CURRENT REVIEW

AlgU, a Conserved Sigma Factor Regulating Abiotic Stress Tolerance and Promoting Virulence in *Pseudomonas syringae*

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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, or behavior, singly or in combination, 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.

Keywords: bacterial gene regulation, ECF sigma factor, immunity evasion, plant niche, plant pathogen, stress response

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 (Kennelly et al. 2007; Martin et al. 1993a; Scortichini et al. 2012; Xin et al. 2018). 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 and 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 the success of *P. syringae* 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, upregulate production of alginate exopolysaccharide, adjust intracellular compatible solute levels, and adjust their metabolism to utilize apoplastic nutrients (Lovelace et al. 2018; Nobori et al. 2018, 2020; Yu et al. 2013).

ECF SIGMA FACTORS BRIDGE ENVIRONMENTAL SIGNALS AND TRANSCRIPTIONAL REGULATION

P. syringae have 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 (Staroń 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).

Haibi Wang and Zichu Yang contributed equally to this work.

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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 and 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 (Fig. 1).

The ECF sigma factor AlgU (synonym RpoE in bacteria outside the pseudomonads) is broadly conserved among bacteria (Starón et al. 2009). The considerable variation in the naming conventions of this sigma factor (Box 1) 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 *Escherichia coli* (Cheng et al. 2008; Markel et al. 2016) and typically upregulates 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 and Chakrabarty 1995). In *P. syringae*, AlgU regulates between 800 and 1,000 genes and makes important contributions to the

Fig. 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 = *P. stutzeri* 28a24, NZ_CP007441; Pfl = *P. fluorescens* F113, NC_016830; Pto = *P. syringae* pv. *tomato* DC3000, NC_004578; Psy = *P. syringae* pv. *syringae* B728a, NC_007005; Pmac = *P. syringae* pv. *maculicola* ES4326, CP047260; Pgly = *P. 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* GM11000, NC_003295; Brkc = *Burkholderia cepacia* ATCC 25416, NZ_CP007746; Acv = *Acidovorax citrulli* AAC00-1, NC_008752. Homologous genes have been assigned the same color.

regulation of virulence genes as well as the repression of flagellin (Bao et al. 2020; Markel et al. 2016; Schreiber and Desveaux 2011).

**AlgU IS KEPT INACTIVE
BY THE ANTI-SIGMA FACTOR MucA UNDER
NONINDUCING CONDITIONS**

AlgU (RpoE) is encoded in an operon that contains up to four regulatory genes named *mucA*, *mucB*, *mucC*, and *mucD* (synonym *rseA*, *rseB*, *rseC*, and *degP*). The *mucA* gene is near universally associated with *algU*, but the presence of *mucB*, *mucC*, and *mucD* varies (Fig. 1). In *P. syringae* pv. *glycinea* PG4180, the *mucC* gene is absent, *algU*, *mucA*, and *mucB* are cotranscribed, 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 (Fig. 2). The N- and C-termini of MucA (RseA) are in the cytoplasm and periplasm, respectively, in both *P. aeruginosa* and *E. coli* (Hayden and Ades 2008; Mathee et al. 1997). 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 noninducing conditions (Rowen and Deretic 2000). It was proposed that remaining free AlgU allows for basal-level expression from AlgU promoters (Rowen and 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 and 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 (Schreiber and Desveaux 2011; Yu et al. 1995). Under inducing conditions, the RIP cascade eliminates MucA and releases AlgU to direct upregulation from corresponding promoters (Rowen and 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 and 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) (Chaba et al. 2011; de Regt et al. 2014, 2015; Pandey et al. 2018; Sohn et al. 2007; Tashiro et al. 2009; Walsh et al. 2003). 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 (Bass et al. 1996; Waller and Sauer 1996; Wilken et al. 2004). DegS in *E. coli* remains inactive until amino acid sequences (e.g., YxF motifs) exposed in misfolded proteins

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 (Cochran et al. 2000; Flynn and Ohman 1988). AlgU was also named AlgT (DeVries and Ohman 1994). Outside of Pseudomonadales, the RpoE name is typically applied (Erickson and 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 *Pseudomonas aeruginosa* (Mathee et al. 1997) or sigma 24 (σ^{24}) in *Escherichia coli* (Raina et al. 1995). In gram-positive *Bacillus subtilis*, the AlgU homolog sigma factor is named sigma W (σ^W , sigW) (Schöbel 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 Peñas et al. 1997), and the *Bacillus subtilis* cognate anti-sigma factor is rsiW (regulator of sigma W) (Schöbel et al. 2004).

