This is a pre- or post-print of an article published in

Brouwer, S., Pustelny, C., Ritter, C., Klinkert, B., Narberhaus, F., Häussler, S.
The PqsR and RhlR transcriptional regulators determine the level of Pseudomonas quinolone signal synthesis in Pseudomonas aeruginosa by producing two different pqsABCDE mRNA Isoforms
(2014) Journal of Bacteriology, 196 (23), pp. 4163-4171.

The PqsR and RhlR transcriptional regulators determine the level of PQS synthesis in *Pseudomonas aeruginosa* by producing two different *pqsABCDE* mRNA isoforms

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Keywords: Quorum sensing, PQS signaling, posttranscriptional regulation, RNA structure

Abstract

Regulation of gene expression plays a key role in bacterial adaptability to changes in the environment. An integral part of this gene regulatory network is achieved via quorum sensing (QS) systems that coordinate bacterial responses under high cellular densities. In the nosocomial pathogen *Pseudomonas aeruginosa* the 2-alkyl-4-quinolone (*pqs*) signaling pathway is crucial for bacterial survival under stressful conditions. Biosynthesis of the *Pseudomonas* Quinolone Signal (PQS) is dependent on the *pqsABCDE* operon, which is positively regulated by the LysR-family regulator PqsR and repressed by the transcriptional regulator protein RhlR. However, the molecular mechanisms underlying this inhibition have remained elusive. Here, we demonstrate that not only PqsR but also RhlR activates transcription of *pqsA*. The latter uses an alternative transcriptional start site and induces expression of a longer transcript that forms a secondary structure in the 5'-untranslated leader region. As a consequence access of the ribosome to the Shine-Dalgarno sequence is restricted and translation efficiency reduced. Our finding of a competition between RhlR and PqsR for transcription initiation of the *pqsA-E* operon underlines that *P. aeruginosa* uses various levels of regulation to fine-tune PQS synthesis.

Introduction

The opportunistic pathogen *Pseudomonas aeruginosa* is a ubiquitous bacterium which is able to thrive in a wide variety of environments. It is frequently associated with severe nosocomial infections and is found to be the leading cause of morbidity and mortality among people with cystic fibrosis (1). The remarkable ecological success of *P. aeruginosa* can be attributed to its large metabolic versatility and its sophisticated quorum sensing (QS) network. This cell-to-cell communication enables *P. aeruginosa* to control expression of numerous virulence factors and is involved in biofilm formation, thus facilitating establishment of acute and chronic infections.

QS in *P. aeruginosa* is tightly regulated by at least three different systems organized in a hierarchical manner. The AHL-dependent *las* system is considered to stand at the top of the hierarchy. It is composed of the LuxRI homologues LasR and LasI. The signal synthase LasI directs the synthesis of *N*-(3-oxododecanoyl)-L-homserine lactone (3-oxo-C12-HSL), which is the ligand of the LasR receptor (2, 3). The *las* system is interconnected with the *rhl* system as LasR bound to its autoinducer 3-oxo-C12-HSL induces expression of RhlRI (4). LasR-3-oxo-C12-HSL and RhlR-C4-HSL direct transcription of several QS-regulated virulence factors, including pyocyanin, hydrogen cyanide and exotoxin A (5, 6). In addition, *P. aeruginosa* possesses a third QS system, *pqs*, based on 2-alkyl-4-quinolone (AQ) signal molecules (7). The *Pseudomonas* Quinolone Signal (PQS) and its direct precursor 2-heptyl-4-quinolone (HHQ) are active members of the over 50 different AQs produced by *P. aeruginosa* (8). Like AHLs, they play an important role in the expression of several virulence factors as well as in inducing a protective stress response towards deteriorating environmental conditions (9–11). Investigations of PQS biosynthesis revealed that the major synthase genes are arranged in a polycistronic operon (*pqsABCDE*) and that transcription of this operon is under control of PqsR, the Lys-R type transcriptional regulator of the *pqs* system (12, 13). PqsR, which is activated by PQS binding (14), plays a critical role in the pathogenicity of *P. aeruginosa* and is regulated by both the *las* and *rhl* system (13, 14) and the small RNA PhrS (15). While LasR activates *pqsR* transcription and subsequently enhances *pqsA-E* expression, RhlR was found to be a repressor of *pqsR* transcription (14). Interestingly, RhlR was also found to inhibit *pqsA-E* expression by binding to a *las/rhl* box centred at -311 bp upstream of the *pqsA* transcriptional initiation site (16). Recently, we could show that there is an alternative transcriptional start site (*pqsA*-339) just downstream of the RhlR-binding site (17), indicating that repression of PqsA expression via RhlR might be posttranscriptional.

