

11-11-2012

A dynamic and intricate regulatory network determines *Pseudomonas aeruginosa* virulence

Deepak Balasubramania

Department of Biological Sciences and Herbert Wertheim College of Medicine, Florida International University,
dbalasu@fiu.edu

Lisa Schneper

Herbert Wertheim College of Medicine, Florida International University, schnepel@fiu.edu

Hansi Kumari

Herbert Wertheim College of Medicine, Florida International University, hkumari@fiu.edu

Kalai Mathee

Herbert Wertheim College of Medicine, Florida International University, matheek@fiu.edu

Follow this and additional works at: https://digitalcommons.fiu.edu/cas_bio



Part of the [Biology Commons](#)

Recommended Citation

Balasubramania, Deepak; Schneper, Lisa; Kumari, Hansi; and Mathee, Kalai, "A dynamic and intricate regulatory network determines *Pseudomonas aeruginosa* virulence" (2012). *Department of Biological Sciences*. 144.

https://digitalcommons.fiu.edu/cas_bio/144

This work is brought to you for free and open access by the College of Arts, Sciences & Education at FIU Digital Commons. It has been accepted for inclusion in Department of Biological Sciences by an authorized administrator of FIU Digital Commons. For more information, please contact dcc@fiu.edu.

SURVEY AND SUMMARY

A dynamic and intricate regulatory network determines *Pseudomonas aeruginosa* virulence

Deepak Balasubramanian^{1,2}, Lisa Schneper², Hansi Kumari² and Kalai Mathee^{2,*}

¹Department of Biological Sciences, College of Arts and Science and ²Department of Molecular Microbiology and Infectious Diseases, Herbert Wertheim College of Medicine, Florida International University, Miami, FL 33199, USA

Received August 14, 2012; Revised October 2, 2012; Accepted October 6, 2012

ABSTRACT

Pseudomonas aeruginosa is a metabolically versatile bacterium that is found in a wide range of biotic and abiotic habitats. It is a major human opportunistic pathogen causing numerous acute and chronic infections. The critical traits contributing to the pathogenic potential of *P. aeruginosa* are the production of a myriad of virulence factors, formation of biofilms and antibiotic resistance. Expression of these traits is under stringent regulation, and it responds to largely unidentified environmental signals. This review is focused on providing a global picture of virulence gene regulation in *P. aeruginosa*. In addition to key regulatory pathways that control the transition from acute to chronic infection phenotypes, some regulators have been identified that modulate multiple virulence mechanisms. Despite of a propensity for chaotic behaviour, no chaotic motifs were readily observed in the *P. aeruginosa* virulence regulatory network. Having a 'birds-eye' view of the regulatory cascades provides the forum opportunities to pose questions, formulate hypotheses and evaluate theories in elucidating *P. aeruginosa* pathogenesis. Understanding the mechanisms involved in making *P. aeruginosa* a successful pathogen is essential in helping devise control strategies.

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative bacterium that has the ability to thrive in most natural and man-made environments. It is found in diverse habitats, including soil, water, plants and animals, and can infect multiple hosts (1,2). *Pseudomonas aeruginosa* causes a wide variety of acute (short duration, typically severe)

and chronic (persisting for a long time, often refractory to treatment, severity varying with pathogen) human infections, including in patients with severe burn wounds, urinary tract infections, AIDS, lung cancer, chronic obstructive pulmonary disease, bronchiectasis and cystic fibrosis (CF) (3–6).

Metabolic versatility, intrinsic and acquired antibiotic resistance, biofilm formation and production of multiple virulence (disease-causing) factors make *P. aeruginosa* a formidable pathogen. The virulence machinery of *P. aeruginosa* comprises both cell-associated determinants (such as lipopolysaccharides, pili, flagella) and numerous secreted factors (such as elastases, proteases, exotoxins, pyocyanin, extracellular polysaccharides). One of the mechanisms by which *P. aeruginosa* senses external signals is using sensor proteins that, through phosphotransfer or phosphorelay, activate specific transcriptional regulators. These sensor–regulator protein pairs are called two-component systems (TCS). The *P. aeruginosa* PAO1 genome encodes ~127 TCS members, compared with 60 in *Escherichia coli* (7) and 70 in *Bacillus subtilis* (8), reflecting the adaptability of *P. aeruginosa*. TCS and their modifications also feed into major regulatory pathways and play a critical role in allowing cells to modulate gene expression in response to environmental conditions (9,10). Many of the secreted virulence factors and phenotypes, such as biofilm formation, are under the control of a cell density recognition mechanism called quorum sensing (QS) that aids in the coordinated expression of genes (11,12). QS is a key to virulence gene expression in many bacteria and serves as an attractive target for antibacterial chemotherapy (13).

In humans, acute *P. aeruginosa* infections in specific sites, such as the CF lung, eventually lead to chronic infections. This is caused by adaptive modifications in the infecting clonal type, resulting in diverse morphotypes (14). Acute virulence factors include the Type 2 and Type 3 secretion systems, flagella, type IV pili and QS-regulated

*To whom correspondence should be addressed. Tel: +1 305 348 0628; Fax: +1 305 348 2913; Email: Kalai.Mathee@fiu.edu

virulence factors (proteases, elastase, pyocyanin) (15). On establishing a chronic infection, *P. aeruginosa* overproduces extracellular polysaccharides, forms biofilms and small colony variants and upregulates the Type 6 secretion system (15–18). Antibiotic resistance plays a major role in both types of infection, although the cells display higher levels of resistance in chronic infections (18,19). The transition to a chronic infection phase is the result of numerous changes in cellular physiology in response to external stimuli (20). The changes include downregulation of acute virulence genes with a concomitant upregulation of chronic infection phenotypes and antibiotic resistance, facilitating recalcitrant infections (15,20). Host invasion, establishment of acute infection and the subsequent transition to the chronic phase involves tightly regulated expression of many genes associated with metabolism, virulence and antibiotic resistance. Several key players in these transition processes have been identified and include transcriptional and post-transcriptional regulators (21–23). Many of these will be discussed in subsequent sections of this review.

Gene regulation in *P. aeruginosa* is a complex process involving numerous transcriptional regulators, regulatory RNAs (rgRNA) and σ factors. The *P. aeruginosa* genome is >6 Mb (24), approaching that of lower eukaryotes. The genome is plastic and has acquired genes and undergone extensive rearrangements to adapt to specific niches (25). The large genome of *P. aeruginosa* supports a multitude of regulatory networks, with ~8% of the total genome dedicated to the regulatory proteins (26). *Pseudomonas aeruginosa* PAO1 encodes 434 transcriptional regulators, 24 σ factors and 34 small RNAs, many of which remain to be characterized (24,27–29). Moreover, predicted regulatory networks indicate that there is an extensive crosstalk between the different transcriptional regulators (27,30). These networks, however, are based in part on *in silico* analyses, and their validity needs to be established. This review makes an effort to consolidate the empirically proven major virulence regulatory networks in *P. aeruginosa* with the hope of providing a framework for future studies to better understand pathogenic processes in *P. aeruginosa* and in related bacteria.

MAJOR VIRULENCE REGULATORY SYSTEMS IN *P. AERUGINOSA*

The experimentally established virulence regulatory network in *P. aeruginosa* is depicted in Figure 1. Our group and others have previously performed *in silico* analyses of the *P. aeruginosa* transcriptional regulatory network (27,30,31). Comparing those networks with the network depicted in Figure 1 clearly demonstrates the gap in knowledge between predicted networks and established ones. An important contributing factor to this discrepancy is the fact that the functions of the majority of the genes in the PAO1 genome remain unknown. Deep sequencing, transcriptome metaanalysis (32,33) and complementary studies will aid in assigning functions to the hypothetical genes and undoubtedly narrow this knowledge gap.

Cis regulatory elements (CREs) form a critical part of transcription. CREs are non-coding DNA sequences

present in or near a gene, and they often contain binding sites for transcription factors and/or other regulators of transcription (34). The two major CREs are promoters and enhancers (35,36). The promoters contain the binding sites for transcription factors and other regulatory molecules, such as σ factors and regulatory RNAs (37–39). Enhancers, once thought to be part of only eukaryotes, are found widely in prokaryotes also, and they function in conjunction with the σ^{54} -RNA polymerase (40–42). The known *P. aeruginosa* transcription factor binding sites are listed in Table 1. This section will focus on the transcriptional and post-transcriptional regulation of critical pathways that determine *P. aeruginosa* pathogenesis.

QS

QS is a signalling mechanism that bacteria use to regulate gene expression in a population density-dependent manner, and it was first demonstrated in *Vibrio fischeri* (58). In QS, the bacteria produce and secrete small molecules called autoinducers or quorumones. When these molecules reach a concentration threshold, they diffuse back into the cell to elicit a coordinated response promoting group survival (59). *Pseudomonas aeruginosa* uses QS to regulate production of various virulence determinants, such as extracellular proteases, iron chelators, efflux pump expression, biofilm formation, motility and the response to host immune signals (60). This is achieved using two types of autoinducers, *N*-acyl-homoserine lactones (AHLs) and 2-alkyl-4 quinolones (AQs) (61).

AHL-mediated QS

Pseudomonas aeruginosa has two canonical AHL QS signalling pathways, the *las* and *rhl* systems. Together, these pathways affect expression of ~10% of the *P. aeruginosa* transcriptome (62). The *lasI* (PA1432) and *rhlI* (PA3476) genes encode the *N*-3-oxododecanoyl homoserine lactone (3-oxo-C12-AHL) synthetase (63,64) and *N*-butyrylhomoserine lactone (C4-AHL) synthetase, respectively (65–68). The resulting AHLs then bind and activate their cognate LuxR family regulators, LasR (PA1430) (64) or RhlR (PA3477) (67). LasR and RhlR multimerize in the presence of their cognate AHL (69,70). In *in vitro* studies, LasR–DNA interaction is cooperative and non-cooperative in the presence or absence of a dyad symmetry in the binding sites, respectively (71). Rhl-regulated promoters have binding sites with a dyad symmetry (72).

AQ-mediated QS

Pseudomonas aeruginosa synthesizes two AQ QS signals, 2-heptyl-3-hydroxy-4-quinolone (PQS) and its precursor, 2-heptyl-4-quinolone (HHQ) (73). Both PQS and HHQ enhance *in vitro* binding of the LysR-type transcription regulator, MvfR (also known as PqsR, PA1003), to the promoter of the *pqsABCDE* operon (PA0996–PA1000), suggesting that they function as MvfR effectors (74). Microarray analysis identified 141 genes differentially expressed in an *mvfR* mutant strain, including *lasR*, *algT/U* (PA0762), *rsmA* (PA0905) and *rsaL* (PA1431) (75). PQS also acts independently of MvfR to induce expression of

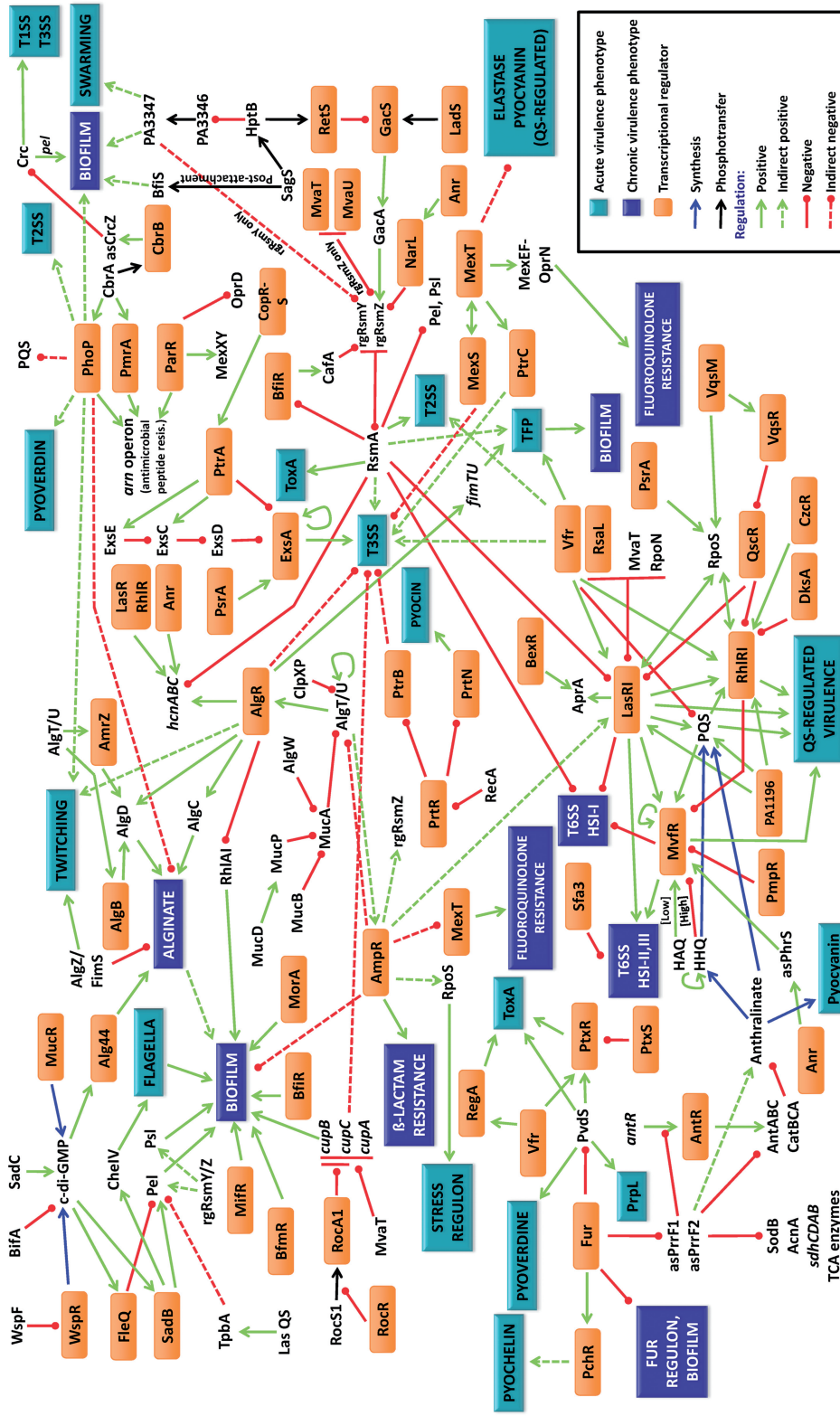


Figure 1. The *P. aeruginosa* virulence regulatory network. The pathogenic potential of *P. aeruginosa* is dictated by multiple virulence systems that are regulated transcriptionally, post-transcriptionally and post-translationally. The central mechanism for *P. aeruginosa* virulence regulation is QS, which controls expression of many virulence factors in a population density-dependent manner. Key activators of this system are LasR, RhlR, MvfR, VqsR, the cAMP receptor protein RpoS, the stationary phase σ factor RpoN, Las system repressors include RsaL, the H-NS protein MvaT, the σ factor RpoN and the sRNA-binding protein RsmA, whereas others like QscR repress both the Las and Rhl systems. Other regulators such as AmpR affect QS genes by an unknown mechanism. QS plays a role in regulating critical pathogenic mechanisms, including biofilm formation, secretion systems, production of numerous virulence factors, efflux pumps, antibiotic resistance and motility. Acute *P. aeruginosa* infections can lead to chronic infections in response to largely unidentified signals. A key regulatory pathway that controls this lifestyle switch is the RetS-LadS-GacSA-RsmA pathway. RetS and LadS are hybrid sensor proteins that, in response to external signals, either activate or repress the GacSA TCS. The GacA regulator then activates transcription of two sRNAs, rgRsmZ and rgRsmY that sequester and inhibit activity of the sRNA-binding protein, RsmA. RsmA is a key activator/repressor that post-transcriptionally regulates numerous acute and chronic infection phenotypes, including multiple QS-regulated virulence factors, biofilm formation, Type 2, Type 3 and Type 6 secretion systems and motility. Another major phenotypic change associated with the switch from acute to chronic phases of infection is the formation of biofilm. This is associated with extensive changes in transcription. Three key TCS involved in activating biofilm formation are BfSR, MifR and BfmSR. Cyclic-di-GMP is another major player influencing this process, whose levels are controlled by diguanylate cyclases and phosphodiesterases. QS and the *cpx* genes enhance biofilms, whereas regulators like AmpR repress it. An important component of *P. aeruginosa* biofilms are extracellular polysaccharides, such as alginate, Pel and Psl. Alginate production is under the control of the master regulator ECF AlgT/U, whose activity is regulated transcriptionally by AmpR, post-translationally by MucA and MucB and by regulated intermembrane proteolysis involving MucP, AlgW, ClpXP and others. AlgT/U activates the alginate biosynthetic operon through AlgR, AlgB and AmrZ. In addition, biofilm formation is also affected by iron concentration, a process governed by the master repressor of iron uptake, Fur. Fur controls uptake of iron by regulating the σ factor PvdS, thereby modulating siderophore levels. Fur also modulates transcription of two key regulatory RNAs, asPrfF1 and asPrfF2. These two sRNAs are involved not only in regulating iron-uptake-related genes but also enzymes of the trichloroacetic acid cycle and genes involved in anthranilate synthesis. Anthranilate, a precursor for

(continued)

Table 1. *Cis* regulatory elements in *P. aeruginosa* transcriptional regulation

| Transcription factor | <i>Cis</i> regulatory element | Major virulence phenotype regulated | Reference |
|------------------------|--|--|-----------|
| AlgR | ACCGTTCGTC | Alginate production, biofilm formation, T3SS | (43) |
| AlgZ | GGCCATTACCAGCC | Alginate production | (44) |
| Anr | TTGATN ₄ ATCAA | Anaerobic regulator of QS | (45) |
| ArgR | TGTCGCN ₈ AA | Carbon and nitrogen catabolism | (46) |
| ExsA | TNAAAANA | T3SS | (47) |
| FleQ | Box 1: CGCCTAAAAATTGACAGTT Box 2: CATTAGATTGACGTTAATC | Motility, biofilm formation | (48) |
| Fur | GATAATGATAATCATTATC | Iron uptake | (49) |
| LasR (<i>las</i> box) | NHCTRNSNNDHNDKNNAGNB | QS | (50) |
| MexT | ATCAN ₃ GTCGATN ₄ ACYAT | Antibiotic resistance, T3SS, QS | (51) |
| MvfR | TTCGACTCCGAA | QS | (52) |
| PsrA | G/CAAAACN ₂₋₄ GTTTG/C | Stress regulon (RpoS), T3SS | (53) |
| RcsB ^a | TTA-GAAACGTCCTAAA | Fimbriae | (54) |
| RhlR (<i>lux</i> box) | CCTGTGAAT/ATCC/TGGT/CAGTT | QS | (55) |
| Vfr | AATTGACTAATCGTTCACATTTG | QS | (56) |
| VqsR | TCGCCN ₈ GGCGA | QS | (57) |

H = C/T/A; R = A/G; S = C/G; D = G/A/T; K = G/T; B = C/G/T; N = A/C/G/T.

