AlgU, a conserved sigma factor regulating abiotic stress tolerance and promoting virulence in *Pseudomonas syringae*.

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Abstract

Pseudomonas syringae can rapidly deploy specialized functions to deal with abiotic and biotic stresses. Host niches pose specific sets of environmental challenges driven in part by immune defenses. Bacteria use a "just-in-time" strategy of gene regulation, meaning that they only produce the functions necessary for survival as needed. Extracytoplasmic function (ECF) sigma factors transduce a specific set of environmental signals and change gene expression patterns by altering RNA polymerase promoter specificity, to adjust bacterial physiology, structure, and/or behavior to improve chances of survival. The broadly conserved ECF sigma factor, AlgU, affects virulence in both animal and plant pathogens. *Pseudomonas syringae* AlgU controls expression of more than 800 genes, some of which contribute to suppression of plant immunity and bacterial fitness in plants. This review discusses AlgU activation mechanisms, functions controlled by AlgU, and how these functions contribute to *P. syringae* survival in plants.

Introduction

Pseudomonas syringae is a species complex divided into more than fifty pathovars, each of which is able to grow and cause disease in a limited range of plants (Baltrus et al., 2017). *P. syringae* is responsible for economically significant disease outbreaks in many crops worldwide (Xin et al., 2018, Scortichini et al., 2012, Kennelly et al., 2007, Martin et al., 1993a). Because of its agricultural importance and tractability, *P. syringae* has become a popular model organism for understanding mechanisms of plant disease and plant immune defenses (Mansfield et al., 2012, Xin & He, 2013).

P. syringae is adept at surviving diverse and dynamic conditions such as atmospheric, aquatic, terrestrial, and plant host environments (Berge et al., 2014), each presenting specific challenges and opportunities. The ability to quickly and accurately adjust gene expression according to changes in the environment underpins *P. syringae's* success as a plant pathogen. Take the foliar invasion process as an example. *P. syringae* enters the apoplast by moving from leaf surfaces through stomata or wounds using chemotaxis and flagellar motility. Flagellar synthesis is suppressed after entering the apoplastic space, minimizing detection by the plant immune system (Bao et al., 2020). Once in the apoplast, the bacteria also express and assemble the type III secretion system (T3SS) to translocate protein effectors into plant cells to suppress plant immunity, up-regulate production of alginate exopolysaccharide, adjust intracellular compatible solute levels, and adjust their metabolism to utilize apoplastic nutrients (Nobori et al., 2018, Lovelace et al., 2018, Yu et al., 2013, Nobori et al., 2020).

ECF sigma factors bridge environmental signals and transcriptional regulation

P. syringae has the ability to detect their entrance into plant tissues and make appropriate transcriptional adjustments. This is carried out using cell-surface signaling systems that sense and transduce environmental information across the cytoplasmic membrane and through the cytoplasm to adjust gene expression. ExtraCytoplasmic Function (ECF) sigma factors are one of the principal mechanisms that bacteria have for adjusting gene expression patterns in response to external stimuli (Staron et al., 2009). Sigma factors are exchangeable subunits of RNA polymerase (RNAP) that transiently interact with the RNAP core enzyme to coordinate binding and transcription initiation at corresponding promoter sequences (Ishihama, 2000). Accordingly, competition between sigma factor subunits changes the set of promoters transcribed and the sets of functions expressed.

ECF sigma factors are a class of alternative (i.e., non-housekeeping) sigma factors that typically function with anti-sigma factors as part of cell surface signaling systems (Lonetto et al., 1994, Stacey & Pritchett, 2016). Canonical anti-sigma factors are located at the cytoplasmic

membrane and are regulated by extracellular cues. Anti-sigma factors suppress the activity of ECF sigma factors by sequestering them from RNAP and target promoters. With appropriate cues, ECF sigma factors are activated and released from anti-sigma factors (Potvin et al., 2008). It is common for ECF sigma factors to be coexpressed with their cognate anti-sigma in an operon (Figure 1).

The ECF sigma factor AlgU (synonym, RpoE in bacteria outside of Pseudomonads) is broadly conserved among bacteria (Staron et al., 2009). The considerable variation in the naming conventions of this sigma factor (Box 1) which stems from multiple independent discoveries and reflects its importance in diverse bacteria. AlgU (RpoE) recognizes the consensus promoter sequence (gGAACt-16/17-GTCnAA) among *P. syringae*, *P. aeruginosa*, and *E. coli* (Markel et al., 2016, Cheng et al., 2008), and typically up-regulates expression of genes conferring tolerance to osmotic, oxidative, and heat stresses (Table 1). In Pseudomonads, AlgU also controls the synthesis and export of alginate, a hydroscopic exopolysaccharide composed of D-mannuronic and L-guluronic acid polymers (Boyd & Chakrabarty, 1995). In *P. syringae*, AlgU regulates between 800 and 1000 genes, and makes important contributions to the regulation of virulence genes as well as the repression of flagellin (Markel et al., 2016, Bao et al., 2020, Schreiber & Desveaux, 2011).

AlgU is kept inactive by the anti-sigma factor MucA under non-inducing conditions

AlgU (RpoE) is encoded in an operon that contains up to four regulatory genes named *mucABCD* (synonym, *rseABC* and *degP*). The *mucA* gene is near universally associated with *algU*, but the presence of *mucB*, *mucC,* and *mucD* varies (Figure 1). In *P. syringae* pv. glycinea PG4180, the *mucC* gene is absent *algU* and *mucAB* are co-transcribed. and *mucD* is transcribed independently (Schenk et al., 2006). The *muc* genes code for proteins that determine the strength of AlgU membrane-sequestration and activation. They are named for the mucoid colony phenotype associated with *mucA* mutants, which overproduce alginate (Martin et al., 1993b).