Posttranscriptional repression events in prokaryotes mainly occur either by binding of small regulatory RNAs to target mRNA molecules or by formation of secondary structures in the mRNA, which play an important role in posttranscriptional regulation of gene expression in bacteria (18, 19). One common form of an RNA regulatory element is the so called riboswitch. These regulatory elements usually reside in the non-coding region of the mRNA and regulate gene expression by forming alternative structures in response to binding of a specific metabolite (20). Bacteria commonly mask the Shine-Dalgarno (SD) sequence to block access of the 30S ribosomal subunit. With this, translation initiation becomes highly dependent on the folding structure of the initiation region of the mRNA (21). A well-studied example of such a regulatory mechanism includes RNA thermosensors. They form a zipper-like structure which unwinds with increasing temperature allowing successful binding of the ribosome (22). For example, in *Yersinia* the virulence factor LcrF is expressed at 37°C, but access to the SD sequence is abolished at 26°C (23, 24). Thermosensors are also known to occur in *Pseudomonas* species. Recently, the bacterial small heat shock protein IbpA was found to be under control of two temperature-sensitive hairpin structures in the 5'-UTR of *ibpA* in *P. aeruginosa* (25).

In the present study, we show that repression of PQS biosynthesis by RhlR in *P. aeruginosa* is due to conformational masking of the translation initiation site of the *pqsA-E* transcript. We demonstrate that RhlR promotes transcription of *pqsA-E* from an alternative transcriptional start site. The resulting long 5'-UTR folds into a structure, which hinders association of the 30S ribosomal subunit with the mRNA and impedes translation initiation.

Results

RhlR induces transcription of the PQS biosynthetic operon via an alternative transcriptional start site

In an early attempt to investigate regulation of PQS synthesis, RhlR-C4-HSL was found to negatively regulate *pqsR* transcription and thus to inhibit PQS synthesis (14). Later, Xiao and colleagues showed binding of RhlR to a *las/rhl* box in the 5'-leader sequence of *pqsA* (Fig.1A) (15). Deletion of the entire *las/rhl* box significantly increased *pqsA* transcription in *P. aeruginosa* wild-type but not in *rhlR*$^-$ mutant cells. It was therefore suggested that binding of RhlR to the *las/rhl* box lowers *pqsA* transcription (16).

To address this further, we generated *luxCDABE* promoter fusions that contained the recently predicted *pqsA*-339 (17) (p339), and the *pqsA*-71 (p71) transcription initiation site of the *pqsA-E* operon respectively (Fig.1B,C). Expression of *lux* in the *P. aeruginosa* PA14 parental strain and *rhlR*$^-$ and *pqsR*$^-$ mutant strains was monitored during exponential growth phase where the promoters have been predicted to be active (17). Consistent with Dötsch and colleagues (17), we found expression from the region containing *pqsA*-339 (Fig.2). RhlR acts as transcriptional activator of *pqsA*-339 and not as a repressor of *pqsA* transcription. While both promoters were active in the parental strain, p71 luminescence was only detected in PA14_*pqsR* while absent in PA14_*rhlR*. Vice versa *pqsA*-71 was only active in PA14_*rhlR* but silent in the absence of PqsR, demonstrating that induction of *pqsA*-339 is strictly dependent on RhlR.