Gene names	Synonymous or analogous genes
<i>algU</i>	<i>rpoE</i> , <i>algT</i>
<i>mucA</i>	<i>rseA</i>
<i>mucB</i>	<i>rseB</i> , <i>algN</i>
<i>mucC</i>	<i>rseC</i>
<i>mucD</i>	<i>degP</i> , <i>algY</i> , <i>htrA</i>
<i>algW</i>	<i>degS</i>
<i>mucP</i>	<i>rseP</i>
<i>algO</i>	<i>prc</i> , <i>tsp</i>

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 (Ades et al. 1999; Sohn et al. 2007). In addition to the YxF motif, *P. aeruginosa* AlgW can also bind and be activated by C-terminal WVF, LVF, WIF, WVV (MucE homologs), FTF (PilA¹⁰⁸), and GYYYTVV (internal motif of CupB5) (de Regt et al. 2014; Qiu et al. 2007; 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 (Damron et al. 2009b; Figueroa-Bossi et al. 2006); Wood and Ohman 2009. 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 and 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 and 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* PA14 all reduced in-plant growth under certain conditions (Wang et al. 2019; Yorgey et al. 2001). *P. aeruginosa* MucD can degrade animal host immune factors (Okuda et al. 2011) and is required to produce a *Caenorhabditis 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 noninducing 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 can tetramerize 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).

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 and 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 RseP to 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 Δ *algO* *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 MucA is recognized 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 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 and Sauer 2012; Joshi and 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 coregulate accessory functions that assist growth in conditions that are idiosyncratic to their specific lifestyles (Rhodius et al. 2006). 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 1,000 genes (Markel et al. 2016; Yu et al. 2014), 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*) T3SS and type III secreted effector (T3E) genes. In *P. syringae* pv. *tomato* DC3000, AlgU also contributes to downregulation of flagellar expression, which has a clear role in minimizing plant immune activation through host flagellin detection (Bao et al. 2020; Markel et al. 2016).

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 at which 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 in which 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 (Brenic and 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 and Venturi 2006). This is in contrast to the AlgU homologs of *B. cenocepacia* and *B. pseudomallei*, which contribute to stress tolerance and animal virulence (Flannagan and 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 (Staroń 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 enterocolitica*, *E. coli*, and *Vibrio cholerae*, *RpoE* is considered essential (Davis and Waldor 2009; De Las Penas et al. 1997b; Heusipp et al. 2003). Recovery of *rpoE* mutations in these strains requires either specialized growth conditions or compensating secondary mutations. In *Salmonella* spp., *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

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

Bacteria	Osmotic stress	Envelope stress	Oxidative stress	Heat shock	Biofilm formation	Stationary phase survival
<i>Pseudomonas aeruginosa</i>	Yes (Harty et al. 2019; Behrends et al. 2010)	Yes (Wood et al. 2006; Harty et al. 2019)	Yes (Yu et al. 1996)	Yes (Schurr et al. 1995)	Yes (Bazire et al. 2010)	Yes (Behrends et al. 2010; Harty et al. 2019; Waite et al. 2006)
	No (Wood et al. 2006)		No (Wood et al. 2006; Damron et al. 2009a)			
<i>P. syringae</i>	Yes (Keith and Bender 1999; Markel et al. 2016)	NA	Yes (Keith and Bender 1999; Markel et al. 2016)	Yes (Keith and Bender 1999)	Yes (Laue et al. 2006)	NA
<i>Escherichia coli</i>	Yes (Kocharunchitt et al. 2014)	Yes (Xue et al. 2015)	Yes (Egler et al. 2005; Desnues et al. 2003)	Yes (Hiratsu et al. 1995)	Yes (Serra et al. 2016)	Yes (Costanzo and Ades 2006)
<i>Salmonella enterica</i> (Shi et al. 2018; Amar et al. 2018)	Yes	Yes	Yes	No	Yes	Yes
<i>Yersinia enterocolitica</i> (Heusipp et al. 2003)	Yes	No	NA	No	NA	NA
<i>Xanthomonas campestris</i> (Bordes et al. 2011)	No	No	Yes	Yes	NA	Yes
<i>Xylella fastidiosa</i> (da Silva Neto et al. 2007)	No	Yes	No	Yes	NA	No
<i>Burkholderia pseudomallei</i> (Korbsrisate et al. 2005)	Yes	Yes	Yes	NA	Yes	Yes
<i>B. cepacia</i> (Devescovi and Venturi 2006)	No	NA	No	Yes	No	NA

^a In-vitro inducers commonly used for osmotic stress = NaCl, sorbitol, envelope stress = ethanol, penicillin G, zinc (Mellies et al. 2012), oxidative stress = paraquat, H₂O₂, copper (Thurman et al. 1989), heat shock = up to 50°C. Yes or no mean that AlgU is required or upregulated in association with this condition or not required or upregulated under this condition. NA = no applicable literature was identified.