The anti-sigma factor MucA is a 21 kDa protein with a single transmembrane domain (Xie et al., 1996). MucA sequesters AlgU at the cytoplasmic membrane and prevents RNAP and DNA interactions through direct protein-protein interaction, thereby suppressing AlgU activity (Figure 2). The N- and C-termini of MucA (RseA) are in the cytoplasm and periplasm, respectively, in both *P. aeruginosa* and *E. coli* (Mathee et al., 1997, Hayden & Ades, 2008). X-ray crystal structure analysis shows that the *P. aeruginosa* MucA N-terminus interacts with the RNAP-binding and DNA-binding domains of AlgU (Li et al., 2019). In *P. aeruginosa*, about 2/3 of total AlgU are sequestered by MucA under non-inducing conditions (Rowen & Deretic, 2000). It was proposed that remaining free AlgU allows for basal-level expression from AlgU promoters

(Rowen & Deretic, 2000). The MucA C-termini also interacts directly with periplasmic protein MucB (synonym, RseB, AlgN). Crystal structures show that *P. aeruginosa* MucB is composed of two domains separated by a flexible linker that form a binding pocket for MucA (Li et al., 2019). This interaction provides an additional mechanism of environmental-sensing (discussed below) (Cezairliyan & Sauer, 2009, Schurr et al., 1996).

Transitioning from an AlgU-inactive to an AlgU-activated state is mediated by degradation of MucA via a regulated intramembrane proteolysis (RIP) cascade. The structure of the AlgU (RpoE) regulatory cascade has been extensively studied in *P. aeruginosa* and *E. coli*. The high degree of similarity in RIP regulation between these two species suggests broad conservation (Yu et al., 1995, Schreiber & Desveaux, 2011). Under inducing conditions, the RIP cascade eliminates MucA and releases AlgU to direct up-regulation from corresponding promoters (Rowen & Deretic, 2000). The RIP cascade was shown to be very responsive. In *P. aeruginosa*, cell wall stress can stimulate MucA degradation within ten minutes (Wood & Ohman, 2009). The short response time is critical for surviving dynamic environments.

RIP step 1: proteolytic cleavage of MucA by AlgW

AlgU activation is carried out through a series of proteolytic cleavages that inactivate MucA and release AlgU to interact with core RNAP and initiate transcription of its regulon. Envelope stresses activate specific proteases and result in MucA cleavage. One well characterized indicator of envelope stress are misfolded outer member proteins (OMPs) (Pandey et al., 2018, Tashiro et al., 2009, de Regt et al., 2015, de Regt et al., 2014, Walsh et al., 2003, Sohn et al., 2007, Chaba et al., 2011). Stress induced malfunction of OMP transport, folding, or assembly, can increase unfolded OMPs in the periplasm (Walsh et al., 2003). The serine protease AlgW (synonym, DegS) is activated through interactions with peptide motifs in the C-termini of misfolded OMPs (Wilken et al., 2004, Bass et al., 1996, Waller & Sauer, 1996). DegS in *E. coli* remains inactive until amino acid sequences (e.g., YxF motifs) exposed in misfolded proteins interact with its periplasmic PDZ domain. This interaction converts DegS into its proteolytically active conformation (de Regt et al., 2015).

In *E. coli*, the degree of DegS activation depends on the amino acid sequences upstream of the YxF motif, providing a mechanism for fine-tuning DegS activity according to the abundance and diversity of unfolded OMPs present (Sohn et al., 2007, Ades et al., 1999). In addition to the YxF motif, *P. aeruginosa* AlgW can also bind and be activated by C-terminal WVF, LVF, WIF, WVW (MucE homologs), FTF ($PilA^{108}$), and GYYYTVV (internal motif of CupB5) (Qiu et al., 2007, de Regt et al., 2014, Ryan Withers et al., 2013). An increase of misfolded OMPs can also result from loss of the histidine kinase KinB, the periplasmic protease MucD, or the sRNA binding chaperon protein Hfq (Wood & Ohman, 2009, Figueroa-Bossi et al., 2006, Damron et al., 2009b). The

essentiality of AlgW (DegS) varies from species to species, but its function is usually related to virulence in studied species (Mathur et al., 2007, Rowley et al., 2005, Schreiber & Desveaux, 2011).

MucD (synonym, DegP, AlgY, HtrA), a product of the *algU-muc* operon, is a periplasmic/secreted serine endoprotease (Wang et al., 2019). In *P. aeruginosa,* MucD suppresses RIP activation by degrading peptides that accumulate during stress (Qiu et al., 2007). The absence of MucD increases AlgW-dependent MucA degradation (Wood & Ohman, 2009). MucD protein levels are regulated by a *mucD* antisense transcript and feedback from misfolded OMPs, as less misfolded OMPs lead to less induction of the whole *algU-mucABCD* operon in *P. aeruginosa* (Knight et al., 2010, Tashiro et al., 2009, Yang et al., 2011). MucD plays an important role in virulence and colonization in plants. The deletion of *mucD* in *P. syringae* pv. *tomato* DC3000, *P. fluorescens* SBW25, and *P. aeruginosa* strain PA14 all reduced *in planta* growth under certain conditions (Yorgey et al., 2001, Wang et al., 2019). *P. aeruginosa* MucD can degrade animal host immune factors (Okuda et al., 2011) and is required to produce a *C. elegans* killing extracellular toxin (Yorgey et al., 2001), indicating that MucD has functions beyond regulating stress signals related to RIP.