The RhlR-dependent long 5'-UTR of *pqsA* blocks the translation initiation site

Since RhlR is an activator of *pqsA* transcription but represses PQS production, we hypothesized that base-pairing in the translation initiation region of the longer transcript might result in gene silencing due to inaccessibility of the SD sequence to the 30S ribosomal subunit. To test this hypothesis, we predicted the secondary structures of the *pqsA*-71 induced 5'-leader sequence (Fig.3A, short) and of a longer construct containing six additional nucleotides present in the *pqsA*-339 transcript (Fig.3A, long). In contrast to the short mRNA the long RhlR-induced transcript is predicted to sequester the SD sequence.

We next analyzed the thermal stability of 71–mer single-stranded DNA oligonucleotides equivalent to the sequence of the mRNAs (Fig.3B). To equate the expansion at the 5'-end of

the 'long' RhlR-induced primer (which comprises the entire sequence predicted to participate in hairpin formation), six nucleotides (TTCTGT) were added to the 3'-end of the 'short' primer. Both unfolding curves can be best explained by two separate unfolding events. Strikingly, the 'long' primer underwent thermal unfolding at significantly higher temperatures (44°C and 46°C) and with greater cooperativity than the 'short' oligonucleotide (26°C and 44°C). This strongly supports formation of a stable secondary structure in the RhlR-mediated transcript of *pqsA-E*. This effect could be reversed by site-specific mutagenesis of five nucleotides upstream of *pqsA*-71, responsible for base pairing with the SD region, with random non-complementary nucleotides (CGTTC replaced by AAGAA).

To examine a potential inhibitory role of the secondary structure in the 5'-UTR of *pqsA* on translation efficiency, we generated additional promoter-fusion constructs (Fig.1C). The construct pshort contains both transcriptional start sites. plong harbors additionally the 70 nucleotides downstream of *pqsA*-71 containing the authentic RBS. Intriguingly, the presence of the 70 nucleotides significantly reduced ($p < 0.05$) luminescence in the wild-type strain and completely abolished luminescence in PA14_*pqsR* (Fig.4). In contrast, PqsR-dependent transcription in the *rhlR*⁻ mutant fully recovered luminescence and both constructs displayed similar activity. Interestingly, activation of *pqsA*-71 by PqsR in pshort was significantly enhanced in the absence of RhlR as compared to the wild-type ($p < 0.01$). The present data support our hypothesis, that RhlR mediates posttranscriptional control on *pqsA-E* expression by extending the 5'-UTR of *pqsA* that leads to alterations in the folding pattern of the mRNA in a way that inhibits efficient translation.

RhlR-induced transcription of the *pqs* operon abolishes efficient translation of the mRNA

Our data imply that formation of a secondary structure within the RhlR-induced long *pqsA* transcript inhibits *pqsA* translation. This effect should be reversed by site-specific mutagenesis liberating the SD sequence as depicted in Fig.3A. To test this, we analyzed PqsA expression *in vivo* using his-tagged *pqsA* whose expression was under control of the *lac* promoter but dependent on accessibility of the native SD sequence. Levels of PqsA-His were monitored during exponential growth in both *P. aeruginosa* PA14 and *E. coli* BL21 (Fig.5A). Strikingly, we were unable to detect PqsA-His in cells harboring the native RhlR-induced sequence while secondary structure destabilization in the derepressed construct drastically

177 restored PqsA production in both bacterial strains indicating that it is a general phenomenon
178 not dependent on a *Pseudomonas*-specific factor.