^aRcsB is found only in *P. aeruginosa* PA14, not in *P. aeruginosa* PAO1.

the Fur regulon through its ability to bind iron (73,76) and membrane vesicle formation by inducing membrane curvature (77,78). PmpR (PA0964), a YebC member, negatively regulates MvfR (Figure 1) (79).

QS regulation

The *las*, *rhl* and PQS/HHQ/MvfR systems exhibit positive feed-forward autoregulation (52,80). In addition, the *P. aeruginosa* AHL systems function in a hierarchical manner, as the 3-oxo-C12-AHL-LasR complex positively regulates *rhlI*, *rhlR* and *mvfR* expression as well as *lasI* (81–83). Exceptions to this have been noted. RhlR has been shown to regulate LasR-dependent genes in strains lacking *lasR* (84), and timing of *lasI*, *lasR*, *rhlI* and *rhlR* expression can vary drastically depending on growth conditions (85).

Many global regulators have been shown to modulate QS-dependent genes. RpoS (PA3622), the stationary phase σ factor affects ~40% of the QS regulon (72,86). RpoS binding sites have been identified in several of the QS-dependent promoters. RpoS also affects *lasR* and *rhlR* expression, and LasR binding sites have been identified in promoters of other transcriptional regulators in the QS regulon, including *PA2588*, *PA4778*, *pvdS* (*PA2426*), *vqsR* (*PA2591*) and *rsaL* (87). Chromatin immunoprecipitation studies have shown occupancy by histone-like silencers MvaT (PA4315) and MvaU (PA2667) on *lasI*, *lasR*, *mvfR*, *rpoS* and *rsaL* (88). RsaL plays an important role in *las* signalling homeostasis, by binding to the *lasI* promoter and preventing LasR-mediated activation (89). In addition to affecting gene expression through *las* regulation, microarray analyses indicate that RsaL affects expression of 130 genes, including direct regulation of

pyocyanin and hydrogen cyanide genes (89). RsaL also seems to be important in regulating the transition from planktonic to a sessile state, as *rsaL* mutants exhibit increased swarming motility and fail to form biofilms (90). RsaL expression is under the control of LysR-type regulator OxyR (PA5344) (91). The *lasI* promoter region has also been shown to be bound by CzcR (PA2523), which is required for expression of *rhlI* and *rhlR* in addition to *lasI* (Figure 1) (92). CzcR is part of the CzcRS TCS, which is shown to be involved in carbapenem and heavy metal resistance (93).

VqsR (PA2591), which is induced by H₂O₂ or human serum (94) and is under LasR regulation (95), regulates QS through inhibition of the LuxR-type regulator, QscR (PA1898, Figure 1) (96). Although QscR binds to 3-oxo-C12-AHL, its specificity is not as stringent as LasR (97). The QscR regulon partially overlaps that ascribed to the *las* and *rhl* systems, but also has unique targets (98). In the absence of AHL, QscR can multimerize and form heterodimers with LasR and RhlR (99). QscR also plays a role in LasI homeostasis, as mutations in *qscR* result in premature *lasI* expression (100). An AraC family member VqsM (PA2227) regulates VqsR in addition to numerous genes involved in QS, including RsaL, PprB (PA4296), MvfR, RpoS as well as AlgT/U and MexR (PA0424) (101).

Additionally, *pqsH* (PA2587), which encodes the enzyme responsible for oxidation of HHQ to form PQS, is positively regulated by the *las* system (102,103) and is negatively regulated by the *rhl* system (52). PQS is derived from anthranilate, which is synthesized by the kynurenine pathway (104). Kynurenine pathway anthranilate is also required for *N*-decanoyl-homoserine lactone (C10-AHL) dependent signalling, which is independent of *las*, *rhl* and

Figure 1. Continued

synthesis of PQS, is a key regulatory molecule of the PQS signalling system in *P. aeruginosa*, which is involved in expression of QS-regulated virulence factors. Details on the individual interactions and the appropriate references can be found in the text. Some of the interactions labelled as indirect are regulated by unknown mechanisms and warrant further investigation. In the figure, some regulators and phenotypes have been mentioned more than once.

qscR (104). The receptor for this signalling is yet to be identified (105). Besides potential heterodimerization with QscR, additional post-transcriptional regulation of QS has been described. In one such mechanism, QteE (PA2593) destabilizes LasR and RhlR, and in the absence of *qteE*, the quorum threshold-requirement for activation of QS-dependent genes is lost (106). RsmA negatively regulates *rhl* and *las* signalling, resulting in reduced AHL levels (107). Moreover, it has been shown the RNA chaperone Hfq (PA4944) positively regulates *rhlI* translation through *rsmY* and RsmA (108).

Recently, our laboratory has established a role for the β -lactamase regulator AmpR (PA4109) in activating QS-regulated genes (23). The production of QS-regulated secreted virulence factors, such as LasA (PA1871) and LasB (PA3724) proteases, and pyocyanin production is significantly impaired in AmpR-deficient strains. Further, loss of *ampR* reduced virulence in the *Caenorhabditis elegans* toxicity assay (23,109). In addition, AmpR regulates non- β -lactam resistance by repressing activity of the MexEF-OprN (PA2493-PA2495) efflux pump, the alginate master regulator AlgT/U (110) and biofilm formation (23), suggesting that it plays a role in maintaining the acute mode of infection.

TCS

TCS are sophisticated signalling mechanisms marked by a highly modular design that have been adapted and integrated into a wide variety of cellular signalling circuits. The archetypical TCS is composed of a membrane integrated sensory histidine kinase (HK) and a cytoplasmic response regulator (RR) (111). The HK contains a periplasmic N-terminal domain that detects specific stimuli (sensing domain) and a C-terminal cytoplasmic transmitter domain that comprises a dimerization domain, a conserved histidine and an adenosine triphosphate catalytic domain (112). HKs can have two or more transmembrane domains with little or no periplasmic domain, whereas others are completely cytoplasmic. The cognate RR contains a conserved receiver domain and a variable output domain (113). On receiving a signal, two HK monomers dimerize and cross-phosphorylate at the conserved histidine residue, and the phosphate is subsequently transferred to an aspartate residue in the receiver domain of the cognate RR (114). The phosphotransfer is catalysed by the receiver domain, and it results in a conformational change that activates the output domain, which often binds DNA and modulates gene expression or enzymatic activity (9,113,115). Variations to this model occur in phosphorelays, where a sensor kinase first transfers the phosphoryl group to an RR that has no output domain. This P~RR then transfers the phosphoryl group to a histidine-containing phosphotransfer protein, and this in turn serves as a phosphate donor to a terminal RR, which has an output domain mediating a cellular response (10). In other cases, the sensor kinase and the RR lacking an output domain are fused into one protein (hybrid sensor kinase) (116). Other variations include the TCS connectors, a group of proteins that modulate the phosphorylation

state and activity of sensor HK and RR and establish regulatory links between otherwise independent signal transduction pathways (117).

Pseudomonas aeruginosa, equipped with 55 HKs, 89 RRs and 14 HK-RR hybrids, possesses one of the largest pool of TCS proteins identified in any microorganism analysed thus far (24). This provides the bacterium with a sophisticated capability to regulate diverse metabolic adaptations, virulence and antibiotic resistance processes that are hallmark of *P. aeruginosa* infections. One of the critical TCSs is GacSA (GacS-PA0928, GacA-PA2586), which is central to expression of virulence factors, secondary metabolites, biofilm formation and QS (107,118) and is the switch between acute and chronic infections (1,119). GacS is a hybrid sensor HK that contains an HK domain, an RR domain and a histidine phosphotransfer (Hpt) domain (21,120). GacS phosphorylation is under the control of two hybrid sensor kinases, RetS (PA4856) (21) and LadS (PA3974) (22) (Figure 1). RetS can directly interact with GacS and prevent GacS phosphorylation (22,121), whereas LadS phosphorylates GacS (22). Phosphorylated GacA positively regulates the transcription of two small regulatory RNAs, rgRsmZ (PA3621.1) and rgRsmY (PA0527.1), which block the negative regulator RNA-binding protein RsmA (PA0905). RsmA positively regulates genes of the Type 3 secretion system, type IV pili formation and iron homeostasis while repressing QS, Type 6 secretion and potentially other transcription factors (122-124). The GacSA TCS is also involved in antibiotic resistance to three different families of antibiotics, tobramycin, ciprofloxacin and tetracycline (125), apparently through RsmA/rgRsmZ.

In *P. aeruginosa*, PhoPQ (PA1179-PA1180) together with PmrAB (PA4776-PA4777) are two TCSs that respond to limiting concentrations of cations, and regulate resistance to polymyxin B and cationic antimicrobial peptides through the regulation of the *arnBCADTEF-pmrE* (PA3552-PA3559) LPS modification operon (126,127). PhoQ is involved in swarming and twitching motility as well as in biofilm formation and is required for virulence without affecting the T3SS or QS systems (Figure 1) (128). The HK PhoQ activates the RR PmrA independently of PmrB, suggesting an interaction between these TCSs (129). In addition, increased resistance to antibiotics, including polymyxin B, aminoglycosides and quinolones in *phoQ* mutants suggests crosstalk between PhoPQ and other TCSs (130,131).

Formation of biofilms

Biofilms are surface-associated multicellular bacterial communities encapsulated in a self-produced extracellular matrix composed of polysaccharides, proteins and nucleic acids that mediate cell-to-cell and cell-to-surface interactions (132). *Pseudomonas aeruginosa* biofilms, typically associated with poor patient prognosis, signify the switch from an acute to a chronic infection. Biofilms can be formed on abiotic (environment) or biotic (wounds, surgical implants, CF lung) surfaces (133). Biofilm formation and maintenance is tightly regulated in response to environmental cues, conferring enhanced resistance

against antimicrobial agents and immune defence mechanisms on the biofilm bacteria (12). Formation of biofilms is a multi-stage process that is initiated by the surface attachment of planktonic bacteria to form a monolayer, clonal growth/aggregation leading to the formation of microcolonies, maturation to form mushroom-shaped structures and dispersal (134–136). As can be imagined, this complex transition in the bacterial lifestyle is accompanied by drastic changes in gene regulation.

Surface attachment by *P. aeruginosa* to form microcolonies has been attributed to type IV pili, flagella, free DNA, alginate and Pel and Psl polysaccharides, although pili, alginate and flagella mutants also form biofilms (136,137). Attachment is a reversible process, and the commitment to form biofilms is partly under positive SadB (PA5346) regulation (138). SadB upregulates both Pel polysaccharide production and the chemotaxis-like cluster CheIV (PA0408–PA0417), which is thought to regulate flagellar motion by an unknown mechanism (139).

The Cup fimbriae, encoded by three distinct gene clusters *cupA* (PA2128–PA2133), *cupB* (PA4081–PA4086) and *cupC* (PA0992, PA0993, PA0994) in *P. aeruginosa* PAO1, have been demonstrated to play a role in different stages of biofilm formation on biotic and abiotic surfaces (140). Regulation of the *cup* genes is complex, involving a phase variation-dependent repression of *cupA* expression by an H-NS member MvaT (141,142). MvaT also regulates the *cupB* and *cupC* loci to a lesser extent (141). The *cupB* and *cupC* clusters are under the primary regulation of the RocS1–RocR–RocA1 (PA3946–PA3948) three-component system (143). This system is similar to the *Bordetella pertussis* BvgASR system (144) and consists of the hybrid sensor kinase RocS1, the response regulator RocA1 and the RocA1-repressor RocR (143,145). RocR has been hypothesized to bind to c-di-GMP through its EAL (diguanylate phosphodiesterase) domain and prevents phosphotransfer from RocS1 to RocA1, thus preventing RocA1 activation (143,145). *Pseudomonas aeruginosa* PA14 has a fourth *cup* cluster (*cupD*) on the pathogenicity island PAPI-I, which is controlled positively by the response regulator RcsB (PA4080) and negatively by the EAL-domain containing response regulator PvrR (146). In addition, diguanylate cyclases and phosphodiesterases of the *wsp* gene cluster (PA3702–PA3708) (147,148) MorA (PA4601) (149) and TpbA–TpbB (PA3885, PA1120) (150) modulate intracellular levels of c-di-GMP to exert a regulatory effect on the *cup* gene clusters (Figure 1).

The main components of the extracellular polymeric substance matrix of biofilms are Pel and Psl polysaccharides, alginate and free DNA (12,136). Both *pel* and *psl* gene loci are post-transcriptionally regulated by the RetS–LadS (PA4856 and PA3974, respectively) system through *rsmY* and *rzmZ* (21,22,121) and by c-di-GMP levels, either directly (147) or by binding the transcriptional regulator FleQ (PA1097) (151). The *pel* operon is also repressed by the *las* QS system through the tyrosine phosphatase TpbA (PA3885) (150). A membrane-bound sensor, PpyR (PA2663) enhances biofilm formation through the *psl* operon and virulence through modulating QS (152). Although alginate is a major component of

biofilms and affects biofilm structure, it is not essential for biofilm formation (136). Alginate regulation is discussed in a separate section (see later in the text). QS regulates cell lysis in biofilms (153–155), thereby controlling the release of extracellular DNA, a major component of the biofilm matrix (156,157). The QS system also regulates rhamnolipid production (67) that promotes motility and, hence, formation of the cap in the mushroom structure of mature biofilms (158), and maintenance of biofilm channels (159). BfiRS (PA4196–PA4197), BfmRS (PA4101–PA4102) and MifR (PA5511) are TCSs shown to regulate biofilm development and maturation by sequential phosphorylation (160). They activate biofilm formation at different transition stages, reversible to irreversible attachment (BfiRS), irreversible attachment to maturation stage-1 (BfmRS) and maturation stage-1 to mushroom structure formation (MifR) (Figure 1) (160). The BfiRS system may function in conjunction with the GacSA TCS and feed into the Rsm loop of regulation to control biofilm formation (160). SagS (PA2824), the cognate sensor of HptB (PA3345), modulates biofilm development (by controlling BifS phosphorylation) and other virulence phenotypes (by modulating rgsRmZ levels) depending on whether the cells are in the planktonic or biofilm phase (161).

Analyses of clinical isolates reveal a positive correlation between expression of *lasR*, *rhlR* and acute virulence factors (162,163), suggesting that QS is required for virulence *in vivo*. QS is also important when *P. aeruginosa* grows as biofilms in the CF lung (164). *In vivo* studies show that *lasI* and *rhlII* mutants produce milder chronic lung infections compared with their wild-type counterparts (165) and form more susceptible biofilms (166). However, in some *in vitro* studies, there was no apparent difference in the biofilms formed by the QS mutants and the wild-type strains (167,168). This discrepancy in the requirement of QS for biofilm formation and establishment of a successful chronic infection is probably not surprising, as QS regulates many different functions. Further, it has been demonstrated that the CF environment selects for strains with *lasR* mutations, although the *rhl* system is intact (169). Although *lasR* is higher up in the QS hierarchy, studies have shown that secondary mutations can re-establish *rhl* expression in *las* mutants (170). This suggests that in CF biofilms, the *rhl* system is more important, and *lasR* inactivation serves to downregulate the acute virulence factors (171).

A major cause of antibiotic resistance in biofilms has recently been attributed to the phenomenon of persistence. Persister cells are small subpopulations of antibiotic-sensitive cells that have acquired transient antibiotic tolerance (172). When the antibiotic levels drop, the persisters grow into a population of sensitive cells, again with a small sub-population of persisters (173,174). Many genes involved in the formation of persisters have been identified in *P. aeruginosa* PA14, including two transcriptional regulators AlgR (PA5261) and PilH (PA0409) (175). However, this topic is outside the scope of this review but has been extensively reviewed elsewhere (176,177).

Alginate production

In the lungs, especially in patients with CF, *P. aeruginosa* can convert from a non-mucoid to an alginate-overproducing mucoid phenotype signalling chronic infection (178). Chronic *P. aeruginosa* infection seems to be localized to foci within the anaerobic mucus environment in the lung's respiratory zone (179–182). These foci lead to tissue damage decreasing lung function, and the appearance of the mucoid phenotype correlates with poor patient prognosis (183,184). The exopolysaccharide alginate is a linear polymer of β -D-mannuronic acid and α -L-guluronic acid (185), which stimulates production of IgG and IgA antibodies (186). Although production of alginate is metabolically taxing, it protects the bacteria from phagocytosis and antibodies, thus conferring a survival advantage (187,188). Conversion to mucoidy occurs when biofilms are treated with activated polymorphonuclear leucocytes (189), hydrogen peroxide (189), antibiotics (190) and nutrient starvation (191,192).

A complex regulatory pathway controls alginate biosynthesis. The central player is the σ^E family extracytoplasmic function σ factor AlgT/U (PA0762) (193,194), whose activity is inhibited post-transcriptionally by the anti- σ factor MucA (PA0763) and by MucB (PA0764) (195–197). Loss of function mutations in *mucA* or *mucB* result in a mucoid phenotype (195,198,199) because of release of AlgT/U from MucA by a regulated intramembrane proteolytic pathway [reviewed in (200,201)]. It was recently demonstrated that MucA proteolysis is regulated not only by AlgW (PA4446) but also by MucD (PA0766) by activating the MucP protease (PA3649) (202). In addition, AmpR links alginate production with antibiotic resistance and QS by negatively regulating *algT/U* expression (Figure 1) (110). AlgT/U regulates alginate production at least in part by autoregulation (193), controlling expression of the transcriptional regulators *algR* (203,204), *algB* (198,204), *amrZ* (205) and the *algD* (PA3540) alginate biosynthetic operon (204,206,207). AlgB (208), AlgR (43,209,210) and AmrZ (211) directly bind to the *algD* operon to activate transcription. The alternative σ factor RpoN (PA4462) is also required for high levels of *algT/U* and *algD* expression (212).

The *algB* (PA5483) and *algR* (PA5261) genes encode NtrC and LytR subfamily, respectively, of TCS RRs (213). Interestingly, aspartic acid phosphorylation in the regulatory domain is not essential for alginate production (214). Transcriptome analysis of a PAO*mucA22* mucoid strain (PDO300) (196) identified seven predicted transcriptional regulators, PA1235, PA1261, PA1637 (KdpE), PA2881, PA3420, PA3771 and PA5431, and one sensor kinase, EraS (PA1979), whose expression was down-regulated in an *algB* mutant but not in a strain containing a mutation in its cognate TCS sensor, KinB (PA5484) (208). In addition to regulating the *algD* operon, AlgR directly activates transcription of *algC* (PA5322), which encodes a phosphomannomutase/phosphoglucomutase essential for Psl, alginate and rhamnolipid synthesis (Figure 1) (215–218). AlgR also is important for mature biofilm formation, possibly by directly repressing *rhl*-QS (219), type IV pilus formation by binding to the

fimTU-pilVWXYIY2E promoter (220,221) and hydrogen cyanide production by binding to the *hcnA* (PA2193) promoter (222). Interestingly, in contrast to alginate production, the phosphorylation site is required for regulating cyanide production and twitching motility (220,223). AlgR has also been shown to indirectly regulate the cyclic AMP/Vfr-dependent pathway (224). The AlgR regulon has been characterized by several transcriptome studies (219,222,225). Two other regulators of alginate production in *P. aeruginosa* are Alg44 (PA3542) (226) and a diguanylate cyclase, MucR (PA1727) (227). MucR produces a pool of c-di-GMP in the vicinity of the PilZ domain of Alg44 (PA3542), which then positively regulates alginate production (Figure 1) (226–228).