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

Fig. 2. A graphic summary of the AlgU (RpoE) regulated intramembrane proteolysis (RIP) pathway model (from *Escherichia coli* and *Pseudomonas 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 outer member proteins, 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 a 1:1:1 ratio. MucB large and small domains are indicated as L and S, respectively. MucB protects the AlgW cleavage site. AlgU RNA polymerase (RNAP)-binding and DNA-binding domains are labeled as R and D, respectively. 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 RNAP and guides the induction of stress response and virulence pathways. The capsulation and osmotic stress response help bacteria survive in the plant apoplastic environment, while suppression of pathogen-associated molecular pattern expression and activation of virulence factors counteract the plant immune system. T3SS = type III secretion system.

solutes, such as glycine betaine, is conserved across kingdoms (Csonka 1989; Roesser and Muller 2001). In *P. syringae*, AlgU upregulates the intake or synthesis of three compatible solutes, namely, the quaternary ammonium compound glycine betaine, the dipeptide *N*-acetylglutaminyglutamine 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 that 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-plant fitness in a strain-specific manner (Freeman et al. 2010, 2013; Kurz et al. 2010).

Alginate production. Regulation of alginate production by AlgU has been extensively studied in pseudomonads (Keith and Bender 1999; Keith et al. 2003; Markel et al. 2016; Schenk et al. 2008). Alginate (copolymer of *O*-acetylated β -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; Mohammadi and Ahmed 2007). Alginate is considered a virulence factor in some plant and animal pathogens (Boyd and Chakrabarty 1995; Keith et al. 2003; Yu et al. 1999); 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 and 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 reactive oxygen species signaling and accumulation (Chang et al. 2007; Keith et al. 2003). 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 and 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 nonhost 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 (Ishiga et al. 2018; Markel et al. 2016; Schenk et al. 2008).

De-flagellation. Interestingly, AlgU can also indirectly coordinate downregulation of gene expression. In the case of pseudomonads, AlgU coordinates downregulation 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*, downregulation of flagellar biosynthesis is thought to correlate with avoidance of host immune detection (Amiel et al. 2010; Moradali et al. 2017; Tart et al. 2006). 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 (Bao et al. 2020; Pfeilmeier et al. 2016). The importance of the AlgU signaling cascade for de-flagellation was observed in *P. syringae* pv. *maculicola* ES4236, in which *Tn* inactivation of AlgW resulted in decreased AlgU activity and increased expression of flagella as well as reduced in-plant growth and disease (Schreiber and 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 downregulation of flagellar expression is conserved across plant-pathogenic, plant-associated, and animal-pathogenic pseudomonads, there are surprising mechanistic differences in how flagellar downregulation is coordinated among these bacteria. In *P. aeruginosa* and *P. fluorescens*, AlgU upregulates the transcriptional regulator AmrZ (AlgZ), which, in turn, suppresses motility by down-regulating FleQ, the master regulator of flagellar and chemotaxis gene expression (Martínez-Granero et al. 2012; Muriel et al. 2019; Tart et al. 2005, 2006). In both *P. syringae* and *P. stutzeri*, AlgU also upregulates AmrZ expression. However, in these bacteria AmrZ acts as a positive regulator of motility rather than a negative regulator (Baltrus et al. 2018; Prada-Ramírez et al. 2016). Additionally, as *fleQ* is not AlgU-regulated in *P. syringae* (Baltrus et al. 2018; Markel et al. 2016), 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 (Bao et al. 2020; Schreiber and Desveaux 2011). The differences in AlgU-dependent flagellar regulatory mechanisms suggest that AlgU-mediated de-flagellation may have emerged multiple times independently among pseudomonads.

T3SS, T3Es, 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 (Bao et al. 2020; Schreiber and Desveaux 2011), AlgU also upregulates 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 upregulation 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, upregulating expression *hrp/hrc* T3SS structural genes that code for the injectisome and the T3SS secreted effector proteins. Based on Chip-seq analysis AlgU likely upregulates *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).

AlgU also upregulates 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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