179 To further assess the exact molecular role of the hairpin loop we performed primer extension
180 inhibition (toeprinting) experiments to examine binding of the 30S ribosomal subunit to an
181 mRNA molecule. In this method, ribosome-mRNA complex formation inhibits the primer
182 extension reaction resulting in a terminated product (toeprint) around position +17 with
183 respect to the translational start site. We used the assay to investigate the ability of the
184 ribosome to recognize the SD sequence and to form a translation initiation complex upstream
185 of *pqsA*. Efficiency of ribosome binding was compared between the following *in vitro*
186 transcribed mRNAs: (1) PqsR-induced *pqsA* transcript, (2) RhlR-induced *pqsA* transcript, and
187 (3) a mutated RhlR-induced mRNA exhibiting a destabilized secondary structure (Fig.5B).
188 Consistent with impaired translation from the long transcript, a toeprint at position +17 was
189 detectable in the PqsR-induced *pqsA* transcript but absent in the RhlR-induced *pqsA* transcript
190 (Fig.5C). Binding of the ribosome was re-established upon destabilization of the secondary
191 structure. This clearly demonstrates that the secondary structure of the RhlR-induced *pqsA*
192 transcript, which comprises the SD sequence, prevents formation of the pre-initiation
193 complex. Additional reverse transcription products are indicative of additional double-
194 stranded regions able to terminate reverse transcription (structures 1 and 2 in Fig.5C).
195 Together these data demonstrate that RhlR mediates post-transcriptional repression of PQS
196 synthesis by initiating a long *pqsA* transcript, in which the SD sequence is sequestered in a
197 secondary structure.

198

199 ## Discussion

200 Pathogens have developed mechanisms to persist and survive in various environments
201 including the human host. In *P. aeruginosa*, the production of the inter-bacterial signal
202 molecule PQS is critical for survival under deteriorating conditions. PQS itself is a
203 multifunctional molecule acting as a QS signal molecule (26), it has an iron-chelating activity
204 and is essential for biofilm formation (6, 10). Furthermore, PQS plays a pivotal role in tuning
205 cellular physiology and has been implicated in cell death under stressful conditions (6, 27).
206 Recently, PQS was suggested to act as both a pro-oxidant and an inducer of an anti-oxidative
207 stress response (9), emphasizing the importance of this molecule in environmental adaptation
208 of *P. aeruginosa*. Therefore, expression of PQS needs to be strictly controlled.

The complex regulatory circuit of PQS synthesis involves the LysR-type transcriptional regulator protein PqsR, which recognizes and binds the signal molecule PQS and subsequently enhances transcription of the 4-quinolone biosynthetic operon *pqsA-D* thus forming a positive autoregulatory loop (14). Expression of *pqsR* in turn is controlled by the *las* and *rhl* QS systems, interconnecting all three QS systems of *P. aeruginosa*. However, the transcriptional regulator of the *rhl* system, RhlR, was also shown to directly exert control on PQS biosynthesis by binding to the *pqsA-E* promoter region (16). The present study provides molecular insights into this mechanism.

The discovery of an alternative transcriptional start site (*pqsA*-339) of the *pqsA-E* operon suggested an additional level of direct transcriptional regulation of the PQS system in *P. aeruginosa* (17). The fact that a RhlR-binding box is located just upstream of this transcriptional start site lead us to hypothesize that RhlR might be the transcriptional regulator of this promoter. Indeed, in the present study we demonstrate that RhlR is actually a transcriptional activator of *pqsA-E* and initiates transcription from *pqsA*-339. Further analyses revealed that RhlR binding to the *pqsA* promoter region induces the transcription of a *pqsA-E* mRNA with an extended 5´-UTR that exhibits a stable secondary structure, which sequesters the SD sequence and inhibits translation initiation of *pqsA*. As a consequence, a PA14_*pqsR* mutant displayed transcriptional activation of *pqsA*-339 but failed to efficiently induce production of PqsA. These data clearly show that RhlR competes with PqsR for binding to the promoter region of *pqsA* (Fig.6). By inducing a longer *pqsA-E* transcript, RhlR prevents translation of the *pqsA* gene, whose product is responsible for priming anthranilate for entry into the PQS biosynthetic pathway and whose deletion is known to impede PQS production (28, 29). Hence, depending on the presence of sufficient intra- and extracellular concentrations of the signal molecules C4-HSL and/or PQS, the PQS signaling pathway can either be induced by PqsR or inhibited by RhlR. The control of PQS expression by two transcriptional regulators of different QS systems thus allows *P. aeruginosa* to fine-tune PQS signaling in response to cell density and environmental stimuli.