MucB interaction with MucA restricts AlgW access to MucA under non-inducing conditions. In *E. coli*, RseB interaction with RseA reduces RseA degradation by 2.4-fold (Ades et al., 1999). Under envelope stresses, outer membrane lipopolysaccharides (LPS) mislocalize to the periplasm. The accumulated periplasmic LPS interacts with RseB and induces it to form a tetramer, which is then released from RseA (Lima et al., 2013). In *P. aeruginosa*, MucB cantetramerize via intermolecular disulfide bonds, which suggests that MucB may serve as a redox sensor under oxidative conditions (Li et al., 2019). As a result, in both *E. coli* and *P. aeruginosa,* the frequency of this first MucA (RseA) cleavage step is a function of MucB (RseB) removal and AlgW (DegS) activation (Chaba et al., 2011, Kim, 2015, Mathee et al., 1997, Desnues et al., 2003).

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RIP step 2: proteolytic cleavage of MucA by MucP

After AlgW cleaves the C-terminal portion of MucA, the transmembrane portion of MucA becomes more susceptible to cleavage by MucP (synonym, RseP). The requirement for MucP (RseP) mediated MucA (RseA) cleavage in *P. aeruginosa* is similar to that in *E. coli* (Damron & Yu, 2011, Qiu et al., 2007), while the biochemical aspect of MucP (RseP) is more extensively studied in *E. coli. E. coli* RseP is an intramembrane zinc metalloprotease that contains two periplasmic PDZ domains. Both the zinc-binding and protease motifs are within the

transmembrane region on the cytoplasmic surface (Kanehara et al., 2002). The PDZ domains can interact with the truncated RseA C-terminus exposed by DegS cleavage (Inaba et al., 2008). This interaction activates RsePto cleave RseA within the transmembrane region, releasing the N-terminus of RseA from the membrane to the cytoplasm (Akiyama et al., 2004). It is not clear how intramembrane peptide bond hydrolysis takes place in a hydrophobic environment, but the glutamic acid residue in the HEXXH Zinc-binding motif is required for hydrolysis (Kanehara et al., 2001). In contrast to DegS, which is highly specific to RseA, RseP can degrade a diverse variety of proteins and may have housekeeping functions (Akiyama et al., 2004). One example is that it processes secreted proteins to remove signal peptides (Saito et al., 2011).

Another periplasmic protease, AlgO (synonym, Prc, Tsp), is also a part of the RIP process. AlgU activation is reduced in *ΔalgO P. aeruginosa* mutants exposed to cell wall stresses (Wood et al., 2006). AlgO cleaves MucA upstream of MucP cleavage, and an excess of MucP can compensate for the loss of *algO* (Delgado et al., 2018). It is generally accepted that AlgO prefers truncated MucA and has minimal effect on full-length MucA. However, it is not clear if AlgW cleavage is directly upstream of AlgO recognition, or if they recognize MucA independently.

RIP final step: degradation of MucA by ClpXP

After MucP (RseP) cleavage, the remaining cytoplasmic (N-terminal) portion of MucA (RseA) is recognized and fed to the protease ClpXP by the adaptor, SspB, in both *P. aeruginosa* and *E. coli* (Flynn et al., 2004, Qiu et al., 2008). ClpXP is a cytoplasmic protease complex of ClpX, an AAA+ unfoldase, and ClpP, a peptidase. ClpXP is highly conserved and has a broader substrate range than MucP. In contrast to other housekeeping proteases, ClpXP determines substrate specificity through adaptors, like SspB, rather than universally recognizing misfolded proteins (Baker & Sauer, 2012, Joshi & Chien, 2016). *P. aeruginosa* encodes two copies of ClpP, and both are required for MucA RIP (Qiu et al., 2008). ClpXP fully degrades the remaining MucA fragment, freeing AlgU.

AlgU/RpoE regulates stress tolerance and promotes virulence in both plant and animal pathogens

Regulating expression of stress tolerance (and alginate production genes in Pseudomonads) are core functions of AlgU (RpoE), enabling rapid physiological adaptation in a wide range of bacteria. While the core role of these sigma factors is conserved, individual species and strains have evolved to use AlgU (RpoE) to co-regulate accessory functions that assist growth in conditions that are idiosyncratic to their specific lifestyles (Rhodius et al., 2005). Recent studies show that AlgU is critical in the transition of *P. syringae* from free-living to pathogenic growth within leaf tissue. In *P. syringae* pv. *tomato* DC3000 and *P. syringae* pv. syringae B728a, AlgU

regulates between 800 and 1000 genes (Yu et al., 2014, Markel et al., 2016), including the core AlgU stress response functions, as well as many genes associated with colonization of plants. Some of these genes are exclusively dedicated to promoting virulence in plants, such as the hypersensitive response and pathogenicity (*hrp*) type III secretion system (T3SS), and type III secreted effector (T3Es) genes. In *P. syringae* pv. *tomato* DC3000, AlgU also contributes to down-regulation of flagellar expression, which has a clear role in minimizing plant immune activation through host flagellin detection (Markel et al., 2016, Bao et al., 2020).

The importance of AlgU in coordinating *P. syringae* transition to the plant niche is also supported by evidence of an arms race centered on AlgU activation. Transcriptional analysis suggests that AlgU activity is suppressed in *P. syringae* pv. *tomato* DC3000 during exposure to pattern triggered immunity (PTI) induced by the flg22 flagellin epitope. PTI exposure correlates with reduced induction of alginate synthetic and osmotolerance genes as well as reduced repression of flagellar genes (Lovelace et al., 2018, Nobori et al., 2018). As described above, AlgU activity is subject to an intricate control mechanism that involves protein-protein interactions and proteolytic processing. MucD, a component of the regulatory cascade, is degraded by *Arabidopsis* immunity-induced secreted aspartic proteases SAP1 and SAP2, which contributes to the antimicrobial effects of PTI (Wang et al., 2019). It is worth noting that there may be several distinct points where plant immune systems interfere with the AlgU signaling cascade. For example, loss of *mucD* is associated with AlgU activation and overproduction of alginate (Qiu et al., 2007, Wang et al., 2019). In contrast, PTI exposure results in reduced expression of alginate production genes suggesting that the repressive effects of PTI on AlgU signaling are potentially independent of MucD degradation.