Regulation of iron uptake

Iron is critical for growth of all organisms, and *P. aeruginosa* is no exception. Transcriptome studies reveal that a large number of genes are regulated in response to iron (229,230). Biologically useful iron (Fe^{2+}) in the environment is scarce and is available mostly in the insoluble Fe^{3+} form. To help scavenge this free iron, bacteria produce siderophores that bind extracellular iron and transport them back into the cell through TonB-dependent receptors on the cell surface (231). *Pseudomonas aeruginosa* produces two siderophores, pyoverdine and pyochelin, and can also subvert siderophores produced by other organisms to take up haem (232,233). However, excess free iron in the cell leads to formation of toxic reactive oxygen species, and, therefore, cells tightly regulate the uptake (234). The ferric uptake regulator (Fur, PA4769) is a conserved protein in *P. aeruginosa* and other Gram-negative bacteria, and it is a major iron acquisition regulator (235). Fur dimerizes rapidly after synthesis, and it takes a minimum of two dimers to bind promoters of genes under Fur regulation in *P. aeruginosa* (236). Fur controls the iron regulon directly by binding the Fur box (237) and indirectly by modulating expression of other regulators, including the pyochelin uptake regulator PchR (PA4227), ECF σ factors like PvdS, TCS regulators and small regulatory RNAs [asPrrF1 (PA4704.1), asPrrF2 (PA4704.2); Figure 1] (237–239).

Iron concentrations in the cell also influence expression of virulence factors in *P. aeruginosa*. PvdS, for example, is critical in linking iron and virulence by controlling the production of pyoverdine, an outer membrane pyoverdine receptor [FpvA (PA2398)] and two important extracellular virulence factors [PrpL (PA4175) and exotoxin A (PA1148); Figure 1] (240–242). Also, *pvdS* mutants showed reduced virulence in a rabbit endocarditis model (243). Human lactoferrin inhibits *P. aeruginosa* biofilm formation, indicating a role for iron in the process (244). Iron chelation by lactoferrin induces twitching motility in *P. aeruginosa* negating colonization and ultimately, biofilm formation (244,245). Intracellular iron concentrations are one of the signals for biofilm development in a process involving Fur but not the iron uptake regulatory RNAs asPrrF1 and asPrrF2 (246). Further, high levels of

iron suppress the PQS system, release of extracellular DNA and biofilm formation (247).

The link between iron and QS systems in *P. aeruginosa* is complex. QS systems are enhanced under limiting iron concentrations (85,248,249), and major QS regulators are also involved in regulating iron responsive genes (75,250,251). MvfR, for example, has been demonstrated to control transcription of iron-related genes, and it has an iron-starvation box in its promoter, a site recognized by PvdS (PA2426) to turn on transcription under low-iron concentrations (229,252). It was recently demonstrated that iron levels affect activity of the MvfR signalling molecule HAQ, adding another layer of complexity to the role of iron in QS (253). Another major QS and virulence regulator, VqsR regulates phenazine production by modulating *phnAB* expression (94). Moreover, the small regulatory RNAs asPrrF1 and asPrrF2, which are negatively regulated by Fur, positively regulate PQS production (Figure 1) (254). PQS has been shown to accumulate in the outer membrane and in membrane vesicles (77). PQS chelates iron, and this facilitates pyochelin and pyoverdinin in scavenging iron (73,76).

Thus, iron uptake regulation in *P. aeruginosa* is a complex affair and involves multiple regulators that affect expression of numerous genes either by themselves or through other regulators. In addition, the interconnections between iron uptake mechanisms and other virulence systems, such as QS and biofilm formation, demonstrate the versatility of this bacterium in being able to pragmatically read environmental signals to accordingly modulate gene expression.

Toxins and exoproteins

Exoproteins are an important component of bacterial survival not only because they allow the bacteria to interact with their immediate environment and other organisms in the vicinity but also because they play a critical role in virulence. *P. aeruginosa* has a large complement of secreted proteins and five (type I, II, III, V and VI) of the seven secretion systems characterized in bacteria (255). A majority of the secreted proteins are toxins that aid in *P. aeruginosa* virulence, most of which, including LasA, LasB, PrpL, ToxA and phospholipases [PlcH (PA0844), PlcN (PA3319), PlcB (PA0026)], is secreted through the Xcp type II secretion system (T2SS) (255). Effector molecules that are crucial for evading the host phagocytic response are secreted through a dedicated T3SS (256), whereas the type I system (T1SS) secretes the alkaline protease AprA (257,258) and the haemophore HasAp (PA3407) (259). Substrates of the recently identified T6SS are just being discovered (260). In addition, c-di-GMP levels, modulated by the diguanylate cyclase WspR (PA3702) is involved in the switch between T3SS and T6SS independent of RetS but is dependent on rgRsmY and rgRsmZ (Figure 1) (261).

HasAp, a T1SS-secreted haem-uptake protein in *P. aeruginosa*, is under QS control (250). QS is also known to regulate PrpL that targets the human lactoferrin (see previous section) (240). Thus, QS in *P. aeruginosa* not only regulates enzymes to degrade the human lactoferrin

but also produces proteins to retrieve the iron from the degraded lactoferrin. The other known T1SS substrate, AprA, is regulated by a novel LTTR named BexR (PA2432) that controls bistability in *P. aeruginosa* (Figure 1) (262). Inactivation of QS has been demonstrated to reduce expression of T2SS-secreted proteases, chitinases and lipases (63,263) because of downregulation of the Xcp T2SS (264,265). The TCS PhoBR (PA5360–PA5361) regulates other T2SS-secreted exoproteins, such as PlcH, PlcC, PlcN and the Hxc T2SS secreted alkaline phosphatase LapA (266,267). Microarray analysis revealed that a novel cell-surface signalling system PUMA3 regulates Hxc T2SS genes (268). The three T6SS systems (HSI-I, HSI-II and HSI-III) in *P. aeruginosa* are differentially regulated by the QS systems (269). Although LasR and MvfR negatively regulate the HSI-I system, they positively regulate expression of the functionally redundant HSI-II and HSI-III (Figure 1) (269). In addition, a putative regulator Sfa3 (SfnR) in *P. aeruginosa* PA14 (an orthologue of PA2359 in PAO1) potentially regulates the HSI-III cluster (269). HSI-I expression is also regulated by RetS through RsmA (270).

Pseudomonas aeruginosa T3SS is regulated in a complex and multi-tiered process, and it is probably the most well understood (271). Expression of T3SS is regulated transcriptionally and post-transcriptionally in response to host cell contact and environmental Ca^{2+} levels (272,273). ExsA (PA1713), an AraC member, regulates expression of the 43 genes that form the T3SS in *P. aeruginosa* by binding as a monomer to an A-rich 8-bp region upstream of the -35 in the promoter of genes under its regulation (272,47). ExsA autoregulates its own expression and is also activated by PsrA (PA3006), a member of the TetR family (274,275). Two anti-activators [ExsD (PA1714) and PtrA (PA2808)] also regulate ExsA-mediated activation (Figure 1). T3SS transcription is coupled to secretion and involves the anti-activator ExsD and the anti-anti-activator ExsC (PA1710) that regulate ExsA function. Under non-inducing conditions (high Ca^{2+}), ExsE (PA1711) binds the anti-anti-activator ExsC, allowing the anti-activator ExsD to bind ExsA and inhibit transcription. Under Ca^{2+} delimiting conditions, ExsE is secreted, freeing ExsC to bind ExsD. Free ExsA then activates T3SS expression (Figure 1) (276). Although this is the primary mode of control, T3SS can also be triggered by stress because of DNA damage (RecA-mediated activation of PtrB) (277), high salt (278,279), metabolic stress (123,279,280), alginate regulators AlgT/U, AlgR and MucA (281), the MexEF–OprN efflux pump regulator MexT (PA2492) through PtrC (282) and the RetS/LadS/Gac-Rsm TCSs (21,22,283). Under low oxygen conditions, the anaerobic regulator Anr activates the response regulator NarL (PA3879), which in turn represses rgRsmY and rgRsmZ expression, allowing RsmA to activate T3SS (284). Expression of T3SS, however, happens only in a subset of the population even under inducing conditions (279,285). Multiple levels of control allow fine-tuning of T3SS expression, allowing *P. aeruginosa* to sense various environmental conditions

and regulate expression in conjunction with other virulence factors.

Regulatory RNAs in *P. aeruginosa* virulence

RNAs other than messenger RNAs, transfer RNAs or ribosomal RNAs are termed small RNAs (sRNAs), and they affect all steps in gene expression pathways in both prokaryotes and eukaryotes (286). In general, sRNA-mediated regulation occurs in one of two ways, base pairing with DNA or mRNA, or by affecting the activity of a protein or protein complex (286). Not surprisingly, virulence gene expression in *P. aeruginosa* also relies on small rgRNA-mediated post-transcriptional regulation. The importance of rgRNAs in regulation of bacterial virulence is well established (39,287,288). Most of the *P. aeruginosa* rgRNAs that have been characterized play a role in virulence gene regulation (discussed later in the text). A recent study identified ~150 novel sRNAs using sRNA-Seq in *P. aeruginosa* PAO1 and PA14, which includes both strain-specific and shared ones (289).

Perhaps the most well characterized system involves the rgRNAs, rgRsmY and rgRsmZ (whose roles in virulence regulation have been discussed in the 'TCS' and 'Toxins and Exoproteins' sections). These are two functionally redundant rgRNAs in *P. aeruginosa* that play a critical role in the switch between acute and chronic infections (21,290). GacA of the GacSA TCS positively regulates expression of rgRsmY and rgRsmZ, which then bind to and sequester the sRNA-binding protein RsmA through the GGA motif (291–293), leading to derepression of the genes that RsmA represses (107,124,294,295). The consequences of RsmA sequestration result in dysregulation of the expression of numerous virulence factors, as discussed in the 'TCS' section. Given the importance of this regulatory process in *P. aeruginosa* pathogenesis, regulation of expression of rgRsmY and rgRsmZ is multi-tiered. The histidine phosphotransfer protein HptB is phosphorylated by a phosphorelay involving the three sensor kinases PA2824, PA1611 and PA1976 (296). Phosphorylated HptB then transfers the phosphate to an anti-anti- σ factor PA3374, to negatively regulate expression of *rsmY* (296,297). In another mode of regulation, the BfiSR TCS activates expression of the ribonuclease CafA (PA4477), which specifically targets rgRsmZ (298). Further regulation is achieved by the global regulators of the H-NS family of proteins MvaT and MvaU, which bind to AT-rich regions upstream of the *rsmZ* gene repressing their expression (299). In addition to all this, there is a negative autoregulatory feedback mechanism, the details of which have not been elucidated yet (300). On synthesis, rgRsmY is stabilized by Hfq binding, either alone or in conjunction with RsmA (108,301).

Another example of post-transcriptional regulation by sequestering a RNA-binding protein links virulence with metabolism. *Pseudomonas aeruginosa* Crc (PA5332) is a RNA-binding protein that recognizes CA-motifs around the ribosome binding sites of the mRNA of carbon compound catabolism genes. Crc thus represses genes whose products help utilize less preferred carbon sources (302–304). When less preferred substrates, such as

mannitol, have to be utilized, expression of catabolic genes is achieved by sequestration of Crc by the rgRNA, rgCrcZ (305). Expression of rgCrcZ is under the control of the TCS CbrAB (PA4725–PA4726), which in conjunction with Crc plays a role in carbon compound catabolism, biofilm formation, antibiotic resistance, secretion systems and swarming (306–312).

Pseudomonas aeruginosa antisense sRNAs (asRNAs) can also act by base pairing with target mRNAs, thus inhibiting translation (313). One such example is asPhrS (PA3305.1), which plays a role in PQS and pyocyanin expression (314). Transcriptome studies indicate an extensive overlap between the genes that are positively regulated by the transcriptional regulator PqsR (also known as MvfR, PA1003) and asPhrS, suggesting that asPhrS regulates *pqsR* mRNA (75,314). Interestingly, it was shown that asPhrS specifically targets a region in the RBS of a small ORF (*uof*), which is present upstream of PqsR (314). As translation of *pqsR* and *uof* are coupled, asPhrS regulates *pqsR* translation by modulating translation of *uof* (314). Expression of asPhrS is under the control of the oxygen responsive regulator Anr (314). Hfq controls asPhrS expression indirectly by regulating Anr expression, whose mechanism of action is yet to be elucidated (314,315).

Small asRNAs also play a role in regulation of iron uptake and involve base pairing by the sRNAs asPrrF1 and asPrrF2, which are the *P. aeruginosa* orthologues of *E. coli* RyhB (237,316). Expression of asPrrF1 and asPrrF2 is repressed by Fur when iron concentrations are high (237). Under iron-starvation conditions, asPrrF1 and asPrrF2 are expressed and base pair with the mRNA of target genes, which include the superoxide dismutase *sodB* (PA4366), genes involved in the trichloroacetic acid cycle and anthranilate and catechol degradation (317). Thus, asPrrF1 and asPrrF2 link carbon metabolism, iron uptake and QS-mediated virulence. Another asRNA gene asPrrH is located in the same locus as asPrrF1 and asPrrF2. The asPrrH asRNA (at 325 nt) is longer than asPrrF1 (116 nt) and asPrrF2 (114 nt), and the coding region of asPrrH overlaps with the asPrrF1 terminator, the intergenic region between asPrrF1 and asPrrF2 and the 5'-end of the asPrrF2 ORF (318). The expression of asPrrH is maximal in the stationary phase of growth, similar to asPrrF1 and asPrrF2, and under iron-deplete conditions (318). Haem represses asPrrH expression, and this involves the outer membrane haem receptors PhuR (PA4710) and HasR (PA3408) (318). Interestingly, under conditions of haem starvation, asPrrH expression leads to the repression of *achAB* and *sdhCDAB*, which are also targets of the PrrF asRNAs (318). In addition to these targets, asPrrH also represses NirL, a protein involved in biosynthesis of haem, under haem and iron limitation (318).

CONCLUSIONS AND PERSPECTIVES

Pseudomonas aeruginosa is a versatile bacterium that can thrive in a wide range of habitats. This is achieved by an intricately interlinked regulatory system of transcriptional regulators, σ factors, sRNAs and their regulons.

The exquisite control of gene expression is exemplified in the virulence regulatory network (Figure 1), which demonstrates that none of the virulence mechanisms are isolated. Expression of individual virulence networks is under transcriptional and post-transcriptional regulation of multiple-regulatory systems, either directly or indirectly. Furthermore, some signalling cascades inversely regulate the acute and chronic virulence phenotypes depending on the signals sensed. The next critical phase of research should focus on the signals that the bacteria recognizes to achieve gene regulation.

The extent of cross-regulation between the transcriptional regulators highlights the global nature of the regulation, where individual subnetworks (such as the QS network, alginate network and so forth) are interlinked to form a hyperconnected network (Figure 1). Given the complexity of the connections, one can expect the response of a cell to be elaborate even when faced with a simple stress condition. A fundamental point in a network setting is that one should evaluate the role of individual players (such as a regulator) not in isolation, but with the knowledge that the entire network will react to what it does. In other words, local changes can have global effects. This, in turn, results in subtle cause-effect relationships. Studying the functions of a regulator by generating deletion or overexpression strains is often performed under the assumption that other regulators will remain static. In reality, however, such modifications can lead to aberrant changes across the network in ways that were initially unintended. Moreover, there is a possibility that such changes can occur because the network connections might not always be obvious. This can be attributed, in part, to as yet unelucidated implicit players in the network that dictate or otherwise influence cellular response. This is a likely explanation for the many 'global' regulators in *P. aeruginosa* and in similar bacteria that have complex regulatory networks. In such cases, many of the phenotypes observed with single regulator mutant strains can be part of a ripple effect that propagates through the network affecting disparate phenotypes.

A simplified model of gene regulatory network treats genes as being on or off, that is, taking binary values. It is, therefore, no surprise that Boolean networks (discrete dynamical network models) have been used to model and study gene regulatory circuits (319–321). Probabilistic Boolean networks, which take into account molecular and genetic noise (322,323), and stochastic Boolean networks, which permit the modelling of gene perturbations (324), provide important insights into the dynamical behaviour of the system. Although they are computationally complex, they are a valuable addition to the numerous other programs that are available to analyse gene regulatory networks (31,325,326).

Dynamical systems theory helps us to analyse the behaviour of complex systems that can frequently be expressed by time-differential equations. When the behaviour of a dynamical system depends sensitively on small changes in initial conditions, then the system is said to be chaotic, that is, capable of exhibiting chaotic behaviour. Researchers have investigated whether regulatory

networks can have subsystems that are capable of exhibiting chaotic behaviour (327). It has been shown that competition between two or more subnetworks of comparable importance can lead to chaos (328–330). In fact, chaos has been shown to be possible in biochemical systems with only two feedback loops, and positive feedback is known to be necessary for chaotic behaviour (331). So, one would expect chaotic subsystems in a regulatory network as complex as the one that controls *P. aeruginosa* virulence (Figure 1). Despite of this predisposition, gene regulatory networks seldom exhibit chaotic behaviour. This could be because the competitions between opposing nodes are not strong enough (332) or that chaotic behaviours are short-lived because of triggering of other pathways, such as cell-cell communication (333). Another possibility is that the natural random variability of biochemical systems masks the chaotic behaviour (332). However, maintaining a low level of chaos in such a complex network is probably a combination of the aforementioned and, potentially, as yet unknown factors.