Many bacterial mRNAs have been described to harbor structured elements in their 5′-leader sequence that control translation (30). Here, to our knowledge, we report for the first time on the modulation of bacterial protein levels via the production of two mRNA isoforms. Those isoforms form variable secondary structures and thus are translated at variable efficiency. We found that the competition between two transcription factors for transcription initiation result in alternative transcripts that have profound effects on cell-signaling dependent phenotypes.

Although not previously described in bacteria, different mRNA isoforms are well-known to play an important role in the regulation of translation in eukaryotes. In mammalians up to 10-18% of all genes use multiple promoters (31). For instance, the *axin2* gene, which is involved in early post-natal development and tumor suppression, has three promoters whose expression is strictly tissue-specific (32, 33). The 5´-UTR of each mRNA isoform affects translation efficiency and mRNA stability due to the formation of different secondary structures. Another example in eukaryotes is the tumor suppressor gene *brca1* where transcription is induced from two separate promoters. Here, as observed for *pqsA* mRNA translation, a longer 5´-UTR of the *brca1* mRNA is translated at lower efficiency due to formation of a stable secondary structure (34). Taken together, this study sheds light on the posttranscriptional regulation of PQS synthesis and illustrates that the promoter region of *pqsA* represents a major site of transcriptional and translational control. Differential secondary structures in mRNA isoforms that directly impact on translational efficiency of genes - a common mechanism of regulation in eukaryotes - might represent an underestimated mechanism of posttranscriptional control in bacteria and might play a more important role in bacterial adaptation than previously anticipated.

Materials and Methods

Bacterial strains and growth conditions

Unless otherwise noted, bacterial strains listed in Table 1 were grown in Luria broth (LB) medium at 37°C and shaking at 180 rpm. *E. coli* DH5*α* was routinely used for subcloning and propagation. For plasmid selection and maintenance, antibiotics were added at the following final concentrations (mg/ml): for *E. coli*, ampicillin 100; tetracycline 12.5; gentamicin 15; for *P. aeruginosa*, carbenicillin 400; tetracycline 100; gentamicin 30.

Table 1. Bacterial strains and plasmids

Strain or plasmid	Relevant feature(s)	Source or reference
Strains		
E. coli DH5α	F$^-$ *endA1 gln*V44 *thi-1 recA1 relA1 gyr*A96 *deoR nupG* Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*) U169, *hsdR*17 ($r_K^-m_K^+$), λ-	(35)
E. coli BL21 [DE3]	F- *ompT hsdS*$_B$ (r_B^- m_B^-) gal dcm	Stratagene
E. coli S17-1	Mobilizing strain for RP4 Mob-containing plasmids	
PA14	Wild-type	(36)
PA14_*pqsR*	*pqsR* knockout mutant	This study
PA14_*rhlR*	*rhlR* knockout mutant	This study
Plasmids		
pEX18Ap	Gene replacement vector with MCS from pUC18, *oriT*$^+$ *sacB*$^+$, Amp^R	(37)
pEX18Ap2	pEX18TAp derivative, 845bp fragment containing 5S rRNA and *lacZ*-alpha genes and MCS removed by inverse PCR, novel MCS generated with unique restriction sites for XhoI, PstI, SmaI/XmaI, XbaI, SacI, HindIII, NheI, NotI, MluI, KpnI, BamHI, EcoRI, Amp^R	(38)
pEX18Ap-Δ*pqsR*::FRT-Gm	Gene replacement vector for PA14 *pqsR* containing a FRT-Gm cassette, Amp^R	This study
pEX18Ap2-Δ*rhlR*::FRT-Gm	Gene replacement vector for PA14 *rhlR* containing a FRT-Gm cassette, Amp^R	This study
pFLP3	FLP expression vector, *sacB*$^+$ *oriT*$^+$, Amp^R Tc^R	(39)
pBBR1-MCS5-Terminator-RBS-Lux (pMTRL)	Broad-host-range low-copy-number vector pBBR1-MCS5 harboring *luxCDABE* and terminators lambda T0 rrnB1 T1 for plasmid-based transcriptional fusions, Gm^R	(40)
p339	-501 to -338 fragment upstream of *pqsA* cloned into pMTRL	This study