AlgU homologs are present in most bacterial lineages. Despite accumulating evidence indicating an important role in regulating virulence gene expression, the function of AlgU homologs in most plant pathogenic bacteria have been understudied. However, there are a few examples where the roles of AlgU homologs have been examined in plant pathogens or plant-associated bacteria. In *Xanthomonas campestris* pv. *campestris,* as in *P. syringae,* the AlgU homolog stimulates expression of T3SS and T3E genes and significantly contributes to virulence (Bordes et al., 2011, Cheng et al., 2008, Yang et al., 2018). This is an interesting convergence in AlgU regulation as *P. syringae* and *X. campestris* use very different regulatory cascades to control expression of their respective *hrp* virulence regulons(Brencic & Winans, 2005). In *Xylella fastidiosa*, the AlgU homolog is the only known ECF sigma factor. It is induced in xylem fluid and contributes to heat stress tolerance, biofilm formation, and virulence in grapevines (da Silva Neto et al., 2007, Shi et al., 2007). However, AlgU homologs do not contribute to plant disease in all plant pathogens. In *Burkholderia cepacia*, the AlgU homolog does not contribute to virulence on onion, or to the canonical stress responses observed in other bacteria (Devescovi

& Venturi, 2006). This is in contrast to the AlgU homolog of *B. cenocepacia* and *B. pseudomallei*, which contributes to stress tolerance and animal virulence (Flannagan & Valvano, 2008, Korbsrisate et al., 2005). In the soft rot necrotroph *Dickeya dadantii* 3937, *in vitro* transcription of T3SS-associated promoters was not influenced by mutation of the *algU* homolog (Li et al., 2010). *Alphaproteobacteria* are one of only a few groups of bacteria that notably often lack AlgU family members (Staron et al., 2009). AlgU sigma factors were not identified in the *Rhizobiales* oncogenic pathogen *Agrobacterium tumefaciens* C58 and are sporadically distributed among related root-nodulating symbionts (*e.g. Bradyrhizobium, Rhizobium, Sinorhizobium*). The phloem-limited pathogen *Ca. Liberibacter asiaticus*, causative agent of Huanglongbing (yellow dragon disease, citrus greening), encodes only three sigma factors, none of which are ECF sigma factors (Hartung et al., 2011).

The role of AlgU homologs in the physiology of human bacterial pathogens has been much more extensively studied. In most cases, *algU* mutants adhere to the pattern of having reduced stress tolerance and reduced virulence. In *P. aeruginosa,* it is common for strains to accumulate mutations in *mucA* during chronic lung infection resulting in constitutive AlgU activity and mucoid phenotype on agar media (Yu et al., 1996). In *Yersinia*, *E. coli*, and *Vibrio*, RpoE is considered essential (Heusipp et al., 2003). Recovery of *rpoE* mutations in these strains requires either specialized growth conditions or compensating secondary mutations. In *Salmonella*, *rpoE* is not essential, but *rpoE* mutants epistatically require a functional LPS O-antigen to survive (Amar et al., 2018).

AlgU-regulated pathways in P. syringae

Osmotic stress response: The AlgU regulon in *P. syringae* pv. *tomato* DC3000 is enriched for genes whose functions pertain to responding to and surviving osmotic stress (Markel et al., 2016). Osmotic stress, in the form of an abundance of solutes or lack of water, results in water loss from the cytoplasm, which is deleterious to cellular function and potentially lethal. To cope with osmotic stress, bacteria increase the concentration of compatible solutes like trehalose or glycine betaine through biosynthesis or increased uptake (Wood, 2015). These compatible solutes are useful osmoprotectants because they can accumulate to high concentrations in the cytoplasm without interfering with cellular functions. The use of certain compatible solutes such as glycine betaine is conserved across kingdoms (Csonka, 1989, Roesser & Muller, 2001). In *P. syringae*, AlgU up-regulates the intake or synthesis of three compatible solutes: the quaternary ammonium compound (QAC) glycine betaine, the dipeptide N-acetylglutaminylglutamine amide (NAGGN) (Kurz et al., 2010) and the disaccharide trehalose (α-d-glucopyranosyl-α-d-glucopyranoside) (Freeman et al., 2010, Markel et al., 2016). Betaine based osmotic protection is hypothesized to be part of the acute osmotic stress response which is later replaced by NAGGN based protection (Li et al., 2013). *P. syringae* strains vary in their

capacity for both synthesis and uptake of compatible solutes and their degrees of osmotolerance. These variations in osmotolerance differentially affect epiphytic fitness of strains on the leaf surface (Chen et al., 2013, Yu et al., 2013). Accumulation of specific compatible solutes in the bacterial cytoplasm contributes to *in planta* fitness in a strain specific manner (Freeman et al., 2010, Freeman et al., 2013, Kurz et al., 2010).

***Alginate production*:** Regulation of alginate production by AlgU has been extensively studied in Pseudomonads (Keith & Bender, 1999, Keith et al., 2003, Schenk et al., 2008, Markel et al., 2016). Alginate (co-polymer of O-acetylated beta-1,4-linked D-mannuronic acid and L-guluronic acid) is an exopolysaccharide that was first described in brown algae and its production is conserved across many *Pseudomonas* species (Fett et al., 1986, Muhammadi & Ahmed, 2007). Alginate is considered a virulence factor in some plant and animal pathogens (Boyd & Chakrabarty, 1995, Yu et al., 1999, Keith et al., 2003), however its specific role in virulence is variable (Markel et al., 2016).