In gene regulatory networks, a particular dynamical system is characterized by time-evolving variables (chemical concentrations, gene expression and so forth) and by parameters (temperature, ambient chemical concentrations and so forth). A network can exhibit chaotic or non-chaotic behaviour depending on the parameters that influence it (334). Environmental factors, such as the temperature or the nutritional status of the cells, parameterize the relationship between transcription factors and the genes that they regulate. Although it is understood that some choices of parameters can induce chaotic behaviours, the parameter may not even be achievable, such as high temperatures (334). Mutations can also alter relationships in regulatory networks by causing changes in existing links or forming new ones. In a dynamically robust (non-chaotic) system, small finite changes in the parameters lead to only qualitative changes in the dynamical behaviour. However, there are boundaries in the parameter space where the behaviour of the system changes qualitatively and may include the possibility of chaotic dynamics. Predicting whether a network will be stable or chaos-prone under some conditions has been proven to be difficult and remains poorly characterized. Recent work has identified the minimum number, types and interactions among three and four nodes/subnetworks that can lead to chaos in a gene regulatory network (332). Such minimal subnetworks have been termed 'chaotic motifs', and networks with these motifs can exhibit chaotic behaviour under the right parameters (332). Analysis of the network in Figure 1 does not readily show such chaotic motifs. This could be because the network is incomplete (lack of data on the interactions among the *P. aeruginosa* regulators) or because of errors in the inferred interactions. Although *P. aeruginosa* virulence regulation has been extensively studied, there is yet much to learn. Thus, absence of empirical evidence does not preclude a propensity to chaos and is worth further investigation.

Depending on an elaborate network to achieve gene regulation is likely an adaptive mechanism by

P. aeruginosa. Possessing alternate pathways to regulate the same phenotype ensures a rapid response to stimuli even if one of the pathways is affected, thus enhancing survival. Such examples can be seen throughout the network. As discussed in the 'Toxins and Exoproteins' section, expression of T3SS genes can be regulated at multiple levels, in response to various different signals and stress conditions, and it is not entirely dependent on any one signal. However, the extent of contributions of the individual regulators and, consequently, the fine balance that exists in some regulatory cascades within the network, are sometimes not easily apparent. Network dependence is also a probable reason for regulator genes being non-essential, in the sense that deleting a transcriptional regulator gene typically does not affect cell viability because of the presence of alternate regulatory mechanisms. Having key regulators modulate different related phenotypes has the added advantage in allowing the cells to adapt to external signals by modulating one or a few regulators instead of individually regulating different virulence systems. A case in point is AmpR that positively regulates acute virulence factors while downregulating chronic infection phenotypes (23). Also of importance is the co-regulation of metabolism and virulence. Studies have identified regulators like CbrB that, with its cognate sensor CbrA, not only regulate carbon metabolism but also virulence phenotypes through the rgRNA, rgCrcZ and the RNA-binding protein Crc (306,307). Moreover, there is crosstalk between CbrA and regulators other than CbrB, highlighting the complexity of the system (306).

The plethora of transcriptome studies using microarrays or deep sequencing will add to the database of genes that are differentially expressed in response to regulator mutations or specific growth conditions. Differentiating the direct effect of a change from a ripple effect can, at least partly, be achieved by meta-analysis studies that look at multiple transcriptomes, identifying effects unique to each condition and differentiating them from the so-called ripple (32,33). Network analyses can help us understand the relationship between different regulators, group them based on function and, more importantly, help identify critical nodes and prominent players. This can serve as a means of target identification in attempting to deal with *P. aeruginosa* infections. In Figure 1, we see that some parts of the network are more densely connected than others, with central cores containing most of the links. A case in point is LasR of the QS subnetwork. It is well known that QS is central to virulence regulation in *P. aeruginosa* and targeting key regulators will have a better chance of therapeutic success. Recently, inhibitors of a key QS regulator were shown to reduce pathogenicity in *Vibrio cholera* (335).

With the extensive use of high-throughput transcriptomics, gene regulation studies are now focusing on the role of non-coding RNAs in bacteria. rgRNAs have been shown to be extensively involved in gene regulation in *P. aeruginosa* and other bacteria (124,237,299,305,314,336). Techniques such as RNA-seq allow for the entire transcriptome to be sequenced, giving us an unprecedented insight into non-coding RNAs, asRNAs and

sRNAs involved in regulation. Preliminary studies using prediction software and complementary experiments have already advanced our understanding (29,108,124,299,314,337). Given the many different ways in which small RNAs can modulate gene expression (313) and potentially undiscovered ones, we can look forward to exciting new discoveries in bacterial gene regulation in the coming years.

ACKNOWLEDGEMENTS

The authors are extremely grateful to Giri Narasimhan (Florida International University) and Edward Celarier (NASA Goddard) for extensive discussions and critical comments on chaos theory.

FUNDING

National Institutes of Health-Minority Medical Research Support SCORE [S06 GM08205 and 5SC1AI081376 to K.M.]; FIU Research Assistantship (Herbert Werthiem College of Medicine to D.B.). Funding for open access charge: National Institutes of Health-Minority Medical Research Support SCORE [5SC1AI081376 to K.M.].

Conflict of interest statement. None declared.

REFERENCES

- Rahme,L.G., Ausubel,F.M., Cao,H., Drenkard,E., Goumnerov,B.C., Lau,G.W., Mahajan-Miklos,S., Plotnikova,J., Tan,M.W., Tsongalis,J. *et al.* (2000) Plants and animals share functionally common bacterial virulence factors. *Proc. Natl Acad. Sci. USA*, **97**, 8815–8821.
- Mahajan-Miklos,S., Rahme,L.G. and Ausubel,F.M. (2000) Elucidating the molecular mechanisms of bacterial virulence using non-mammalian hosts. *Mol. Microbiol.*, **37**, 981–988.
- Kerr,K.G. and Snelling,A.M. (2009) *Pseudomonas aeruginosa*: a formidable and ever-present adversary. *J. Hosp. Infect.*, **73**, 338–344.
- Valderrey,A.D., Pozuelo,M.J., Jimenez,P.A., Macia,M.D., Oliver,A. and Rotger,R. (2010) Chronic colonization by *Pseudomonas aeruginosa* of patients with obstructive lung diseases: cystic fibrosis, bronchiectasis, and chronic obstructive pulmonary disease. *Diagn. Microbiol. Infect. Dis.*, **68**, 20–27.
- Bouza,E., Burillo,A. and Munoz,P. (2002) Catheter-related infections: diagnosis and intravascular treatment. *Clin. Microbiol. Infect.*, **8**, 265–274.
- Manfredi,R., Nanetti,A., Ferri,M. and Chiodo,F. (2000) *Pseudomonas* spp. complications in patients with HIV disease: an eight-year clinical and microbiological survey. *Eur. J. Epidemiol.*, **16**, 111–118.
- Mizuno,T. (1997) Compilation of all genes encoding two-component phosphotransfer signal transducers in the genome of *Escherichia coli*. *DNA Res.*, **4**, 161–168.
- Fabret,C., Feher,V.A. and Hoch,J.A. (1999) Two-component signal transduction in *Bacillus subtilis*: how one organism sees its world. *J. Bacteriol.*, **181**, 1975–1983.
- Gooderham,W.J. and Hancock,R.E. (2009) Regulation of virulence and antibiotic resistance by two-component regulatory systems in *Pseudomonas aeruginosa*. *FEMS Microbiol. Rev.*, **33**, 279–294.
- Buelow,D.R. and Raivio,T.L. (2010) Three (and more) component regulatory systems - auxiliary regulators of bacterial histidine kinases. *Mol. Microbiol.*, **75**, 547–566.
- Ng,W.L. and Bassler,B.L. (2009) Bacterial quorum-sensing network architectures. *Annu. Rev. Genet.*, **43**, 197–222.

12. Harmsen, M., Yang, L., Pamp, S.J. and Tolker-Nielsen, T. (2010) An update on *Pseudomonas aeruginosa* biofilm formation, tolerance, and dispersal. *FEMS Immunol. Med. Microbiol.*, **59**, 253–268.
13. Njoroge, J. and Sperandio, V. (2009) Jamming bacterial communication: new approaches for the treatment of infectious diseases. *EMBO Mol. Med.*, **1**, 201–210.
14. Burns, J.L., Gibson, R.L., McNamara, S., Yim, D., Emerson, J., Rosenfeld, M., Hiatt, P., McCoy, K., Castile, R., Smith, A.L. *et al.* (2001) Longitudinal assessment of *Pseudomonas aeruginosa* in young children with cystic fibrosis. *J. Infect. Dis.*, **183**, 444–452.
15. Hogardt, M. and Heesemann, J. (2012) Microevolution of *Pseudomonas aeruginosa* to a chronic pathogen of the cystic fibrosis lung. *Curr. Top. Microbiol. Immunol.*, Feb 8 (doi: 10.1007/82_2011_199; epub ahead of print).
16. Coggan, K.A. and Wolfgang, M.C. (2012) Global regulatory pathways and cross-talk control *Pseudomonas aeruginosa* environmental lifestyle and virulence phenotype. *Curr. Issues Mol. Biol.*, **14**, 47–70.
17. Hoboth, C., Hoffmann, R., Eichner, A., Henke, C., Schmoldt, S., Imhof, A., Heesemann, J. and Hogardt, M. (2009) Dynamics of adaptive microevolution of hypermutable *Pseudomonas aeruginosa* during chronic pulmonary infection in patients with cystic fibrosis. *J. Infect. Dis.*, **200**, 118–130.
18. Hogardt, M., Hoboth, C., Schmoldt, S., Henke, C., Bader, L. and Heesemann, J. (2007) Stage-specific adaptation of hypermutable *Pseudomonas aeruginosa* isolates during chronic pulmonary infection in patients with cystic fibrosis. *J. Infect. Dis.*, **195**, 70–80.
19. Oliver, A. and Mena, A. (2010) Bacterial hypermutation in cystic fibrosis, not only for antibiotic resistance. *Clin. Microbiol. Infect.*, **16**, 798–808.
20. Hasset, D.J., Sutton, M.D., Schurr, M.J., Herr, A.B., Caldwell, C.C. and Matu, J.O. (2009) *Pseudomonas aeruginosa* hypoxic or anaerobic biofilm infections within cystic fibrosis airways. *Trends Microbiol.*, **17**, 130–138.
21. Goodman, A.L., Kulasekara, B., Rietsch, A., Boyd, D., Smith, R.S. and Lory, S. (2004) A signaling network reciprocally regulates genes associated with acute infection and chronic persistence in *Pseudomonas aeruginosa*. *Dev. Cell*, **7**, 745–754.
22. Ventre, I., Goodman, A.L., Vallet-Gely, I., Vasseur, P., Soscia, C., Molin, S., Bleves, S., Lazdunski, A., Lory, S. and Filloux, A. (2006) Multiple sensors control reciprocal expression of *Pseudomonas aeruginosa* regulatory RNA and virulence genes. *Proc. Natl Acad. Sci. USA*, **103**, 171–176.
23. Balasubramanian, D., Schnepfer, L., Merighi, M., Smith, R., Narasimhan, G., Lory, S. and Mathee, K. (2012) The regulatory repertoire of *Pseudomonas aeruginosa* AmpC β -lactamase regulator AmpR includes virulence genes. *PLoS One*, **7**, e34067.
24. Stover, C.K., Pham, X.Q., Erwin, A.L., Mizoguchi, S.D., Warriner, P., Hickey, M.J., Brinkman, F.S., Hufnagle, W.O., Kowalik, D.J., Lagrou, M. *et al.* (2000) Complete genome sequence of *Pseudomonas aeruginosa* PA01, an opportunistic pathogen. *Nature*, **406**, 959–964.
25. Mathee, K., Narasimhan, G., Valdes, C., Qiu, X., Matewish, J.M., Koehrsen, M., Rokas, A., Yandava, C.N., Engels, R., Zeng, E. *et al.* (2008) Dynamics of *Pseudomonas aeruginosa* genome evolution. *Proc. Natl Acad. Sci. USA*, **105**, 3100–3105.
26. Winsor, G.L., Lam, D.K., Fleming, L., Lo, R., Whiteside, M.D., Yu, N.Y., Hancock, R.E. and Brinkman, F.S. (2011) *Pseudomonas* genome database: improved comparative analysis and population genomics capability for *Pseudomonas* genomes. *Nucleic Acids Res.*, **39**, D596–D600.
27. Balasubramanian, D., Murugapiran, S.K., Silva-Herzog, E., Schnepfer, L., Yang, X., Tatke, G., Narasimhan, G. and Mathee, K. (2013) In: Babu, M.M. (ed.), *Bacterial Gene Regulation and Transcriptional Networks*. Caister Academic Press, United Kingdom.
28. Potvin, E., Sanschagrin, F. and Levesque, R.C. (2008) Sigma factors in *Pseudomonas aeruginosa*. *FEMS Microbiol. Rev.*, **32**, 38–55.
29. Livny, J., Brenic, A., Lory, S. and Waldor, M.K. (2006) Identification of 17 *Pseudomonas aeruginosa* sRNAs and prediction of sRNA-encoding genes in 10 diverse pathogens using the bioinformatic tool sRNAPredict2. *Nucleic Acids Res.*, **34**, 3484–3493.
30. Galan-Vasquez, E., Luna, B. and Martinez-Antonio, A. (2011) The regulatory network of *Pseudomonas aeruginosa*. *Microb. Inform. Exp.*, **1**, 3.
31. Babu, M.M., Teichmann, S.A. and Aravind, L. (2006) Evolutionary dynamics of prokaryotic transcriptional regulatory networks. *J. Mol. Biol.*, **358**, 614–633.
32. Balasubramanian, D. and Mathee, K. (2009) Comparative transcriptome analyses of *Pseudomonas aeruginosa*. *Hum. Genomics*, **3**, 349–361.
33. Goodman, A.L. and Lory, S. (2004) Analysis of regulatory networks in *Pseudomonas aeruginosa* by genomewide transcriptional profiling. *Curr. Opin. Microbiol.*, **7**, 39–44.
34. Ong, C.T. and Corces, V.G. (2011) Enhancer function: new insights into the regulation of tissue-specific gene expression. *Nat. Rev. Genet.*, **12**, 283–293.
35. Levine, M. (2010) Transcriptional enhancers in animal development and evolution. *Curr. Biol.*, **20**, R754–R763.
36. Bulger, M. and Groudine, M. (2011) Functional and mechanistic diversity of distal transcription enhancers. *Cell*, **144**, 327–339.
37. Ishihama, A. (2010) Prokaryotic genome regulation: multifactor promoters, multitarget regulators and hierarchic networks. *FEMS Microbiol. Rev.*, **34**, 628–645.
38. Storz, G., Vogel, J. and Wassarman, K.M. (2011) Regulation by small RNAs in bacteria: expanding frontiers. *Mol. Cell*, **43**, 880–891.
39. Waters, L.S. and Storz, G. (2009) Regulatory RNAs in bacteria. *Cell*, **136**, 615–628.
40. Reitzer, L.J. and Magasanik, B. (1986) Transcription of *glnA* in *E. coli* is stimulated by activator bound to sites far from the promoter. *Cell*, **45**, 785–792.
41. Popham, D.L., Szeto, D., Keener, J. and Kustu, S. (1989) Function of a bacterial activator protein that binds to transcriptional enhancers. *Science*, **243**, 629–635.
42. Buck, M., Gallegos, M.T., Studholme, D.J., Guo, Y. and Gralla, J.D. (2000) The bacterial enhancer-dependent sigma(54) (sigma(N)) transcription factor. *J. Bacteriol.*, **182**, 4129–4136.
43. Mohr, C.D., Leveau, J.H., Krieg, D.P., Hibler, N.S. and Deretic, V. (1992) AlgR-binding sites within the *algD* promoter make up a set of inverted repeats separated by a large intervening segment of DNA. *J. Bacteriol.*, **174**, 6624–6633.
44. Ramsey, D.M., Baynham, P.J. and Wozniak, D.J. (2005) Binding of *Pseudomonas aeruginosa* AlgZ to sites upstream of the *algZ* promoter leads to repression of transcription. *J. Bacteriol.*, **187**, 4430–4443.
45. Winteler, H.V. and Haas, D. (1996) The homologous regulators ANR of *Pseudomonas aeruginosa* and FNR of *Escherichia coli* have overlapping but distinct specificities for anaerobically inducible promoters. *Microbiology*, **142**, 685–693.
46. Lu, C.D., Winteler, H., Abdelal, A. and Haas, D. (1999) The ArgR regulatory protein, a helper to the anaerobic regulator ANR during transcriptional activation of the *arcD* promoter in *Pseudomonas aeruginosa*. *J. Bacteriol.*, **181**, 2459–2464.
47. Hovey, A.K. and Frank, D.W. (1995) Analyses of the DNA-binding and transcriptional activation properties of ExsA, the transcriptional activator of the *Pseudomonas aeruginosa* exoenzyme S regulon. *J. Bacteriol.*, **177**, 4427–4436.
48. Baraquet, C., Murakami, K., Parsek, M.R. and Harwood, C.S. (2012) The FleQ protein from *Pseudomonas aeruginosa* functions as both a repressor and an activator to control gene expression from the *pel* operon promoter in response to c-di-GMP. *Nucleic Acids Res.*, **40**, 7207–7218.
49. Ochsner, U.A., Vasil, A.I. and Vasil, M.L. (1995) Role of the ferric uptake regulator of *Pseudomonas aeruginosa* in the regulation of siderophores and exotoxin A expression: purification and activity on iron-regulated promoters. *J. Bacteriol.*, **177**, 7194–7201.
50. Wagner, V.E., Bushnell, D., Passador, L., Brooks, A.I. and Iglewski, B.H. (2003) Microarray analysis of *Pseudomonas aeruginosa* quorum-sensing regulons: effects of growth phase and environment. *J. Bacteriol.*, **185**, 2080–2095.
51. Tian, Z.X., Fargier, E., Mac Aogain, M., Adams, C., Wang, Y.P. and O’Gara, F. (2009) Transcriptome profiling defines a novel regulon modulated by the LysR-type transcriptional regulator MexT in *Pseudomonas aeruginosa*. *Nucleic Acids Res.*, **37**, 7546–7559.
52. Xiao, G., He, J. and Rahme, L.G. (2006) Mutation analysis of the *Pseudomonas aeruginosa* *myfR* and *pqsABCDE* gene promoters demonstrates complex quorum-sensing circuitry. *Microbiology*, **152**, 1679–1686.