	using SpeI and PstI sites, Gm^R	
p71	-256 to -70 fragment upstream of *pqsA* cloned into pMTRL using SpeI and PstI sites, Gm^R	This study
pshort	-501 to -70 fragment upstream of *pqsA* cloned into pMTRL using SpeI and PstI sites, Gm^R	This study
plong	-501 to +1 fragment upstream of *pqsA* cloned into pMTRL using SpeI and PstI sites, Gm^R	This study
pUCP20	*Escherichia-Pseudomonas* shuttle vector with beta-lactamase (*bla*) and LacZ alpha peptide (*lacZ* alpha) genes; Ap^R/Cb^R	(41)
pUCP20-TOE*pqsA*_(1)	-72 to +60 fragment of *pqsA* containing a T7 promoter (5'-GAA ATTAATACGACTCACTATAGG-3') and a EcoRI restriction site at the 3'-end cloned into pUCP20 using the SmaI site, Amp^R	This study
pUCP20-TOE*pqsA*_(2)	-140 to +60 fragment of *pqsA* containing a T7 promoter (5'-GAA ATTAATACGACTCACTATAGG -3') and a EcoRI restriction site at the 3'-end cloned into pUCP20 using the SmaI site, Amp^R	This study
pUCP20-TOE*pqsA*_(3)	pUCP20-TOE*pqsA*_long containing a mutation in the 5'-UTR of *psqA* where CGTTC was replaced by AAGAA, Amp^R	This study
pUCP20_ΔRBS	Plasmid-borne ribosomal binding site AGGAAA of pUCP20 was replaced by CCTCGC; Amp^R	This study
pUCP20_ΔRBS-*pqsA*-His6	Fragment containing -80 bp of the 5'-UTR and the entire coding sequence of *pqsA* was cloned into pUCP20_ΔRBS using KpnI and HindIII sites, Amp^R	This study
pUCP20_ΔRBS-Δ*pqsA*-His6	pUCP20_ΔRBS-pqsA-His6 containing a mutation in the 5'-UTR of *psqA* where CGTTC was replaced by AAGAA, Amp^R	This study

Construction of knockout mutants

To create the single knockout mutants in the wild-type PA14 parental strain we used an adapted version of the gene replacement method by (37) with use of plasmid pEX18Ap for *pqsR* and pEX18Ap2 (38) for *rhlR*, and a gentamicin resistance cassette flanked by Flipase Recombination Target (FRT-Gm) originating from plasmid pPS856. The mutant fragments were constructed by PCR extension overlap (42) with the following primers for *pqsR*: upstream region *pqsR*up2FEcoRI/*pqsR*up2RBamHI, downstream region *pqsR*dwFcompup/ *pqsR*dwRHindIII; and for *rhlR*: upstream region up*rhlR*NotI-fw/up*rhlR*NheI-rv, downstream region do*rhlR*NheI-fw/do*rhlR*HindIII-rv (Table 2). The BamHI site was introduced between the upstream and downstream region of *pqsR* and the NheI site for *rhlR* respectively. These restriction sites were used to insert the FRT-Gm cassette. In case of *pqsR*, the primers were designed to target for deletion the 529bp upstream region of *pqsR*, which comprise of all promoter binding sites, as well as 487bp part of the coding sequence, while for *rhlR* the primers were designed to delete the entire coding sequence of the gene. The resulting