In the human pathogen *P. aeruginosa*, alginate is a principal component of the capsule, which provides a passive defense layer that protects against detection by immune cells and oxidative bursts (Boyd & Chakrabarty, 1995), enhances initial adhesion to surfaces, and protects from dehydration (Boyd et al., 1987). However, the general defensive role of alginate for plant pathogens in apoplastic spaces remains an open question. It is possible that alginate protects bacterial cells from osmotic stress as well as oxidative stress associated with immune ROS signaling and accumulation (Keith et al., 2003, Chang et al., 2007). Alginate has also been proposed to interfere with plant immune elicitation by chelating and suppressing calcium influx, a key component of the immune signaling cascade (Aslam et al., 2008, Scrase-Field & Knight, 2003). Alginate appears to make strain variable contributions to epiphytic colonization and apoplastic virulence among *P. syringae* strains. In *P. syringae* pv. *syringae* 3525, alginate production promotes survival during epiphytic colonization of non-host tomato (Yu et al., 1999). Conversely, alginate synthesis genes were not observed to make major contributions to bean leaf epiphytic fitness of *P. syringae* pv. *syringae* B728a on bean but are associated with decreased apoplastic fitness (Helmann et al., 2019). However, alginate production in *P. syringae* pv. *tomato* DC3000 and *P. syringae* pv. *glycinea* PG4180 were not associated with reduced virulence phenotypes in tomato or soybean, respectively (Markel et al., 2016, Schenk et al., 2008, Ishiga et al., 2018).

***De-flagellation*:** Interestingly, AlgU can also indirectly coordinate down-regulation of gene expression. In the case of Pseudomonads, AlgU coordinates down-regulation of genes involved in motility and assembly of the flagella. Peptide epitopes within flagellin monomers are detected by both plant and animal immune pattern recognition receptors and can trigger an immune response (Hajam et al., 2017, Hybiske et al., 2004). In *P. aeruginosa*, down-regulation

of flagellar biosynthesis is thought to correlate with avoidance of host immune detection (Tart et al., 2006, Amiel et al., 2010, Moradali et al., 2017). Most plants encode pattern recognition receptors that recognize various flagellin epitopes and activate PTI (Zipfel et al., 2004). Wild-type *P. syringae* pv. *tomato* DC3000 has a similar apoplastic virulence as *fliC* mutants or mutants with reduced *fliC* expression, suggesting that *P. syringae* pv. *tomato* DC3000 may undergo de-flagellation during its interactions with plants (Pfeilmeier et al., 2016, Bao et al., 2020). The importance of the AlgU signaling cascade for de-flagellation was observed in *P. syringae* pv. *maculicola* ES4236, where *Tn* inactivation of AlgW resulted in decreased AlgU activity and increased expression of flagella as well as reduced *in planta* growth and disease (Schreiber & Desveaux, 2011). AlgU-driven repression of flagellin in *P. syringae* pv. *tomato* DC3000 reduces PTI activation and promotes bacterial fitness in tomato (Bao et al., 2020).

Although AlgU-dependent down-regulation of flagellar expression is conserved across plant pathogenic, plant-associated, and animal pathogenic Pseudomonads, there are surprising mechanistic differences in how flagellar down-regulation is coordinated among these bacteria. In *P. aeruginosa* and *P. fluorescens*, AlgU up-regulates the transcriptional regulator AmrZ (AlgZ), which in turn suppresses motility by down-regulating FleQ, the master regulator of flagellar and chemotaxis gene expression (Tart et al., 2006, Tart et al., 2005, Muriel et al., 2019, Martinez-Granero et al., 2012). In both *P. syringae* and *P. stutzeri*, AlgU also up-regulates AmrZ expression. However, in these bacteria AmrZ acts as a positive regulator of motility rather than a negative regulator (Baltrus et al., 2018, Prada-Ramirez et al., 2016). Additionally, as *fleQ* is not AlgU-regulated in *P. syringae* (Markel et al., 2016, Baltrus et al., 2018), the role of AlgU in *P. syringae* de-flagellation likely occurs downstream of FleQ in the flagellar regulatory network. Regardless of these differences in the flagellar regulatory networks, the final output of increased AlgU-driven expression in *P. syringae* is reduced flagellar expression (Schreiber & Desveaux, 2011, Bao et al., 2020). The differences in AlgU-dependent flagellar regulatory mechanisms suggest that AlgU-mediated de-flagellation may have emerged multiple times independently among Pseudomonads.

***T3SS/T3SEs and other virulence factors*:** Successful colonization of plants is a multifaceted and dynamic process. Avoiding plant immunity is a major part of this process, and mounting evidence supports a key role for AlgU in helping *P. syringae* survive in that context. In addition to suppressing flagellin expression (Schreiber & Desveaux, 2011, Bao et al., 2020), AlgU also up-regulates expression of many genes dedicated to actively suppressing plant immunity and promoting successful colonization and disease (Markel et al., 2016). The mechanistic details of AlgU-dependent regulation of these virulence systems are not fully understood, but appear to include up-regulation of *hrpL* expression, which is the master regulator of pathogenicity in *P. syringae*. HrpL is itself an ECF sigma factor, which recognizes and helps initiate transcription

from *hrp* box promoters, up-regulating expression *hrp/hrc* T3SS structural genes that code for the injectisome and the T3SS secreted effector proteins. Based on Chip-seq analysis AlgU likely up-regulates *hrpL* expression indirectly, presumably by directly driving expression of *hrpR* and *hrpS*, the regulators immediately upstream of HrpL (Markel et al., 2016). For a more comprehensive review of P. syringae T3SS regulation, we would point the reader to Xie et al., 2019(Xie et al., 2019).