53. Kojic, M., Aguilar, C. and Venturi, V. (2002) TetR family member *psrA* directly binds the *Pseudomonas rpoS* and *psrA* promoters. *J. Bacteriol.*, **184**, 2324–2330.
54. Nicastro, G.G., Boechat, A.L., Abe, C.M., Kaihama, G.H. and Baldini, R.L. (2009) *Pseudomonas aeruginosa* PA14 *cupD* transcription is activated by the RcsB response regulator, but repressed by its putative cognate sensor RcsC. *FEMS Microbiol. Lett.*, **301**, 115–123.
55. Pearson, J.P., Pesci, E.C. and Iglewski, B.H. (1997) Roles of *Pseudomonas aeruginosa las* and *rhl* quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. *J. Bacteriol.*, **179**, 5756–5767.
56. Dasgupta, N., Ferrell, E.P., Kanack, K.J., West, S.E. and Ramphal, R. (2002) *fleQ*, the gene encoding the major flagellar regulator of *Pseudomonas aeruginosa*, is sigma70 dependent and is downregulated by Vfr, a homolog of *Escherichia coli* cyclic AMP receptor protein. *J. Bacteriol.*, **184**, 5240–5250.
57. Liang, H., Deng, X., Ji, Q., Sun, F., Shen, T. and He, C. (2012) The *Pseudomonas aeruginosa* global regulator VqsR directly inhibits QscR to control quorum-sensing and virulence gene expression. *J. Bacteriol.*, **194**, 3098–3108.
58. Nealson, K.H., Platt, T. and Hastings, J.W. (1970) Cellular control of the synthesis and activity of the bacterial luminescent system. *J. Bacteriol.*, **104**, 313–322.
59. Stevens, A.M., Schuster, M. and Rumbaugh, K.P. (2012) Working together for the common good: cell-cell communication in bacteria. *J. Bacteriol.*, **194**, 2131–2141.
60. Williams, P. and Camara, M. (2009) Quorum sensing and environmental adaptation in *Pseudomonas aeruginosa*: a tale of regulatory networks and multifunctional signal molecules. *Curr. Opin. Microbiol.*, **12**, 182–191.
61. Jimenez, P.N., Koch, G., Thompson, J.A., Xavier, K.B., Cool, R.H. and Quax, W.J. (2012) The multiple signaling systems regulating virulence in *Pseudomonas aeruginosa*. *Microbiol. Mol. Biol. Rev.*, **76**, 46–65.
62. Schuster, M. and Greenberg, E.P. (2006) A network of networks: quorum-sensing gene regulation in *Pseudomonas aeruginosa*. *Int. J. Med. Microbiol.*, **296**, 73–81.
63. Passador, L., Cook, J.M., Gambello, M.J., Rust, L. and Iglewski, B.H. (1993) Expression of *Pseudomonas aeruginosa* virulence genes requires cell-to-cell communication. *Science*, **260**, 1127–1130.
64. Pearson, J.P., Gray, K.M., Passador, L., Tucker, K.D., Eberhard, A., Iglewski, B.H. and Greenberg, E.P. (1994) Structure of the autoinducer required for expression of *Pseudomonas aeruginosa* virulence genes. *Proc. Natl Acad. Sci. USA*, **91**, 197–201.
65. Brint, J.M. and Ohman, D.E. (1995) Synthesis of multiple exoproducts in *Pseudomonas aeruginosa* is under the control of RhlR-RhlI, another set of regulators in strain PAO1 with homology to the autoinducer-responsive LuxR-LuxI family. *J. Bacteriol.*, **177**, 7155–7163.
66. Latifi, A., Winson, M.K., Foglino, M., Bycroft, B.W., Stewart, G.S., Lazdunski, A. and Williams, P. (1995) Multiple homologues of LuxR and LuxI control expression of virulence determinants and secondary metabolites through quorum sensing in *Pseudomonas aeruginosa* PAO1. *Mol. Microbiol.*, **17**, 333–343.
67. Ochsner, U.A. and Reiser, J. (1995) Autoinducer-mediated regulation of rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*. *Proc. Natl Acad. Sci. USA*, **92**, 6424–6428.
68. Pearson, J.P., Passador, L., Iglewski, B.H. and Greenberg, E.P. (1995) A second N-acylhomoserine lactone signal produced by *Pseudomonas aeruginosa*. *Proc. Natl Acad. Sci. USA*, **92**, 1490–1494.
69. Kiratisin, P., Tucker, K.D. and Passador, L. (2002) LasR, a transcriptional activator of *Pseudomonas aeruginosa* virulence genes, functions as a multimer. *J. Bacteriol.*, **184**, 4912–4919.
70. Lamb, J.R., Patel, H., Montminy, T., Wagner, V.E. and Iglewski, B.H. (2003) Functional domains of the RhlR transcriptional regulator of *Pseudomonas aeruginosa*. *J. Bacteriol.*, **185**, 7129–7139.
71. Schuster, M., Urbanowski, M.L. and Greenberg, E.P. (2004) Promoter specificity in *Pseudomonas aeruginosa* quorum sensing revealed by DNA binding of purified LasR. *Proc. Natl Acad. Sci. USA*, **101**, 15833–15839.
72. Schuster, M. and Greenberg, E.P. (2007) Early activation of quorum sensing in *Pseudomonas aeruginosa* reveals the architecture of a complex regulon. *BMC Genomics*, **8**, 287.
73. Diggle, S.P., Matthijs, S., Wright, V.J., Fletcher, M.P., Chhabra, S.R., Lamont, I.L., Kong, X., Hider, R.C., Cornelis, P., Camara, M. et al. (2007) The *Pseudomonas aeruginosa* 4-quinolone signal molecules HHQ and PQS play multifunctional roles in quorum sensing and iron entrapment. *Chem. Biol.*, **14**, 87–96.
74. Xiao, G., Deziel, E., He, J., Lepine, F., Lesic, B., Castonguay, M.H., Milot, S., Tampakaki, A.P., Stachel, S.E. and Rahme, L.G. (2006) MvfR, a key *Pseudomonas aeruginosa* pathogenicity LTTR-class regulatory protein, has dual ligands. *Mol. Microbiol.*, **62**, 1689–1699.
75. Deziel, E., Gopalan, S., Tampakaki, A.P., Lepine, F., Padfield, K.E., Saucier, M., Xiao, G. and Rahme, L.G. (2005) The contribution of MvfR to *Pseudomonas aeruginosa* pathogenesis and quorum sensing circuitry regulation: multiple quorum sensing-regulated genes are modulated without affecting *lasRI*, *rhlRI* or the production of N-acyl-L-homoserine lactones. *Mol. Microbiol.*, **55**, 998–1014.
76. Bredenbruch, F., Geffers, R., Nimitz, M., Buer, J. and Haussler, S. (2006) The *Pseudomonas aeruginosa* quinolone signal (PQS) has an iron-chelating activity. *Environ. Microbiol.*, **8**, 1318–1329.
77. Mashburn, L.M. and Whiteley, M. (2005) Membrane vesicles traffic signals and facilitate group activities in a prokaryote. *Nature*, **437**, 422–425.
78. Schertzer, J.W. and Whiteley, M. (2012) A bilayer-couple model of bacterial outer membrane vesicle biogenesis. *MBio*, **3**, e00297–11.
79. Liang, H., Li, L., Dong, Z., Surette, M.G. and Duan, K. (2008) The YebC family protein PA0964 negatively regulates the *Pseudomonas aeruginosa* quinolone signal system and pyocyanin production. *J. Bacteriol.*, **190**, 6217–6227.
80. Wagner, V.E., Li, L.L., Isabella, V.M. and Iglewski, B.H. (2007) Analysis of the hierarchy of quorum-sensing regulation in *Pseudomonas aeruginosa*. *Anal. Bioanal. Chem.*, **387**, 469–479.
81. Latifi, A., Foglino, M., Tanaka, K., Williams, P. and Lazdunski, A. (1996) A hierarchical quorum-sensing cascade in *Pseudomonas aeruginosa* links the transcriptional activators LasR and RhlR (VsmR) to expression of the stationary-phase sigma factor RpoS. *Mol. Microbiol.*, **21**, 1137–1146.
82. McGrath, S., Wade, D.S. and Pesci, E.C. (2004) Dueling quorum sensing systems in *Pseudomonas aeruginosa* control the production of the *Pseudomonas aeruginosa* quinolone signal (PQS). *FEMS Microbiol. Lett.*, **230**, 27–34.
83. Pesci, E.C., Pearson, J.P., Seed, P.C. and Iglewski, B.H. (1997) Regulation of *las* and *rhl* quorum sensing in *Pseudomonas aeruginosa*. *J. Bacteriol.*, **179**, 3127–3132.
84. Dekimpe, V. and Deziel, E. (2009) Revisiting the quorum-sensing hierarchy in *Pseudomonas aeruginosa*: the transcriptional regulator RhlR regulates LasR-specific factors. *Microbiology*, **155**, 712–723.
85. Duan, K. and Surette, M.G. (2007) Environmental regulation of *Pseudomonas aeruginosa* PAO1 Las and Rhl quorum-sensing systems. *J. Bacteriol.*, **189**, 4827–4836.
86. Schuster, M., Hawkins, A.C., Harwood, C.S. and Greenberg, E.P. (2004) The *Pseudomonas aeruginosa* RpoS regulon and its relationship to quorum sensing. *Mol. Microbiol.*, **51**, 973–985.
87. Gilbert, K.B., Kim, T.H., Gupta, R., Greenberg, E.P. and Schuster, M. (2009) Global position analysis of the *Pseudomonas aeruginosa* quorum-sensing transcription factor LasR. *Mol. Microbiol.*, **73**, 1072–1085.
88. Castang, S., McManus, H.R., Turner, K.H. and Dove, S.L. (2008) H-NS family members function coordinately in an opportunistic pathogen. *Proc. Natl Acad. Sci. USA*, **105**, 18947–18952.
89. Rampioni, G., Schuster, M., Greenberg, E.P., Bertani, I., Grasso, M., Venturi, V., Zennaro, E. and Leoni, L. (2007) RsaL provides quorum sensing homeostasis and functions as a global regulator of gene expression in *Pseudomonas aeruginosa*. *Mol. Microbiol.*, **66**, 1557–1565.
90. Rampioni, G., Schuster, M., Greenberg, E.P., Zennaro, E. and Leoni, L. (2009) Contribution of the RsaL global regulator to *Pseudomonas aeruginosa* virulence and biofilm formation. *FEMS Microbiol. Lett.*, **301**, 210–217.

91. Wei,Q., Le Minh,P.N., Dotsch,A., Hildebrand,F., Panmanee,W., Elfarash,A., Schulz,S., Plaisance,S., Charlier,D., Hassett,D. *et al.* (2012) Global regulation of gene expression by OxyR in an important human opportunistic pathogen. *Nucleic Acids Res.*, **40**, 4320–4333.
92. Diepouis,G., Ducret,V., Caille,O. and Perron,K. (2012) The transcriptional regulator CzcR modulates antibiotic resistance and quorum sensing in *Pseudomonas aeruginosa*. *PLoS One*, **7**, e38148.
93. Perron,K., Caille,O., Rossier,C., Van Delden,C., Dumas,J.L. and Kohler,T. (2004) CzcR-CzcS, a two-component system involved in heavy metal and carbapenem resistance in *Pseudomonas aeruginosa*. *J. Biol. Chem.*, **279**, 8761–8768.
94. Juhas,M., Wiehlmann,L., Huber,B., Jordan,D., Lauber,J., Salunkhe,P., Limpert,A.S., von Gotz,F., Steinmetz,I., Eberl,L. *et al.* (2004) Global regulation of quorum sensing and virulence by VqsR in *Pseudomonas aeruginosa*. *Microbiology*, **150**, 831–841.
95. Li,L.L., Malone,J.E. and Iglewski,B.H. (2007) Regulation of the *Pseudomonas aeruginosa* quorum-sensing regulator VqsR. *J. Bacteriol.*, **189**, 4367–4374.
96. Liang,H., Deng,X., Ji,Q., Sun,F., Shen,T. and He,C. (2012) The *Pseudomonas aeruginosa* global regulator VqsR directly inhibits QscR to control quorum-sensing and virulence gene expression. *J. Bacteriol.*, **194**, 3098–3108.
97. Lee,J.H., Lequette,Y. and Greenberg,E.P. (2006) Activity of purified QscR, a *Pseudomonas aeruginosa* orphan quorum-sensing transcription factor. *Mol. Microbiol.*, **59**, 602–609.
98. Lequette,Y., Lee,J.H., Ledgham,F., Lazdunski,A. and Greenberg,E.P. (2006) A distinct QscR regulon in the *Pseudomonas aeruginosa* quorum-sensing circuit. *J. Bacteriol.*, **188**, 3365–3370.
99. Ledgham,F., Ventre,I., Soscia,C., Foglino,M., Sturgis,J.N. and Lazdunski,A. (2003) Interactions of the quorum sensing regulator QscR: interaction with itself and the other regulators of *Pseudomonas aeruginosa* LasR and RhlR. *Mol. Microbiol.*, **48**, 199–210.
100. Chugani,S.A., Whiteley,M., Lee,K.M., D'Argenio,D., Manoel,C. and Greenberg,E.P. (2001) QscR, a modulator of quorum-sensing signal synthesis and virulence in *Pseudomonas aeruginosa*. *Proc. Natl Acad. Sci. USA*, **98**, 2752–2757.
101. Dong,Y.H., Zhang,X.F., Xu,J.L., Tan,A.T. and Zhang,L.H. (2005) VqsM, a novel AraC-type global regulator of quorum-sensing signalling and virulence in *Pseudomonas aeruginosa*. *Mol. Microbiol.*, **58**, 552–564.
102. Deziel,E., Lepine,F., Milot,S., He,J., Mindrinos,M.N., Tompkins,R.G. and Rahme,L.G. (2004) Analysis of *Pseudomonas aeruginosa* 4-hydroxy-2-alkylquinolines (HAQs) reveals a role for 4-hydroxy-2-heptylquinoline in cell-to-cell communication. *Proc. Natl Acad. Sci. USA*, **101**, 1339–1344.
103. Gallagher,L.A., McKnight,S.L., Kuznetsova,M.S., Pesci,E.C. and Manoel,C. (2002) Functions required for extracellular quinolone signaling by *Pseudomonas aeruginosa*. *J. Bacteriol.*, **184**, 6472–6480.
104. Farrow,J.M. 3rd and Pesci,E.C. (2007) Two distinct pathways supply anthranilate as a precursor of the *Pseudomonas* quinolone signal. *J. Bacteriol.*, **189**, 3425–3433.
105. Chugani,S. and Greenberg,E.P. (2010) LuxR homolog-independent gene regulation by acyl-homoserine lactones in *Pseudomonas aeruginosa*. *Proc. Natl Acad. Sci. USA*, **107**, 10673–10678.
106. Siehnel,R., Traxler,B., An,D.D., Parsek,M.R., Schaefer,A.L. and Singh,P.K. (2010) A unique regulator controls the activation threshold of quorum-regulated genes in *Pseudomonas aeruginosa*. *Proc. Natl Acad. Sci. USA*, **107**, 7916–7921.
107. Pessi,G., Williams,F., Hindle,Z., Heurlier,K., Holden,M.T., Camara,M., Haas,D. and Williams,P. (2001) The global posttranscriptional regulator RsmA modulates production of virulence determinants and N-acylhomoserine lactones in *Pseudomonas aeruginosa*. *J. Bacteriol.*, **183**, 6676–6683.
108. Sonnleitner,E., Schuster,M., Sorger-Domenigg,T., Greenberg,E.P. and Blasi,U. (2006) Hfq-dependent alterations of the transcriptome profile and effects on quorum sensing in *Pseudomonas aeruginosa*. *Mol. Microbiol.*, **59**, 1542–1558.
109. Kong,K.F., Jayawardena,S.R., Indulkar,S.D., Del Puerto,A., Koh,C.L., Hoiby,N. and Mathee,K. (2005) *Pseudomonas aeruginosa* AmpR is a global transcriptional factor that regulates expression of AmpC and PoxB beta-lactamases, proteases, quorum sensing, and other virulence factors. *Antimicrob. Agents Chemother.*, **49**, 4567–4575.
110. Balasubramanian,D., Kong,K.F., Jayawardena,S.R., Leal,S.M., Sautter,R.T. and Mathee,K. (2011) Co-regulation of beta-lactam resistance, alginate production and quorum sensing in *Pseudomonas aeruginosa*. *J. Med. Microbiol.*, **60**, 147–156.
111. Rodrigue,A., Quentin,Y., Lazdunski,A., Mejean,V. and Foglino,M. (2000) Two-component systems in *Pseudomonas aeruginosa*: why so many? *Trends Microbiol.*, **8**, 498–504.
112. Raghavan,V. and Groisman,E.A. (2010) Orphan and hybrid two-component system proteins in health and disease. *Curr. Opin. Microbiol.*, **13**, 226–231.
113. Stock,A.M., Robinson,V.L. and Goudreau,P.N. (2000) Two-component signal transduction. *Annu. Rev. Biochem.*, **69**, 183–215.
114. Laub,M.T. and Goulian,M. (2007) Specificity in two-component signal transduction pathways. *Annu. Rev. Genet.*, **41**, 121–145.
115. Mascher,T., Helmann,J.D. and Uden,G. (2006) Stimulus perception in bacterial signal-transducing histidine kinases. *Microbiol. Mol. Biol. Rev.*, **70**, 910–938.
116. Gao,R. and Stock,A.M. (2010) Molecular strategies for phosphorylation-mediated regulation of response regulator activity. *Curr. Opin. Microbiol.*, **13**, 160–167.
117. Mitrophanov,A.Y. and Groisman,E.A. (2008) Signal integration in bacterial two-component regulatory systems. *Genes Dev.*, **22**, 2601–2611.
118. Kitten,T., Kinscherf,T.G., McEvoy,J.L. and Willis,D.K. (1998) A newly identified regulator is required for virulence and toxin production in *Pseudomonas syringae*. *Mol. Microbiol.*, **28**, 917–929.
119. Coleman,F.T., Mueschenborn,S., Meluleni,G., Ray,C., Carey,V.J., Vargas,S.O., Cannon,C.L., Ausubel,F.M. and Pier,G.B. (2003) Hypersusceptibility of cystic fibrosis mice to chronic *Pseudomonas aeruginosa* oropharyngeal colonization and lung infection. *Proc. Natl Acad. Sci. USA*, **100**, 1949–1954.
120. Hrabak,E.M. and Willis,D.K. (1992) The *lemA* gene required for pathogenicity of *Pseudomonas syringae* pv. *syringae* on bean is a member of a family of two-component regulators. *J. Bacteriol.*, **174**, 3011–3020.
121. Goodman,A.L., Merighi,M., Hyodo,M., Ventre,I., Filloux,A. and Lory,S. (2009) Direct interaction between sensor kinase proteins mediates acute and chronic disease phenotypes in a bacterial pathogen. *Genes Dev.*, **23**, 249–259.
122. Heeb,S. and Haas,D. (2001) Regulatory roles of the GacS/GacA two-component system in plant-associated and other Gram-negative bacteria. *Mol. Plant Microbe Interact.*, **14**, 1351–1363.
123. Wolfgang,M.C., Lee,V.T., Gilmore,M.E. and Lory,S. (2003) Coordinate regulation of bacterial virulence genes by a novel adenylate cyclase-dependent signaling pathway. *Dev. Cell*, **4**, 253–263.
124. Brencic,A. and Lory,S. (2009) Determination of the regulon and identification of novel mRNA targets of *Pseudomonas aeruginosa* RsmA. *Mol. Microbiol.*, **72**, 612–632.
125. Linares,J.F., Gustafsson,I., Baquero,F. and Martinez,J.L. (2006) Antibiotics as intermicrobial signaling agents instead of weapons. *Proc. Natl Acad. Sci. USA*, **103**, 19484–19489.
126. McPhee,J.B., Bains,M., Winsor,G., Lewenza,S., Kwasnicka,A., Brazas,M.D., Brinkman,F.S. and Hancock,R.E. (2006) Contribution of the PhoP-PhoQ and PmrA-PmrB two-component regulatory systems to Mg²⁺-induced gene regulation in *Pseudomonas aeruginosa*. *J. Bacteriol.*, **188**, 3995–4006.
127. Macfarlane,E.L., Kwasnicka,A. and Hancock,R.E. (2000) Role of *Pseudomonas aeruginosa* PhoP-PhoQ in resistance to antimicrobial cationic peptides and aminoglycosides. *Microbiology*, **146**, 2543–2554.
128. Gooderham,W.J., Gellatly,S.L., Sanschagrin,F., McPhee,J.B., Bains,M., Cosseau,C., Levesque,R.C. and Hancock,R.E. (2009)