plasmids pEX18Ap-Δ*pqsR*::FRT-Gm and pEX18Ap2-Δ*rhlR*::FRT-Gm were transferred into *P. aeruginosa* PA14 by two-parental mating using the donor strain *E. coli* S17-1. *P. aeruginosa* cells were selected on nalidixic acid (20µg/ml) and gentamicin (50µg/ml). The occurrence of the double cross-over was checked by plating at least 30 colonies from the mating result on gentamicin and carbenicillin (400µg/ml) containing agar plates. Gentamicin resistant and carbenicillin sensitive bacteria were isolated and the insertion of the FRT-Gm cassette ensured by PCR. Finally the FRT-Gm cassette was removed from the chromosomal DNA with help of flipase encoded on pFLP3 plasmid (39). The knockout mutation was confirmed by PCR using primers annealing outside of any pEX18Ap/pEX18Ap2 mediated deletion regions.

Bioluminescence assays

To generate promoter-*luxCDABE* (*lux*) fusions, *pqsA* promoter fragments were PCR-amplified from PA14 chromosomal DNA, digested with SpeI and PstI, and subcloned into pMTRL (40). P339 was generated from primers *pqsA*-339-SpeI-fw/*pqsA*-339-PstI-rv, p71 from primers *pqsA*-71-SpeI-fw/*pqsA*-71-PstI-rv, pshort from primers *pqsA*-339-SpeI-fw/*pqsA*-71-PstI-rv, and plong from primers *pqsA*-339-SpeI-fw/*pqsA*-ATG-PstI-rv (Table 2). The resulting plasmids were transferred into the *P. aeruginosa* strains PA14 wild-type, PA14_*pqsR* and PA14_*rhlR*. The transcriptional reporter strains were grown overnight with the appropriate antibiotic, then subcultured from an OD_{600} of 0.05 in BM2 medium [7 mM $(NH_4)_2SO_4$, 40 mM K_2HPO_4, 22 mM KH_2PO_4, 0.4% glucose and 2 mM $MgSO_4$] containing 0.01% CAS amino acids and 10 µM $FeSO_4$, and grown at 37°C. Bioluminescence was monitored using an EnSpire Multimode Plate Reader (PerkinElmer). Promoter activities are given as the relative luminescence of 200 µl of the cultures measured in a 96-well plate divided by the OD_{600} (relative light units [RLU] OD_{600}^{-1}). All results represent the mean of at least three independent replicates.

Thermal unfolding of DNA oligonucleotides

UV absorption spectra of 71 bp-long oligonucleotides (Table 2) were recorded on a JASCO J-815 CD spectrometer at a concentration of 10 µM in 50 mM potassium phosphate buffer pH 7.2. Thermal unfolding of secondary structure was monitored as an increase in absorption at 255 nm as a function of temperature in intervals of 1°C and a ramp rate of 4°C per minute. To determine the melting temperatures, raw data were fitted with an equation for a dual step

unfolding of a monomer with corrections for linear changes of the CD signal before and after the unfolding transition (43).