AlgU also up-regulates expression of *P. syringae* pv. *tomato* DC3000 coronatine biosynthetic genes (Ishiga et al., 2018). Coronatine is a phytotoxin sporadically distributed among *P. syringae* strains, with multiple roles in promoting interactions with plants. Coronatine contributes to visible disease symptoms, causing the characteristic chlorotic halos (coronas) around necrotic specks (Bender et al., 1999). Coronatine is a potent structural analog of the plant defense hormone jasmonyl isoleucine that biases defenses to counter necrotrophic pathogens (Geng et al., 2014). Coronatine also drives the reopening of stomata, which close in response to the presence of bacterial flagellin, thus aiding further pathogen invasion into leaf tissues (Geng et al., 2014, Melotto et al., 2017). However, as coronatine expression in *P. syringae* pv. *tomato* DC3000 is itself HrpL-regulated, it is unclear if the role of AlgU on coronatine gene expression is direct or acts indirectly through activation of *hrpL* expression (Sreedharan et al., 2006).

Concluding remarks

The ECF sigma factor AlgU (RpoE) and its regulatory cascade represent an evolutionarily ancestral and broadly conserved response pathway for stress tolerance in bacteria. The capacity of MucB and AlgW to respond to different environmental stress cues presumably allows robust and tuned regulation of the RIP cascade to appropriately control the level of free AlgU in response to multiple signals. Some bacteria appear to have tailored their AlgU (RpoE)-mediated signaling responses in ways that promote virulence during the process of adaptation to a pathogenic lifestyle. In the case of *P. syringae*, AlgU contributes to multiple host-interaction pathways including host attack via the T3SS, immune evasion through de-flagellation and niche acclimation through the regulation of alginate and compatible solute concentrations. However, key questions remain. What is the apoplastic environmental cue that leads to efficient activation of AlgU in *P. syringae*? What is the full regulatory cascade in *P. syringae* for AlgU driven deflagellation? How does PTI interfere with AlgU activity? A deeper mechanistic understanding of AlgU (RpoE) as a key control point for bacterial-host interactions will provide useful insights into bacterial adaptation to, and modulation of, the host niche. These insights have great potential to inform novel management strategies for bacterial diseases of plants and potentially human diseases as well.

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Box 1: Naming conventions and synonyms for AlgU/RpoE signaling pathways. There is considerable confusion in the naming conventions of AlgU and RpoE sigma factors, reflecting both their importance and multiple independent discoveries in different bacteria. The AlgU name is mainly used in the *Pseudomonadales* based on its alginate-associated phenotypes (Flynn & Ohman, 1988, Cochran et al., 2000). AlgU was also named AlgT (DeVries & Ohman, 1994). Outside of *Pseudomonadales*, the RpoE name is typically applied (Erickson & Gross, 1989). AlgU and RpoE are also called sigma E or *sigE* (σ^E) to denote its function as a sigma factor. Based on molecular weight-based naming conventions AlgU and RpoE were also known as either sigma 22 (σ^{22}) in *P. aeruginosa* (Mathee et al., 1997) or sigma 24 (σ^{24}) in *E. coli* (Raina et al., 1995) . In Gram positive *Bacillus subtilis,* the AlgU homolog sigma factor is named sigma W (σ^W, *sigW*) (Schobel et al., 2004). Anti-sigma factors follow their own naming conventions reflecting independent discovery in *P. aeruginosa* and *E. coli*. The *Pseudomonadales* anti-sigma factors and signal cascade components are often named "*muc*" or "*alg*" based on the mucoid alginate-associated phenotypes of corresponding mutants (Schurr et al., 1994). *Enterobacterales* anti-sigma factors are typically named *rse* (regulator of sigma E) (De Las Penas et al., 1997), and the *Bacillus subtilis* cognate anti-sigma factor is *rsiW* (regulator of sigma W) (Schobel et al., 2004).

Gene names	synonymous or analogous genes
algU	*rpoE, algT*
mucA	*rseA*
mucB	*rseB, algN*
mucC	*rseC*
mucD	*degP, algY, htrA*
algW	*degS*
mucP	*rseP*
algO	*prc, tsp*

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Tables

Table 1. AlgU/RpoE involvement in various cellular response pathways in different bacteria.

	osmotic stress[a]	**envelope stress**	**oxidative stress**	**heat shock**	**biofilm formation**	**stationary phase survival**
P. aeruginosa	√[b] (Harty et al., 2019, Behrends et al., 2010) x[c] (Wood et al., 2006)	√ (Wood et al., 2006, Harty et al., 2019)	√ (Yu et al., 1996) x (Wood et al., 2006, Damron et al., 2009a)	√ (Schurr et al., 1995)	√ (Bazire et al., 2010)	√ (Behrends et al., 2010, Harty et al., 2019, Waite et al., 2006)
P. syringae	√ (Keith & Bender, 1999, Markel et al., 2016)	na[d]	√ (Keith & Bender, 1999, Markel et al., 2016)	√ (Keith & Bender, 1999)	√ (Laue et al., 2006)	na
E. coli	√ (Kocharunchitt et al., 2014)	√ (Xue et al., 2015)	√ (Egler et al., 2005, Desnues et al., 2003)	√ (Hiratsu et al., 1995)	√ (Serra et al., 2016)	√ (Costanzo & Ades, 2006)
S. enterica (Shi et al., 2018, Amar et al., 2018)	√	√	√	x[d]	√	√
Y. enterocolitica (Heusipp et al., 2003)	√	x	na	x	na	na
X. campestris (Bordes et al., 2011)	x	x	√	√	na	√
X. fastidiosa (da Silva Neto et al., 2007)	x	√	x	√	na	x
B. pseudomallei (Korbsrisate et al., 2005)	√	√	√	na	√	√
B. cepacia (Devescovi & Venturi, 2006)	x	na	x	√	x	na

[a] *In vitro* inducers commonly used. Osmotic stress: NaCl, sorbitol; envelope stress: ethanol, penicillin G, zinc (Mellies et al., 2012); oxidative stress: paraquat, H_2O_2, copper (Thurman et al., 1989); heat shock: up to 50°C.

