterica</i> <sup>b</sup>	Mouse	[25,26]
	<i>gacAS</i>	<i>P. aeruginosa</i>	Mouse, <i>Arabidopsis</i>	[10,30,32,48]
	<i>PA3946</i> <sup>e</sup>	<i>P. aeruginosa</i>		[31]
	<i>PA4380</i> <sup>e</sup>	<i>P. aeruginosa</i>		[32]

<sup>a</sup>Other models, in addition to *C. elegans*, in which the gene was also necessary for full virulence.

<sup>b</sup>serovar Typhimurium.

<sup>c</sup>*sigB* was necessary infection in a mouse catheter infection model, but not in an intravenous model.

<sup>d</sup>In contrast to the nematode model, overexpression of *srrAB* resulted in decreased virulence in the rabbit model.

<sup>e</sup>Putative gene identified in *P. aeruginosa*.