- The sensor kinase PhoQ mediates virulence in *Pseudomonas aeruginosa*. *Microbiology*, **155**, 699–711.
129. McPhee, J.B., Lewenza, S. and Hancock, R.E. (2003) Cationic antimicrobial peptides activate a two-component regulatory system, PmrA-PmrB, that regulates resistance to polymyxin B and cationic antimicrobial peptides in *Pseudomonas aeruginosa*. *Mol. Microbiol.*, **50**, 205–217.
 130. Kwon, D.H. and Lu, C.D. (2006) Polyamines induce resistance to cationic peptide, aminoglycoside, and quinolone antibiotics in *Pseudomonas aeruginosa* PAO1. *Antimicrob. Agents Chemother.*, **50**, 1615–1622.
 131. Kwon, D.H. and Lu, C.D. (2007) Polyamine effects on antibiotic susceptibility in bacteria. *Antimicrob. Agents Chemother.*, **51**, 2070–2077.
 132. Flemming, H.C. and Wingender, J. (2010) The biofilm matrix. *Nat. Rev. Microbiol.*, **8**, 623–633.
 133. Donlan, R.M. and Costerton, J.W. (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin. Microbiol. Rev.*, **15**, 167–193.
 134. Costerton, J.W., Stewart, P.S. and Greenberg, E.P. (1999) Bacterial biofilms: a common cause of persistent infections. *Science*, **284**, 1318–1322.
 135. Lopez, D., Vlamakis, H. and Kolter, R. (2010) Biofilms. *Cold Spring Harb Perspect. Biol.*, **2**, a000398.
 136. Stapper, A.P., Narasimhan, G., Ohman, D.E., Barakat, J., Hentzer, M., Molin, S., Kharazmi, A., Hoiby, N. and Mathee, K. (2004) Alginate production affects *Pseudomonas aeruginosa* biofilm development and architecture, but is not essential for biofilm formation. *J. Med. Microbiol.*, **53**, 679–690.
 137. Klausen, M., Heydorn, A., Ragas, P., Lambertsen, L., Aaes-Jorgensen, A., Molin, S. and Tolker-Nielsen, T. (2003) Biofilm formation by *Pseudomonas aeruginosa* wild type, flagella and type IV pili mutants. *Mol. Microbiol.*, **48**, 1511–1524.
 138. Caiazza, N.C. and O'Toole, G.A. (2004) SadB is required for the transition from reversible to irreversible attachment during biofilm formation by *Pseudomonas aeruginosa* PA14. *J. Bacteriol.*, **186**, 4476–4485.
 139. Merritt, J.H., Brothers, K.M., Kuchma, S.L. and O'Toole, G.A. (2007) SadC reciprocally influences biofilm formation and swarming motility via modulation of exopolysaccharide production and flagellar function. *J. Bacteriol.*, **189**, 8154–8164.
 140. Vallet, I., Olson, J.W., Lory, S., Lazdunski, A. and Filloux, A. (2001) The chaperone/usher pathways of *Pseudomonas aeruginosa*: identification of fimbrial gene clusters (*cup*) and their involvement in biofilm formation. *Proc. Natl Acad. Sci. USA*, **98**, 6911–6916.
 141. Vallet, I., Diggle, S.P., Stacey, R.E., Camara, M., Ventre, I., Lory, S., Lazdunski, A., Williams, P. and Filloux, A. (2004) Biofilm formation in *Pseudomonas aeruginosa*: fimbrial *cup* gene clusters are controlled by the transcriptional regulator MvaT. *J. Bacteriol.*, **186**, 2880–2890.
 142. Vallet-Gely, I., Donovan, K.E., Fang, R., Joung, J.K. and Dove, S.L. (2005) Repression of phase-variable *cup* gene expression by H-NS-like proteins in *Pseudomonas aeruginosa*. *Proc. Natl Acad. Sci. USA*, **102**, 11082–11087.
 143. Kulasekara, H.D., Ventre, I., Kulasekara, B.R., Lazdunski, A., Filloux, A. and Lory, S. (2005) A novel two-component system controls the expression of *Pseudomonas aeruginosa* fimbrial *cup* genes. *Mol. Microbiol.*, **55**, 368–380.
 144. Merkel, T.J., Barros, C. and Stibitz, S. (1998) Characterization of the *bygR* locus of *Bordetella pertussis*. *J. Bacteriol.*, **180**, 1682–1690.
 145. Kuchma, S.L., Connolly, J.P. and O'Toole, G.A. (2005) A three-component regulatory system regulates biofilm maturation and type III secretion in *Pseudomonas aeruginosa*. *J. Bacteriol.*, **187**, 1441–1454.
 146. Mikkelsen, H., Ball, G., Giraud, C. and Filloux, A. (2009) Expression of *Pseudomonas aeruginosa* CupD fimbrial genes is antagonistically controlled by RcsB and the EAL-containing PvrR response regulators. *PLoS One*, **4**, e6018.
 147. Hickman, J.W., Tifrea, D.F. and Harwood, C.S. (2005) A chemosensory system that regulates biofilm formation through modulation of cyclic diguanylate levels. *Proc. Natl Acad. Sci. USA*, **102**, 14422–14427.
 148. Guvener, Z.T. and Harwood, C.S. (2007) Subcellular location characteristics of the *Pseudomonas aeruginosa* GGDEF protein, WspR, indicate that it produces cyclic-di-GMP in response to growth on surfaces. *Mol. Microbiol.*, **66**, 1459–1473.
 149. Choy, W.K., Zhou, L., Syn, C.K., Zhang, L.H. and Swarup, S. (2004) MorA defines a new class of regulators affecting flagellar development and biofilm formation in diverse *Pseudomonas* species. *J. Bacteriol.*, **186**, 7221–7228.
 150. Ueda, A. and Wood, T.K. (2009) Connecting quorum sensing, c-di-GMP, *pel* polysaccharide, and biofilm formation in *Pseudomonas aeruginosa* through tyrosine phosphatase TpbA (PA3885). *PLoS Pathog.*, **5**, e1000483.
 151. Hickman, J.W. and Harwood, C.S. (2008) Identification of FleQ from *Pseudomonas aeruginosa* as a c-di-GMP-responsive transcription factor. *Mol. Microbiol.*, **69**, 376–389.
 152. Attila, C., Ueda, A. and Wood, T.K. (2008) PA2663 (PpyR) increases biofilm formation in *Pseudomonas aeruginosa* PAO1 through the *psl* operon and stimulates virulence and quorum-sensing phenotypes. *Appl. Microbiol. Biotechnol.*, **78**, 293–307.
 153. D'Argenio, D.A., Calfee, M.W., Rainey, P.B. and Pesci, E.C. (2002) Autolysis and autoaggregation in *Pseudomonas aeruginosa* colony morphology mutants. *J. Bacteriol.*, **184**, 6481–6489.
 154. Allesen-Holm, M., Barken, K.B., Yang, L., Klausen, M., Webb, J.S., Kjelleberg, S., Molin, S., Givskov, M. and Tolker-Nielsen, T. (2006) A characterization of DNA release in *Pseudomonas aeruginosa* cultures and biofilms. *Mol. Microbiol.*, **59**, 1114–1128.
 155. Heurlier, K., Denervaud, V., Haenni, M., Guy, L., Krishnapillai, V. and Haas, D. (2005) Quorum-sensing-negative (*lasR*) mutants of *Pseudomonas aeruginosa* avoid cell lysis and death. *J. Bacteriol.*, **187**, 4875–4883.
 156. Whitchurch, C.B., Tolker-Nielsen, T., Ragas, P.C. and Mattick, J.S. (2002) Extracellular DNA required for bacterial biofilm formation. *Science*, **295**, 1487.
 157. Dominiak, D.M., Nielsen, J.L. and Nielsen, P.H. (2010) Extracellular DNA is abundant and important for microcolony strength in mixed microbial biofilms. *Environ. Microbiol.*, **13**, 710–721.
 158. Pamp, S.J. and Tolker-Nielsen, T. (2007) Multiple roles of biosurfactants in structural biofilm development by *Pseudomonas aeruginosa*. *J. Bacteriol.*, **189**, 2531–2539.
 159. Davey, M.E., Caiazza, N.C. and O'Toole, G.A. (2003) Rhamnolipid surfactant production affects biofilm architecture in *Pseudomonas aeruginosa* PAO1. *J. Bacteriol.*, **185**, 1027–1036.
 160. Petrova, O.E. and Sauer, K. (2009) A novel signaling network essential for regulating *Pseudomonas aeruginosa* biofilm development. *PLoS Pathog.*, **5**, e1000668.
 161. Petrova, O.E. and Sauer, K. (2011) SagS contributes to the motile-sessile switch and acts in concert with BfiSR to enable *Pseudomonas aeruginosa* biofilm formation. *J. Bacteriol.*, **193**, 6614–6628.
 162. Janjua, H.A., Segata, N., Bernabo, P., Tamburini, S., Ellen, A. and Jousson, O. (2012) Clinical populations of *Pseudomonas aeruginosa* isolated from acute infections show a wide virulence range partially correlated with population structure and virulence gene expression. *Microbiology*, **158**, 2089–2098.
 163. Storey, D.G., Ujack, E.E., Rabin, H.R. and Mitchell, I. (1998) *Pseudomonas aeruginosa lasR* transcription correlates with the transcription of *lasA*, *lasB*, and *toxA* in chronic lung infections associated with cystic fibrosis. *Infect. Immun.*, **66**, 2521–2528.
 164. Singh, P.K., Schaefer, A.L., Parsek, M.R., Moninger, T.O., Welsh, M.J. and Greenberg, E.P. (2000) Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. *Nature*, **407**, 762–764.
 165. Wu, H., Song, Z., Givskov, M., Doring, G., Worlitzsch, D., Mathee, K., Rygaard, J. and Hoiby, N. (2001) *Pseudomonas aeruginosa* mutations in *lasI* and *rhlI* quorum sensing systems result in milder chronic lung infection. *Microbiology*, **147**, 1105–1113.
 166. Bjarnsholt, T., Jensen, P.O., Burmolle, M., Hentzer, M., Haagensen, J.A., Hougen, H.P., Calum, H., Madsen, K.G., Moser, C., Molin, S. et al. (2005) *Pseudomonas aeruginosa* tolerance to tobramycin, hydrogen peroxide and

- polymorphonuclear leukocytes is quorum-sensing dependent. *Microbiology*, **151**, 373–383.
167. Purevdorj, B., Costerton, J.W. and Stoodley, P. (2002) Influence of hydrodynamics and cell signaling on the structure and behavior of *Pseudomonas aeruginosa* biofilms. *Appl. Environ. Microbiol.*, **68**, 4457–4464.
 168. Heydorn, A., Ersboll, B., Kato, J., Hentzer, M., Parsek, M.R., Tolker-Nielsen, T., Givskov, M. and Molin, S. (2002) Statistical analysis of *Pseudomonas aeruginosa* biofilm development: impact of mutations in genes involved in twitching motility, cell-to-cell signaling, and stationary-phase sigma factor expression. *Appl. Environ. Microbiol.*, **68**, 2008–2017.
 169. Smith, E.E., Buckley, D.G., Wu, Z., Saenphimmachak, C., Hoffman, L.R., D'Argenio, D.A., Miller, S.I., Ramsey, B.W., Speert, D.P., Moskowitz, S.M. et al. (2006) Genetic adaptation by *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients. *Proc. Natl Acad. Sci. USA*, **103**, 8487–8492.
 170. Van Delden, C., Pesci, E.C., Pearson, J.P. and Iglewski, B.H. (1998) Starvation selection restores elastase and rhamnolipid production in a *Pseudomonas aeruginosa* quorum-sensing mutant. *Infect. Immun.*, **66**, 4499–4502.
 171. Kirisits, M.J. and Parsek, M.R. (2006) Does *Pseudomonas aeruginosa* use intercellular signalling to build biofilm communities? *Cell. Microbiol.*, **8**, 1841–1849.
 172. Lewis, K. (2008) Multidrug tolerance of biofilms and persister cells. *Curr. Top. Microbiol. Immunol.*, **322**, 107–131.
 173. Keren, I., Kaldalu, N., Spoering, A., Wang, Y. and Lewis, K. (2004) Persister cells and tolerance to antimicrobials. *FEMS Microbiol. Lett.*, **230**, 13–18.
 174. Keren, I., Shah, D., Spoering, A., Kaldalu, N. and Lewis, K. (2004) Specialized persister cells and the mechanism of multidrug tolerance in *Escherichia coli*. *J. Bacteriol.*, **186**, 8172–8180.
 175. De Groot, V.N., Verstraeten, N., Fauvart, M., Kint, C.I., Verbeeck, A.M., Beullens, S., Cornelis, P. and Michiels, J. (2009) Novel persistence genes in *Pseudomonas aeruginosa* identified by high-throughput screening. *FEMS Microbiol. Lett.*, **297**, 73–79.
 176. Fauvart, M., De Groot, V.N. and Michiels, J. (2011) Role of persister cells in chronic infections: clinical relevance and perspectives on anti-persister therapies. *J. Med. Microbiol.*, **60**, 699–709.
 177. Lewis, K. (2010) Persister cells. *Annu. Rev. Microbiol.*, **64**, 357–372.
 178. Pressler, T., Bohmova, C., Conway, S., Dumcius, S., Hjelte, L., Hoiby, N., Kollberg, H., Tummler, B. and Vavrova, V. (2011) Chronic *Pseudomonas aeruginosa* infection definition: EuroCareCF Working Group report. *J. Cyst. Fibros.*, **10**, S75–S78.
 179. de Jong, P.A., Nakano, Y., Lequin, M.H., Mayo, J.R., Woods, R., Pare, P.D. and Tiddens, H.A. (2004) Progressive damage on high resolution computed tomography despite stable lung function in cystic fibrosis. *Eur. Respir. J.*, **23**, 93–97.
 180. Meyer, K.C. and Sharma, A. (1997) Regional variability of lung inflammation in cystic fibrosis. *Am. J. Respir. Crit. Care Med.*, **156**, 1536–1540.
 181. Tiddens, H.A. (2002) Detecting early structural lung damage in cystic fibrosis. *Pediatr. Pulmonol.*, **34**, 228–231.
 182. Worlitzsch, D., Tarran, R., Ulrich, M., Schwab, U., Cekici, A., Meyer, K.C., Birrer, P., Bellon, G., Berger, J., Weiss, T. et al. (2002) Effects of reduced mucus oxygen concentration in airway *Pseudomonas* infections of cystic fibrosis patients. *J. Clin. Invest.*, **109**, 317–325.
 183. Doggett, R.G., Harrison, G.M. and Carter, R.E. (1971) Mucoid *Pseudomonas aeruginosa* in patients with chronic illnesses. *Lancet*, **1**, 236–237.
 184. Hoiby, N. (1974) *Pseudomonas aeruginosa* infection in cystic fibrosis. Relationship between mucoid strains of *Pseudomonas aeruginosa* and the humoral immune response. *Acta. Pathol. Microbiol. Scand. [B] Microbiol. Immunol.*, **82**, 551–558.
 185. Evans, L.R. and Linker, A. (1973) Production and characterization of the slime polysaccharide of *Pseudomonas aeruginosa*. *J. Bacteriol.*, **116**, 915–924.
 186. Pedersen, S.S., Espersen, F., Hoiby, N. and Jensen, T. (1990) Immunoglobulin A and immunoglobulin G antibody responses to alginates from *Pseudomonas aeruginosa* in patients with cystic fibrosis. *J. Clin. Microbiol.*, **28**, 747–755.
 187. Pier, G.B., Coleman, F., Grout, M., Franklin, M. and Ohman, D.E. (2001) Role of alginate O acetylation in resistance of mucoid *Pseudomonas aeruginosa* to opsonic phagocytosis. *Infect. Immun.*, **69**, 1895–1901.
 188. Simpson, J.A., Smith, S.E. and Dean, R.T. (1988) Alginate inhibition of the uptake of *Pseudomonas aeruginosa* by macrophages. *J. Gen. Microbiol.*, **134**, 29–36.
 189. Mathee, K., Ciofu, O., Sternberg, C., Lindum, P.W., Campbell, J.I., Jensen, P., Johnsen, A.H., Givskov, M., Ohman, D.E., Molin, S. et al. (1999) Mucoid conversion of *Pseudomonas aeruginosa* by hydrogen peroxide: a mechanism for virulence activation in the cystic fibrosis lung. *Microbiology*, **145**, 1349–1357.
 190. Govan, J.R. and Fyfe, J.A. (1978) Mucoid *Pseudomonas aeruginosa* and cystic fibrosis: resistance of the mucoid form to carbenicillin, flucloxacillin and tobramycin and the isolation of mucoid variants in vitro. *J. Antimicrob. Chemother.*, **4**, 233–240.
 191. Speert, D.P., Farmer, S.W., Campbell, M.E., Musser, J.M., Selander, R.K. and Kuo, S. (1990) Conversion of *Pseudomonas aeruginosa* to the phenotype characteristic of strains from patients with cystic fibrosis. *J. Clin. Microbiol.*, **28**, 188–194.
 192. Terry, J.M., Pina, S.E. and Mattingly, S.J. (1991) Environmental conditions which influence mucoid conversion *Pseudomonas aeruginosa* PAO1. *Infect. Immun.*, **59**, 471–477.
 193. DeVries, C.A. and Ohman, D.E. (1994) Mucoid-to-nonmucoid conversion in alginate-producing *Pseudomonas aeruginosa* often results from spontaneous mutations in *algT*, encoding a putative alternate sigma factor, and shows evidence for autoregulation. *J. Bacteriol.*, **176**, 6677–6687.
 194. Martin, D.W., Holloway, B.W. and Deretic, V. (1993) Characterization of a locus determining the mucoid status of *Pseudomonas aeruginosa*: AlgU shows sequence similarities with a *Bacillus* sigma factor. *J. Bacteriol.*, **175**, 1153–1164.
 195. Martin, D.W., Schurr, M.J., Mudd, M.H. and Deretic, V. (1993) Differentiation of *Pseudomonas aeruginosa* into the alginate-producing form: inactivation of *mucB* causes conversion to mucoidy. *Mol. Microbiol.*, **9**, 497–506.
 196. Mathee, K., McPherson, C.J. and Ohman, D.E. (1997) Posttranslational control of the *algT* (*algU*)-encoded sigma22 for expression of the alginate regulon in *Pseudomonas aeruginosa* and localization of its antagonist proteins MucA and MucB (AlgN). *J. Bacteriol.*, **179**, 3711–3720.
 197. Mathee, K., Kharazami, A.A. and Hoiby, N. (2002) In: McLean, R.J.C. (ed.), *Molecular Ecology of Biofilms*. Horizon, Norfolk, pp. 23–55.
 198. Goldberg, J.B., Gorman, W.L., Flynn, J.L. and Ohman, D.E. (1993) A mutation in *algN* permits trans activation of alginate production by *algT* in *Pseudomonas* species. *J. Bacteriol.*, **175**, 1303–1308.
 199. Martin, D.W., Schurr, M.J., Mudd, M.H., Govan, J.R., Holloway, B.W. and Deretic, V. (1993) Mechanism of conversion to mucoidy in *Pseudomonas aeruginosa* infecting cystic fibrosis patients. *Proc. Natl Acad. Sci. USA*, **90**, 8377–8381.
 200. Ohman, D. (2009) In: Rehm, B.H.A. (ed.), *Alginates: Biology and Applications*, Vol. 13. Springer, Berlin/Heidelberg, pp. 117–133.
 201. Damron, F.H. and Goldberg, J.B. (2012) Proteolytic regulation of alginate overproduction in *Pseudomonas aeruginosa*. *Mol. Microbiol.*, **84**, 595–607.
 202. Damron, F.H. and Yu, H.D. (2011) *Pseudomonas aeruginosa* MucD regulates the alginate pathway through activation of MucA degradation via MucP proteolytic activity. *J. Bacteriol.*, **193**, 286–291.
 203. Martin, D.W., Schurr, M.J., Yu, H. and Deretic, V. (1994) Analysis of promoters controlled by the putative sigma factor AlgU regulating conversion to mucoidy in *Pseudomonas aeruginosa*: relationship to sigma E and stress response. *J. Bacteriol.*, **176**, 6688–6696.
 204. Wozniak, D.J. and Ohman, D.E. (1994) Transcriptional analysis of the *Pseudomonas aeruginosa* genes *algR*, *algB*, and *algD* reveals a hierarchy of alginate gene expression which is modulated by *algT*. *J. Bacteriol.*, **176**, 6007–6014.
 205. Wozniak, D.J., Sprinkle, A.B. and Baynham, P.J. (2003) Control of *Pseudomonas aeruginosa* *algZ* expression by the alternative sigma factor AlgT. *J. Bacteriol.*, **185**, 7297–7300.
 206. Chitnis, C.E. and Ohman, D.E. (1993) Genetic analysis of the alginate biosynthetic gene cluster of *Pseudomonas aeruginosa*