[b] √ : AlgU is required or up-regulated in association with this condition.
[c] x : AlgU is either not required or not up-regulated under this condition.
[d] na : no applicable literature was identified.

Figure legends

Figure 1. Genetic arrangement of the *algU* (*rpoE*) operons in selected plant and animal pathogens and related bacteria. In the *Enterobacteriales*, *degP* is encoded separately from the *rpoE* operon. Gene names or locus numbers are listed as annotated in their corresponding GenBank accession records. Paer = *Pseudomonas aeruginosa* PAO1; NC_002516. Pstz = *Pseudomonas stutzeri* 28a24; NZ_CP007441. Pfl = *Pseudomonas fluorescens* F113; NC_016830. Pto = *Pseudomonas syringae* pv. *tomato* DC3000; NC_004578. Psy = *Pseudomonas syringae* pv. *syringae* B728a; NC_007005. Pmac = *Pseudomonas syringae* pv. *maculicola* ES4326; CP047260. Pgly = *Pseudomonas savastanoi* pv. *glycinea* race 4; NZ_AEGH01000079. Eco = *Escherichia coli* K-12 substr. MG1655; NC_000913. Dda = *Dickeya dianthicola* ME23; CP031560. Patr = *Pectobacterium atrosepticum* SCRI1043; BX950851. Eamy = *Erwinia amylovora* ATCC 49946; FN666575. Pns = *Pantoea stewartii* subsp. *stewartii* DC283; CP017581. Xcc = *Xanthomonas campestris* pv. *campestris* ATCC 33913; NC_003902. Xev = *Xanthomonas euvesicatoria* 85-10; CP017190. Xylf = *Xylella fastidiosa* Temecula1; AE009442. Rsol = *Ralstonia solanacearum* GMI1000; NC_003295. Brkc = *Burkholderia cepacia* ATCC 25416; NZ_CP007746. Acv = *Acidovorax citrulli* AAC00-1; NC_008752. Homologous genes have been assigned the same color.

Figure 2. A graphic summary of the AlgU (RpoE) RIP pathway model (from *E. coli* and *P. aeruginosa*) and several outcomes of AlgU activation in plants. MucD degrades stress peptides but it is not known if they are the same unfolded envelope proteins that activate AlgW. AlgW forms trimers, one monomer is shown in the cartoon. Upon ligand binding to the PDZ domain, AlgW undergoes a conformational change and the peptidase domain adopts an activated form. The ligand can be C-termini of several different types of unfolded proteins, including OMPs, MucE, and type IV pilin. Activators of the MucP PDZ domain are less understood. MucA N- and C-termini are marked as N and C. Percentages indicate approximate amino acid position relative to full length MucA, 100% at the C-terminus. *P. aeruginosa* MucA is 194 amino acids long, and AlgW cuts between A136 and G137. MucA binds to MucB and AlgU at 1:1:1 ratio. MucB large and small domains are indicated as L and S. MucB protects the AlgW cleavage site. AlgU RNAP-binding domain and DNA-binding domain are labeled as R and D. ClpXP consist of ClpX, an unfoldase, and ClpP, a protease. SspB is an adaptor for ClpXP that determines substrate specificity for the MucA cytoplasmic cleavage product. After AlgU is released from MucA, it interacts with RNA polymerase and guides the induction of stress response and virulence

pathways. The capsulation and osmotic stress response help bacteria survive in plant apoplastic environment, while suppression of PAMP expression and activation of virulence factors counteract the plant immune system.

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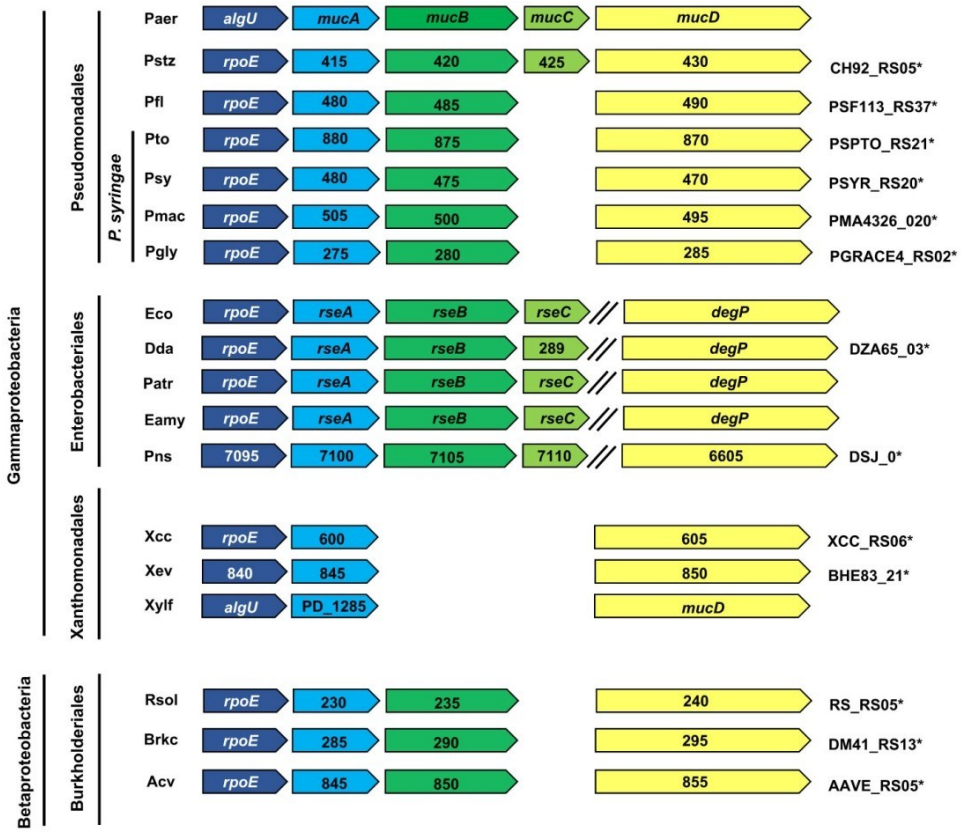