- shows evidence of an operonic structure. *Mol. Microbiol.*, **8**, 583–593.
207. Deretic, V., Gill, J.F. and Chakrabarty, A.M. (1987) Gene *algD* coding for GDPmannose dehydrogenase is transcriptionally activated in mucoid *Pseudomonas aeruginosa*. *J. Bacteriol.*, **169**, 351–358.
 208. Leech, A.J., Sprinkle, A., Wood, L., Wozniak, D.J. and Ohman, D.E. (2008) The NtrC family regulator AlgB, which controls alginate biosynthesis in mucoid *Pseudomonas aeruginosa*, binds directly to the *algD* promoter. *J. Bacteriol.*, **190**, 581–589.
 209. Kato, J. and Chakrabarty, A.M. (1991) Purification of the regulatory protein AlgR1 and its binding in the far upstream region of the *algD* promoter in *Pseudomonas aeruginosa*. *Proc. Natl Acad. Sci. USA*, **88**, 1760–1764.
 210. Mohr, C.D., Martin, D.W., Konyecsni, W.M., Govan, J.R., Lory, S. and Deretic, V. (1990) Role of the far-upstream sites of the *algD* promoter and the *algR* and *rpoN* genes in environmental modulation of mucoidy in *Pseudomonas aeruginosa*. *J. Bacteriol.*, **172**, 6576–6580.
 211. Baynham, P.J., Brown, A.L., Hall, L.L. and Wozniak, D.J. (1999) *Pseudomonas aeruginosa* AlgZ, a ribbon-helix-helix DNA-binding protein, is essential for alginate synthesis and *algD* transcriptional activation. *Mol. Microbiol.*, **33**, 1069–1080.
 212. Damron, F.H., Qiu, D. and Yu, H.D. (2009) The *Pseudomonas aeruginosa* sensor kinase KinB negatively controls alginate production through AlgW-dependent MucA proteolysis. *J. Bacteriol.*, **191**, 2285–2295.
 213. Wood, L.F., Leech, A.J. and Ohman, D.E. (2006) Cell wall-inhibitory antibiotics activate the alginate biosynthesis operon in *Pseudomonas aeruginosa*: Roles of sigma (AlgT) and the AlgW and Prc proteases. *Mol. Microbiol.*, **62**, 412–426.
 214. Ma, S., Selvaraj, U., Ohman, D.E., Quarless, R., Hassett, D.J. and Wozniak, D.J. (1998) Phosphorylation-independent activity of the response regulators AlgB and AlgR in promoting alginate biosynthesis in mucoid *Pseudomonas aeruginosa*. *J. Bacteriol.*, **180**, 956–968.
 215. Zegans, M.E., Wozniak, D., Griffin, E., Toutain-Kidd, C.M., Hammond, J.H., Garfoot, A. and Lam, J.S. (2012) *Pseudomonas aeruginosa* exopolysaccharide Psl promotes resistance to the biofilm inhibitor polysorbate 80. *Antimicrob. Agents Chemother.*, **56**, 4112–4122.
 216. Fujiwara, S., Zielinski, N.A. and Chakrabarty, A.M. (1993) Enhancer-like activity of AlgR1-binding site in alginate gene activation: positional, orientational, and sequence specificity. *J. Bacteriol.*, **175**, 5452–5459.
 217. Ye, R.W., Zielinski, N.A. and Chakrabarty, A.M. (1994) Purification and characterization of phosphomannomutase/phosphoglucomutase from *Pseudomonas aeruginosa* involved in biosynthesis of both alginate and lipopolysaccharide. *J. Bacteriol.*, **176**, 4851–4857.
 218. Zielinski, N.A., Maharaj, R., Roychoudhury, S., Danganan, C.E., Hendrickson, W. and Chakrabarty, A.M. (1992) Alginate synthesis in *Pseudomonas aeruginosa*: environmental regulation of the *algC* promoter. *J. Bacteriol.*, **174**, 7680–7688.
 219. Morici, L.A., Carterson, A.J., Wagner, V.E., Frisk, A., Schurr, J.R., Honer zu Bentrup, K., Hassett, D.J., Iglewski, B.H., Sauer, K. and Schurr, M.J. (2007) *Pseudomonas aeruginosa* AlgR represses the Rhl quorum-sensing system in a biofilm-specific manner. *J. Bacteriol.*, **189**, 7752–7764.
 220. Belete, B., Lu, H. and Wozniak, D.J. (2008) *Pseudomonas aeruginosa* AlgR regulates type IV pilus biosynthesis by activating transcription of the *fimU-pilVWXYIY2E* operon. *J. Bacteriol.*, **190**, 2023–2030.
 221. Lizewski, S.E., Lundberg, D.S. and Schurr, M.J. (2002) The transcriptional regulator AlgR is essential for *Pseudomonas aeruginosa* pathogenesis. *Infect. Immun.*, **70**, 6083–6093.
 222. Carterson, A.J., Morici, L.A., Jackson, D.W., Frisk, A., Lizewski, S.E., Jupiter, R., Simpson, K., Kunz, D.A., Davis, S.H., Schurr, J.R. et al. (2004) The transcriptional regulator AlgR controls cyanide production in *Pseudomonas aeruginosa*. *J. Bacteriol.*, **186**, 6837–6844.
 223. Cody, W.L., Pritchett, C.L., Jones, A.K., Carterson, A.J., Jackson, D., Frisk, A., Wolfgang, M.C. and Schurr, M.J. (2009) *Pseudomonas aeruginosa* AlgR controls cyanide production in an AlgZ-dependent manner. *J. Bacteriol.*, **191**, 2993–3002.
 224. Jones, A.K., Fulcher, N.B., Balzer, G.J., Urbanowski, M.L., Pritchett, C.L., Schurr, M.J., Yahr, T.L. and Wolfgang, M.C. (2010) Activation of the *Pseudomonas aeruginosa* AlgU regulon through *mucA* mutation inhibits cyclic AMP/Vfr signaling. *J. Bacteriol.*, **192**, 5709–5717.
 225. Lizewski, S.E., Schurr, J.R., Jackson, D.W., Frisk, A., Carterson, A.J. and Schurr, M.J. (2004) Identification of AlgR-regulated genes in *Pseudomonas aeruginosa* by use of microarray analysis. *J. Bacteriol.*, **186**, 5672–5684.
 226. Remminghorst, U. and Rehm, B.H. (2006) Alg44, a unique protein required for alginate biosynthesis in *Pseudomonas aeruginosa*. *FEBS Lett.*, **580**, 3883–3888.
 227. Hay, I.D., Remminghorst, U. and Rehm, B.H. (2009) MucR, a novel membrane-associated regulator of alginate biosynthesis in *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol.*, **75**, 1110–1120.
 228. Merighi, M., Lee, V.T., Hyodo, M., Hayakawa, Y. and Lory, S. (2007) The second messenger bis-(3'-5')-cyclic-GMP and its PilZ domain-containing receptor Alg44 are required for alginate biosynthesis in *Pseudomonas aeruginosa*. *Mol. Microbiol.*, **65**, 876–895.
 229. Ochsner, U.A., Wilderman, P.J., Vasil, A.I. and Vasil, M.L. (2002) GeneChip expression analysis of the iron starvation response in *Pseudomonas aeruginosa*: identification of novel pyoverdine biosynthesis genes. *Mol. Microbiol.*, **45**, 1277–1287.
 230. Palma, M., Worgall, S. and Quadri, L.E. (2003) Transcriptome analysis of the *Pseudomonas aeruginosa* response to iron. *Arch. Microbiol.*, **180**, 374–379.
 231. Visca, P., Imperi, F. and Lamont, I.L. (2007) Pyoverdine siderophores: from biogenesis to biosignificance. *Trends Microbiol.*, **15**, 22–30.
 232. Poole, K. and McKay, G.A. (2003) Iron acquisition and its control in *Pseudomonas aeruginosa*: many roads lead to Rome. *Front. Biosci.*, **8**, d661–d686.
 233. Ochsner, U.A., Johnson, Z. and Vasil, M.L. (2000) Genetics and regulation of two distinct haem-uptake systems, *phu* and *has*, in *Pseudomonas aeruginosa*. *Microbiology*, **146**, 185–198.
 234. Touati, D. (2000) Iron and oxidative stress in bacteria. *Arch. Biochem. Biophys.*, **373**, 1–6.
 235. Vasil, M.L. and Ochsner, U.A. (1999) The response of *Pseudomonas aeruginosa* to iron: genetics, biochemistry and virulence. *Mol. Microbiol.*, **34**, 399–413.
 236. Pohl, E., Haller, J.C., Mijovilovich, A., Meyer-Klaucke, W., Garman, E. and Vasil, M.L. (2003) Architecture of a protein central to iron homeostasis: crystal structure and spectroscopic analysis of the ferric uptake regulator. *Mol. Microbiol.*, **47**, 903–915.
 237. Wilderman, P.J., Sowa, N.A., FitzGerald, D.J., FitzGerald, P.C., Gottesman, S., Ochsner, U.A. and Vasil, M.L. (2004) Identification of tandem duplicate regulatory small RNAs in *Pseudomonas aeruginosa* involved in iron homeostasis. *Proc. Natl Acad. Sci. USA*, **101**, 9792–9797.
 238. Cornelis, P., Matthijs, S. and Van Oeffelen, L. (2009) Iron uptake regulation in *Pseudomonas aeruginosa*. *Biometals*, **22**, 15–22.
 239. Ochsner, U.A. and Vasil, M.L. (1996) Gene repression by the ferric uptake regulator in *Pseudomonas aeruginosa*: cycle selection of iron-regulated genes. *Proc. Natl Acad. Sci. USA*, **93**, 4409–4414.
 240. Wilderman, P.J., Vasil, A.I., Johnson, Z., Wilson, M.J., Cunliffe, H.E., Lamont, I.L. and Vasil, M.L. (2001) Characterization of an endoprotease (PrpL) encoded by a PvdS-regulated gene in *Pseudomonas aeruginosa*. *Infect. Immun.*, **69**, 5385–5394.
 241. Ochsner, U.A., Johnson, Z., Lamont, I.L., Cunliffe, H.E. and Vasil, M.L. (1996) Exotoxin A production in *Pseudomonas aeruginosa* requires the iron-regulated *pvdS* gene encoding an alternative sigma factor. *Mol. Microbiol.*, **21**, 1019–1028.
 242. Gaines, J.M., Carty, N.L., Tiburzi, F., Davinic, M., Visca, P., Colmer-Hamood, J.A. and Hamood, A.N. (2007) Regulation of the *Pseudomonas aeruginosa* *toxA*, *regA* and *ptxR* genes by the iron-starvation sigma factor PvdS under reduced levels of oxygen. *Microbiology*, **153**, 4219–4233.
 243. Xiong, Y.Q., Vasil, M.L., Johnson, Z., Ochsner, U.A. and Bayer, A.S. (2000) The oxygen- and iron-dependent sigma factor

- pvdS* of *Pseudomonas aeruginosa* is an important virulence factor in experimental infective endocarditis. *J. Infect. Dis.*, **181**, 1020–1026.
244. Singh, P.K., Parsek, M.R., Greenberg, E.P. and Welsh, M.J. (2002) A component of innate immunity prevents bacterial biofilm development. *Nature*, **417**, 552–555.
245. Singh, P.K. (2004) Iron sequestration by human lactoferrin stimulates *P. aeruginosa* surface motility and blocks biofilm formation. *Biometals*, **17**, 267–270.
246. Banin, E., Vasil, M.L. and Greenberg, E.P. (2005) Iron and *Pseudomonas aeruginosa* biofilm formation. *Proc. Natl Acad. Sci. USA*, **102**, 11076–11081.
247. Yang, L., Barken, K.B., Skindersoe, M.E., Christensen, A.B., Givskov, M. and Tolker-Nielsen, T. (2007) Effects of iron on DNA release and biofilm development by *Pseudomonas aeruginosa*. *Microbiology*, **153**, 1318–1328.
248. Bollinger, N., Hassett, D.J., Iglewski, B.H., Costerton, J.W. and McDermott, T.R. (2001) Gene expression in *Pseudomonas aeruginosa*: evidence of iron override effects on quorum sensing and biofilm-specific gene regulation. *J. Bacteriol.*, **183**, 1990–1996.
249. Kim, E.J., Wang, W., Deckwer, W.D. and Zeng, A.P. (2005) Expression of the quorum-sensing regulatory protein LasR is strongly affected by iron and oxygen concentrations in cultures of *Pseudomonas aeruginosa* irrespective of cell density. *Microbiology*, **151**, 1127–1138.
250. Schuster, M., Lostro, C.P., Ogi, T. and Greenberg, E.P. (2003) Identification, timing, and signal specificity of *Pseudomonas aeruginosa* quorum-controlled genes: a transcriptome analysis. *J. Bacteriol.*, **185**, 2066–2079.
251. Juhás, M., Wiehlmann, L., Salunkhe, P., Lauber, J., Buer, J. and Tummli, B. (2005) GeneChip expression analysis of the VqsR regulon of *Pseudomonas aeruginosa* TB. *FEMS Microbiol. Lett.*, **242**, 287–295.
252. Visca, P., Leoni, L., Wilson, M.J. and Lamont, I.L. (2002) Iron transport and regulation, cell signalling and genomics: lessons from *Escherichia coli* and *Pseudomonas*. *Mol. Microbiol.*, **45**, 1177–1190.
253. Hazan, R., He, J., Xiao, G., Dekimpe, V., Apidianakis, Y., Lesic, B., Astrakas, C., Deziel, E., Lepine, F. and Rahme, L.G. (2010) Homeostatic interplay between bacterial cell-cell signaling and iron in virulence. *PLoS Pathog.*, **6**, e1000810.
254. Oglesby, A.G., Farrow, J.M. 3rd, Lee, J.H., Tomaras, A.P., Greenberg, E.P., Pesci, E.C. and Vasil, M.L. (2008) The influence of iron on *Pseudomonas aeruginosa* physiology: a regulatory link between iron and quorum sensing. *J. Biol. Chem.*, **283**, 15558–15567.
255. Bleves, S., Viarre, V., Salacha, R., Michel, G.P., Filloux, A. and Voulhoux, R. (2010) Protein secretion systems in *Pseudomonas aeruginosa*: a wealth of pathogenic weapons. *Int. J. Med. Microbiol.*, **300**, 534–543.
256. Hauser, A.R. (2009) The type III secretion system of *Pseudomonas aeruginosa*: infection by injection. *Nat. Rev. Microbiol.*, **7**, 654–665.
257. Guzzo, J., Pages, J.M., Duong, F., Lazdunski, A. and Murgier, M. (1991) *Pseudomonas aeruginosa* alkaline protease: evidence for secretion genes and study of secretion mechanism. *J. Bacteriol.*, **173**, 5290–5297.
258. Matsumoto, K. (2004) Role of bacterial proteases in pseudomonal and serratal keratitis. *Biol. Chem.*, **385**, 1007–1016.
259. Wandersman, C. and Deleplaire, P. (2004) Bacterial iron sources: from siderophores to hemophores. *Annu. Rev. Microbiol.*, **58**, 611–647.
260. Hood, R.D., Singh, P., Hsu, F., Guvener, T., Carl, M.A., Trinidad, R.R., Silverman, J.M., Ohlson, B.B., Hicks, K.G., Plemel, R.L. et al. (2010) A type VI secretion system of *Pseudomonas aeruginosa* targets a toxin to bacteria. *Cell Host Microbe*, **7**, 25–37.
261. Moscoso, J.A., Mikkelsen, H., Heeb, S., Williams, P. and Filloux, A. (2011) The *Pseudomonas aeruginosa* sensor RetS switches type III and type VI secretion via c-di-GMP signalling. *Environ. Microbiol.*, **13**, 3128–3138.
262. Turner, K.H., Vallet-Gely, I. and Dove, S.L. (2009) Epigenetic control of virulence gene expression in *Pseudomonas aeruginosa* by a LysR-type transcription regulator. *PLoS Genet.*, **5**, e1000779.
263. Pearson, J.P., Pesci, E.C. and Iglewski, B.H. (1997) Roles of *Pseudomonas aeruginosa las* and *rhl* quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. *J. Bacteriol.*, **179**, 5756–5767.
264. Chapon-Herve, V., Akrim, M., Latifi, A., Williams, P., Lazdunski, A. and Bally, M. (1997) Regulation of the *xcp* secretion pathway by multiple quorum-sensing modulons in *Pseudomonas aeruginosa*. *Mol. Microbiol.*, **24**, 1169–1178.
265. Lazdunski, A.M., Ventre, I. and Sturgis, J.N. (2004) Regulatory circuits and communication in Gram-negative bacteria. *Nat. Rev. Microbiol.*, **2**, 581–592.
266. Filloux, A., Bally, M., Soccia, C., Murgier, M. and Lazdunski, A. (1988) Phosphate regulation in *Pseudomonas aeruginosa*: cloning of the alkaline phosphatase gene and identification of *phoB*- and *phoR*-like genes. *Mol. Gen. Genet.*, **212**, 510–513.
267. Ball, G., Durand, E., Lazdunski, A. and Filloux, A. (2002) A novel type II secretion system in *Pseudomonas aeruginosa*. *Mol. Microbiol.*, **43**, 475–485.
268. Llamas, M.A., van der Sar, A., Chu, B.C., Sparrius, M., Vogel, H.J. and Bitter, W. (2009) A novel extracytoplasmic function (ECF) sigma factor regulates virulence in *Pseudomonas aeruginosa*. *PLoS Pathog.*, **5**, e1000572.
269. Lesic, B., Starkey, M., He, J., Hazan, R. and Rahme, L.G. (2009) Quorum sensing differentially regulates *Pseudomonas aeruginosa* type VI secretion locus I and homologous loci II and III, which are required for pathogenesis. *Microbiology*, **155**, 2845–2855.
270. Mougous, J.D., Cuff, M.E., Raunser, S., Shen, A., Zhou, M., Gifford, C.A., Goodman, A.L., Joachimiak, G., Ordóñez, C.L., Lory, S. et al. (2006) A virulence locus of *Pseudomonas aeruginosa* encodes a protein secretion apparatus. *Science*, **312**, 1526–1530.
271. Yahr, T.L. and Wolfgang, M.C. (2006) Transcriptional regulation of the *Pseudomonas aeruginosa* type III secretion system. *Mol. Microbiol.*, **62**, 631–640.
272. Frank, D.W. (1997) The exoenzyme S regulon of *Pseudomonas aeruginosa*. *Mol. Microbiol.*, **26**, 621–629.
273. Vallis, A.J., Yahr, T.L., Barbieri, J.T. and Frank, D.W. (1999) Regulation of ExoS production and secretion by *Pseudomonas aeruginosa* in response to tissue culture conditions. *Infect. Immun.*, **67**, 914–920.
274. Shen, D.K., Filopon, D., Kuhn, L., Polack, B. and Toussaint, B. (2006) PsrA is a positive transcriptional regulator of the type III secretion system in *Pseudomonas aeruginosa*. *Infect. Immun.*, **74**, 1121–1129.
275. Hogardt, M., Roeder, M., Schreff, A.M., Eberl, L. and Heesemann, J. (2004) Expression of *Pseudomonas aeruginosa* *exoS* is controlled by quorum sensing and RpoS. *Microbiology*, **150**, 843–851.
276. Dasgupta, N., Lykken, G.L., Wolfgang, M.C. and Yahr, T.L. (2004) A novel anti-anti-activator mechanism regulates expression of the *Pseudomonas aeruginosa* type III secretion system. *Mol. Microbiol.*, **53**, 297–308.
277. Wu, W. and Jin, S. (2005) PtrB of *Pseudomonas aeruginosa* suppresses the type III secretion system under the stress of DNA damage. *J. Bacteriol.*, **187**, 6058–6068.
278. Hornef, M.W., Roggenkamp, A., Geiger, A.M., Hogardt, M., Jacobi, C.A. and Heesemann, J. (2000) Triggering the ExoS regulon of *Pseudomonas aeruginosa*: a GFP-reporter analysis of exoenzyme (Exo) S, ExoT and ExoU synthesis. *Microb. Pathog.*, **29**, 329–343.
279. Rietsch, A. and Mekalanos, J.J. (2006) Metabolic regulation of type III secretion gene expression in *Pseudomonas aeruginosa*. *Mol. Microbiol.*, **59**, 807–820.
280. Dacheux, D., Epaulard, O., de Groot, A., Guery, B., Leberre, R., Attree, I., Polack, B. and Toussaint, B. (2002) Activation of the *Pseudomonas aeruginosa* type III secretion system requires an intact pyruvate dehydrogenase *aceAB* operon. *Infect. Immun.*, **70**, 3973–3977.
281. Wu, W., Badrane, H., Arora, S., Baker, H.V. and Jin, S. (2004) MucA-mediated coordination of type III secretion and alginate synthesis in *Pseudomonas aeruginosa*. *J. Bacteriol.*, **186**, 7575–7585.

282. Jin, Y., Yang, H., Qiao, M. and Jun, S. (2011) MexT regulates the type III secretion system through MexS and PtrC in *Pseudomonas aeruginosa*. *J. Bacteriol.*, **193**, 399–410.
283. Mulcahy, H., O'Callaghan, J., O'Grady, E.P., Adams, C. and O'Gara, F. (2006) The posttranscriptional regulator RsmA plays a role in the interaction between *Pseudomonas aeruginosa* and human airway epithelial cells by positively regulating the type III secretion system. *Infect. Immun.*, **74**, 3012–3015.
284. O'Callaghan, J., Reen, F.J., Adams, C. and O'Gara, F. (2011) Low oxygen induces the type III secretion system in *Pseudomonas aeruginosa* via modulation of the small RNAs *rsmZ* and *rsmY*. *Microbiology*, **157**, 3417–3428.
285. Dubnau, D. and Losick, R. (2006) Bistability in bacteria. *Mol. Microbiol.*, **61**, 564–572.
286. Storz, G., Altuvia, S. and Wassarman, K.M. (2005) An abundance of RNA regulators. *Annu. Rev. Biochem.*, **74**, 199–217.
287. Papenfort, K. and Vogel, J. (2009) Multiple target regulation by small noncoding RNAs rewires gene expression at the post-transcriptional level. *Res. Microbiol.*, **160**, 278–287.
288. Gottesman, S. (2004) The small RNA regulators of *Escherichia coli*: roles and mechanisms. *Annu. Rev. Microbiol.*, **58**, 303–328.
289. Ferrara, S., Brugnoli, M., De Bonis, A., Righetti, F., Delvillani, F., Deho, G., Horner, D., Briani, F. and Bertonni, G. (2012) Comparative profiling of *Pseudomonas aeruginosa* strains reveals differential expression of novel unique and conserved small RNAs. *PLoS One*, **7**, e36553.
290. Heurlier, K., Williams, F., Heeb, S., Dormond, C., Pessi, G., Singer, D., Camara, M., Williams, P. and Haas, D. (2004) Positive control of swarming, rhamnolipid synthesis, and lipase production by the posttranscriptional RsmA/RsmZ system in *Pseudomonas aeruginosa* PAO1. *J. Bacteriol.*, **186**, 2936–2945.
291. Heeb, S., Blumer, C. and Haas, D. (2002) Regulatory RNA as mediator in GacA/RsmA-dependent global control of exoproduct formation in *Pseudomonas fluorescens* CHA0. *J. Bacteriol.*, **184**, 1046–1056.
292. Valverde, C., Heeb, S., Keel, C. and Haas, D. (2003) RsmY, a small regulatory RNA, is required in concert with RsmZ for GacA-dependent expression of biocontrol traits in *Pseudomonas fluorescens* CHA0. *Mol. Microbiol.*, **50**, 1361–1379.
293. Kay, E., Dubuis, C. and Haas, D. (2005) Three small RNAs jointly ensure secondary metabolism and biocontrol in *Pseudomonas fluorescens* CHA0. *Proc. Natl Acad. Sci. USA*, **102**, 17136–17141.
294. Heeb, S., Kuehne, S.A., Bycroft, M., Crivii, S., Allen, M.D., Haas, D., Camara, M. and Williams, P. (2006) Functional analysis of the post-transcriptional regulator RsmA reveals a novel RNA-binding site. *J. Mol. Biol.*, **355**, 1026–1036.
295. Kay, E., Humair, B., Denervaud, V., Riedel, K., Spahr, S., Eberl, L., Valverde, C. and Haas, D. (2006) Two GacA-dependent small RNAs modulate the quorum-sensing response in *Pseudomonas aeruginosa*. *J. Bacteriol.*, **188**, 6026–6033.
296. Bordi, C., Lamy, M.C., Ventre, I., Termine, E., Hachani, A., Fillet, S., Roche, B., Blevès, S., Mejean, V., Lazdunski, A. et al. (2010) Regulatory RNAs and the HptB/RetS signalling pathways fine-tune *Pseudomonas aeruginosa* pathogenesis. *Mol. Microbiol.*, **76**, 1427–1443.
297. Hsu, J.L., Chen, H.C., Peng, H.L. and Chang, H.Y. (2008) Characterization of the histidine-containing phosphotransfer protein B-mediated multistep phosphorelay system in *Pseudomonas aeruginosa* PAO1. *J. Biol. Chem.*, **283**, 9933–9944.
298. Petrova, O.E. and Sauer, K. (2010) The novel two-component regulatory system BfiSR regulates biofilm development by controlling the small RNA *rsmZ* through CafA. *J. Bacteriol.*, **192**, 5275–5288.
299. Brencic, A., McFarland, K.A., McManus, H.R., Castang, S., Mogno, L., Dove, S.L. and Lory, S. (2009) The GacS/GacA signal transduction system of *Pseudomonas aeruginosa* acts exclusively through its control over the transcription of the RsmY and RsmZ regulatory small RNAs. *Mol. Microbiol.*, **73**, 434–445.
300. Lapouge, K., Schubert, M., Allain, F.H. and Haas, D. (2008) Gac/Rsm signal transduction pathway of gamma-proteobacteria: from RNA recognition to regulation of social behaviour. *Mol. Microbiol.*, **67**, 241–253.
301. Sorger-Domenigg, T., Sonnleitner, E., Kaberdin, V.R. and Blasi, U. (2007) Distinct and overlapping binding sites of *Pseudomonas aeruginosa* Hfq and RsmA proteins on the non-coding RNA RsmY. *Biochem. Biophys. Res. Commun.*, **352**, 769–773.
302. Moreno, R., Marzi, S., Romby, P. and Rojo, F. (2009) The Crc global regulator binds to an unpaired A-rich motif at the *Pseudomonas putida* *alkS* mRNA coding sequence and inhibits translation initiation. *Nucleic Acids Res.*, **37**, 7678–7690.
303. Moreno, R. and Rojo, F. (2008) The target for the *Pseudomonas putida* Crc global regulator in the benzoate degradation pathway is the BenR transcriptional regulator. *J. Bacteriol.*, **190**, 1539–1545.
304. Moreno, R., Ruiz-Manzano, A., Yuste, L. and Rojo, F. (2007) The *Pseudomonas putida* Crc global regulator is an RNA binding protein that inhibits translation of the AlkS transcriptional regulator. *Mol. Microbiol.*, **64**, 665–675.
305. Sonnleitner, E., Abdou, L. and Haas, D. (2009) Small RNA as global regulator of carbon catabolite repression in *Pseudomonas aeruginosa*. *Proc. Natl Acad. Sci. USA*, **106**, 21866–21871.
306. Yeung, A.T., Bains, M. and Hancock, R.E. (2011) The sensor kinase CbrA is a global regulator that modulates metabolism, virulence, and antibiotic resistance in *Pseudomonas aeruginosa*. *J. Bacteriol.*, **193**, 918–931.
307. Abdou, L., Chou, H.T., Haas, D. and Lu, C.D. (2011) Promoter recognition and activation by the global response regulator CbrB in *Pseudomonas aeruginosa*. *J. Bacteriol.*, **193**, 2784–2792.
308. Li, W. and Lu, C.D. (2007) Regulation of carbon and nitrogen utilization by CbrAB and NtrBC two-component systems in *Pseudomonas aeruginosa*. *J. Bacteriol.*, **189**, 5413–5420.
309. Nishijyo, T., Haas, D. and Itoh, Y. (2001) The CbrA-CbrB two-component regulatory system controls the utilization of multiple carbon and nitrogen sources in *Pseudomonas aeruginosa*. *Mol. Microbiol.*, **40**, 917–931.
310. Rietsch, A., Wolfgang, M.C. and Mekalanos, J.J. (2004) Effect of metabolic imbalance on expression of type III secretion genes in *Pseudomonas aeruginosa*. *Infect. Immun.*, **72**, 1383–1390.
311. Linares, J.F., Moreno, R., Fajardo, A., Martínez-Solano, L., Escalante, R., Rojo, F. and Martínez, J.L. (2010) The global regulator Crc modulates metabolism, susceptibility to antibiotics and virulence in *Pseudomonas aeruginosa*. *Environ. Microbiol.*, **12**, 3196–3212.
312. O'Toole, G.A., Gibbs, K.A., Hager, P.W., Phibbs, P.V. Jr and Kolter, R. (2000) The global carbon metabolism regulator Crc is a component of a signal transduction pathway required for biofilm development by *Pseudomonas aeruginosa*. *J. Bacteriol.*, **182**, 425–431.
313. Sonnleitner, E., Romeo, A. and Blasi, U. (2012) Small regulatory RNAs in *Pseudomonas aeruginosa*. *RNA Biol.*, **9**, 364–371.
314. Sonnleitner, E., Gonzalez, N., Sorger-Domenigg, T., Heeb, S., Richter, A.S., Backofen, R., Williams, P., Huttenhofer, A., Haas, D. and Blasi, U. (2011) The small RNA PhrS stimulates synthesis of the *Pseudomonas aeruginosa* quinolone signal. *Mol. Microbiol.*, **80**, 868–885.
315. Moll, I., Afonyushkin, T., Vytvytska, O., Kaberdin, V.R. and Blasi, U. (2003) Coincident Hfq binding and RNase E cleavage sites on mRNA and small regulatory RNAs. *RNA*, **9**, 1308–1314.
316. Masse, E. and Gottesman, S. (2002) A small RNA regulates the expression of genes involved in iron metabolism in *Escherichia coli*. *Proc. Natl Acad. Sci. USA*, **99**, 4620–4625.
317. Vasil, M.L. (2007) How we learnt about iron acquisition in *Pseudomonas aeruginosa*: a series of very fortunate events. *Biometals*, **20**, 587–601.
318. Oglesby-Sherrouse, A.G. and Vasil, M.L. (2010) Characterization of a heme-regulated non-coding RNA encoded by the *prfF* locus of *Pseudomonas aeruginosa*. *PLoS One*, **5**, e9930.
319. Bornholdt, S. (2008) Boolean network models of cellular regulation: prospects and limitations. *J. R. Soc. Interface*, **5**, S85–S94.
320. Kauffman, S.A. (1969) Metabolic stability and epigenesis in randomly constructed genetic nets. *J. Theor. Biol.*, **22**, 437–467.
321. Thomas, R. (1973) Boolean formalization of genetic control circuits. *J. Theor. Biol.*, **42**, 563–585.
322. Shmulevich, I., Dougherty, E.R. and Zhang, W. (2002) Gene perturbation and intervention in probabilistic Boolean networks. *Bioinformatics*, **18**, 1319–1331.

323. Shmulevich, I., Dougherty, E.R., Kim, S. and Zhang, W. (2002) Probabilistic Boolean Networks: a rule-based uncertainty model for gene regulatory networks. *Bioinformatics*, **18**, 261–274.
324. Liang, J. and Han, J. (2012) Stochastic Boolean networks: an efficient approach to modeling gene regulatory networks. *BMC Syst. Biol.*, **6**, 113.
325. Babu, M.M. (2010) Early Career Research Award Lecture. Structure, evolution and dynamics of transcriptional regulatory networks. *Biochem. Soc. Trans.*, **38**, 1155–1178.
326. Babu, M.M., Lang, B. and Aravind, L. (2009) Methods to reconstruct and compare transcriptional regulatory networks. *Methods Mol. Biol.*, **541**, 163–180.
327. Oestreich, C. (2007) A history of chaos theory. *Dialogues Clin. Neurosci.*, **9**, 279–289.
328. Goldbeter, A. (1996) *Biochemical Oscillations and Cellular Rhythms*. Cambridge University Press, Cambridge, UK.
329. Novak, B. and Tyson, J.J. (2008) Design principles of biochemical oscillators. *Nat. Rev. Mol. Cell. Biol.*, **9**, 981–991.
330. Leloup, J.C. and Goldbeter, A. (1999) Chaos and birhythmicity in a model for circadian oscillations of the PER and TIM proteins in drosophila. *J. Theor. Biol.*, **198**, 445–459.
331. Suguna, C., Chowdhury, K.K. and Sinha, S. (1999) Minimal model for complex dynamics in cellular processes. *Phys. Rev. E Stat. Phys. Plasmas Fluids Relat. Interdiscip. Topics*, **60**, 5943–5949.
332. Zhang, Z., Ye, W., Qian, Y., Zheng, Z., Huang, X. and Hu, G. (2012) Chaotic motifs in gene regulatory networks. *PLoS One*, **7**, e39355.
333. Trosko, J.E. and Ruch, R.J. (1998) Cell-cell communication in carcinogenesis. *Front Biosci.*, **3**, d208–d236.
334. Thomas, R. and D’Ari, R. (1990) *Biological Feedback*. CRC Press, Boca Raton, FL.
335. Ng, W.L., Perez, L., Cong, J., Semmelhack, M.F. and Bassler, B.L. (2012) Broad spectrum pro-quorum-sensing molecules as inhibitors of virulence in vibrios. *PLoS Pathog.*, **8**, e1002767.
336. Wiedenheft, B., Sternberg, S.H. and Doudna, J.A. (2012) RNA-guided genetic silencing systems in bacteria and archaea. *Nature*, **482**, 331–338.
337. Sonnleitner, E., Sorger-Domenigg, T., Madej, M.J., Findeiss, S., Hackermuller, J., Huttenhofer, A., Stadler, P.F., Blasi, U. and Moll, I. (2008) Detection of small RNAs in *Pseudomonas aeruginosa* by RNomics and structure-based bioinformatic tools. *Microbiology*, **154**, 3175–3187.