Figure 1. Genetic arrangement of the *algU* (*rpoE*) operons in selected plant and animal pathogens and related bacteria. In the *Enterobacteriales*, *degP* is encoded separately from the *rpoE* operon. Gene names or locus numbers are listed as annotated in their corresponding GenBank accession records. Paer = *Pseudomonas aeruginosa* PAO1; NC_002516. Pstz = *Pseudomonas stutzeri* 28a24; NZ_CP007441. Pfl = *Pseudomonas fluorescens* F113; NC_016830. Pto = *Pseudomonas syringae* pv. *tomato* DC3000; NC_004578. Psy = *Pseudomonas syringae* pv. *syringae* B728a; NC_007005. Pmac = *Pseudomonas syringae* pv. *maculicola* ES4326; CP047260. Pgly = *Pseudomonas savastanoi* pv. *glycinea* race 4; NZ_AEGH01000079. Eco = *Escherichia coli* K-12 substr. MG1655; NC_000913. Dda = *Dickeya dianthicola* ME23; CP031560. Patr = *Pectobacterium atrosepticum* SCRI1043; BX950851. Eamy = *Erwinia amylovora* ATCC 49946; FN666575. Pns = *Pantoea stewartii* subsp. *stewartii* DC283; CP017581. Xcc = *Xanthomonas campestris* pv. *campestris* ATCC 33913; NC_003902. Xev = *Xanthomonas euvesicatoria* 85-10; CP017190. Xylf = *Xylella fastidiosa* Temecula1; AE009442. Rsol = *Ralstonia solanacearum* GMI1000; NC_003295. Brkc = *Burkholderia cepacia* ATCC 25416; NZ_CP007746. Acv = *Acidovorax citrulli* AAC00-1; NC_008752.Homologous genes have been assigned the same color.

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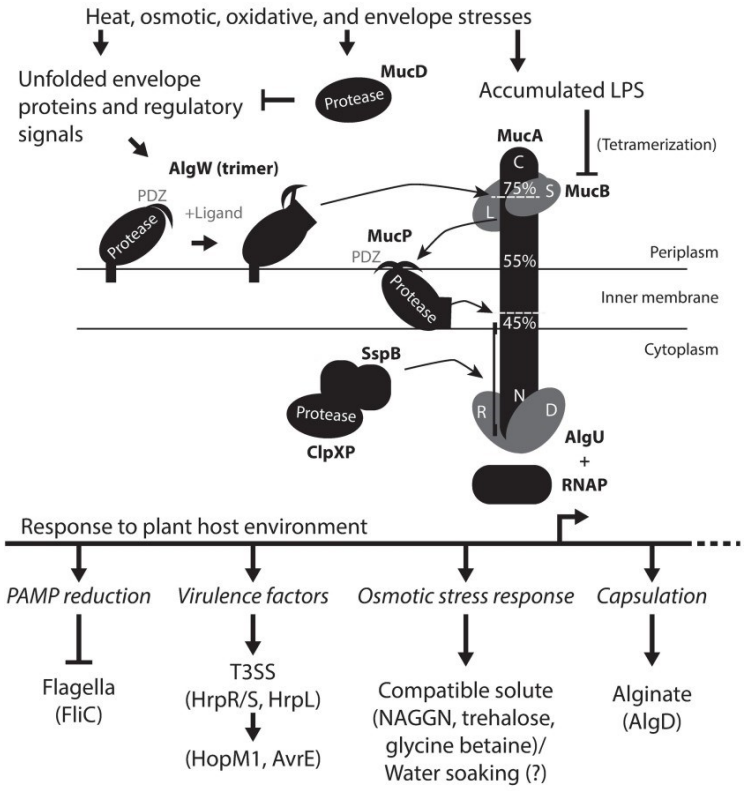

Figure 2. A graphic summary of the AlgU (RpoE) RIP pathway model (from *E. coli* and *P. aeruginosa*) and several outcomes of AlgU activation in plants. MucD degrades stress peptides but it is not known if they are the same unfolded envelope proteins that activate AlgW. AlgW forms trimers, one monomer is shown in the cartoon. Upon ligand binding to the PDZ domain, AlgW undergoes a conformational change and the peptidase domain adopts an activated form. The ligand can be C-termini of several different types of unfolded proteins, including OMPs, MucE, and type IV pilin. Activators of the MucP PDZ domain are less understood. MucA N- and C-termini are marked as N and C. Percentages indicate approximate amino acid position relative to full length MucA, 100% at the C-terminus. *P. aeruginosa* MucA is 194 amino acids long, and AlgW cuts between A136 and G137. MucA binds to MucB and AlgU at 1:1:1 ratio. MucB large and small domains are indicated as L and S. MucB protects the AlgW cleavage site. AlgU RNAP-binding domain and DNA-binding domain are labeled as R and D. ClpXP consist of ClpX, an unfoldase, and ClpP, a protease. SspB is an adaptor for ClpXP that determines substrate specificity for the MucA cytoplasmic cleavage product. After AlgU is released from MucA, it interacts with RNA polymerase and guides the induction of stress response and virulence pathways. The capsulation and osmotic stress response help bacteria survive in plant

apoplastic environment, while suppression of PAMP expression and activation of virulence factors counteract the plant immune system.

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