Generation of His_6-tagged fusion *pqsA* and Immunoblotting

The impact of folding structures in the 5'-UTR on the *in vivo* translation efficiency of *pqsA* in *E. coli* and *P. aeruginosa* was analyzed by cloning C-terminal his-tagged *pqsA* from nucleotide -80 to the stop codon into the KpnI/HindIII sites of pUCP20_ΔRBS, a pUCP20 derivate lacking the plasmid-borne ribosomal binding site generated by site-specific mutagenesis (QuikChange II Site-Directed Mutagenesis Kit, Agilent Technologies) using the primer set pUCP20_RBSmut-fw/pUCP20_RBSmut-rv (Table 2), according to the manufacturer's instructions. pUCP20_ΔRBS-*pqsA*-His_6 was generated using the primer set *pqsA*_KpnI-fw/*pqsA*_6His-rv and primers *pqsA*_mut_KpnI-fw/*pqsA*_6His-rv were used for generation of pUCP20_ΔRBS-Δ*pqsA*-His_6 (Table 2). To prepare samples for Western Blot analysis, whole-cell lysates of cultures grown to exponential growth phase in LB medium were normalized for protein content and 10 µl of an OD_{600} of 10.0 were separated by SDS-PAGE (10% acrylamide) after 15 min incubation at 95°C. As primary antibody we used a His-Tag mouse IgG_1 monoclonal antibody (Novagen) at a dilution of 1:1000. A4a goat anti-mouse IgG & IgM (Dianova) was used as secondary antibody at a dilution of 1:2000. Blots were developed using Lumi-Light Western Blotting Substrate (Roche) and chemiluminescence was detected using a Las-1000 Luminscent Image Analyzer (Fujifilm).

Toeprinting analysis

Primer extension inhibition (toeprinting) assays were performed as described previously (24). Plasmid templates were generated by cloning PCR-amplified fragments comprising a T7 promoter sequence (5'-GAAATTAATACGACTCACTATAGG-3') at the 5´-end, a EcoRV site at the 3'-end, and -140 (2) and -72 (1) to +60 nucleotides of *pqsA*, with usage of primers T7-*pqsA*200nt-fw/Toe_EcoRV-rv and T7-*pqsA*132nt-fw/Toe_EcoRV-rv (Table 2), into SmaI cut pUCP20 (blunt-end treated) resulting in pUCP20-TOE*pqsA*_(2) and pUCP20-TOE*pqsA*_(1), respectively. To generate pUCP20-TOE*pqsA*_(3) site-specific mutagenesis of pUCP20-TOE*pqsA*_(2) was carried out using the QuikChange II Site-Directed Mutagenesis Kit (Agilent Technologies) with the primers 5'UTR*pqsA*_mut-f /5'UTR*pqsA*_mut-r (Table 2), according to the manufacturer's instructions. For *in vitro* transcription with T7 RNA polymerase plasmids were linearized by digestion with EcoRV and the primer Toe_short-rv

344 was used for reverse transcription. The experiments were carried out at 37°C in the presence
345 and absence of *E. coli* 30S ribosomal subunits.
346

347 **Table 2.** Oligonucleotides

Primer	Sequence (5'-3' direction)[a]
Mutagenesis	
*pqsR*up2FEcoRI	GAGAATTCATCCACCGGGCAGCCCAG
*pqsR*up2RBamHI	CGGGATCCGTTAGCGTAGCCACCGGCCAGGC
*pqsR*dwFcompup	TGGCTACGCTAACGGATCCCGTCGGCTACACCAAGGCGTTC
*pqsR*dwRHindIII	CCCAAGCTTGAGAACGCTCTACTCTGGTGCG
up*rhlR*NotI-fw	TATGCGGCCGCTGCAGCGCGCCTACGCG
up*rhlR*NheI-rv	TCAGTCAGTCAGCTAGCTGCAGTAAGCCCTGATCGATAAAATGCA
do*rhlR*NheI-fw	GCTAGCTGACTGACTGAAGCGCAGGGCGCGCCG
do*rhlR*HindIII-rv	TATAAGCTTGGCGGCGTAGCGCGAAAGC
Promoter-*lux* fusions	
pqsA-339-SpeI-fw	TCAGACTAGTGGAGGCTGCAAATGGCA
pqsA-339-PstI-rv	ATACTGCAGGCAAACCAATGACAACCCACTTTGC
pqsA-71-SpeI-fw	TCAGACTAGTTGCCGCCCTTCTTGCTTG
pqsA-71-PstI-rv	ATACTGCAGTCAGGAACGGGAAACTAGCGG
pqsA-ATG-PstI-rv	ATACTGCAGGACAGAACGTTCCCTCTTCAGC
CD Spectroscopy	
short	TGACAAAGCAAGCGCTCTGGCTCAGGTATCTCCTGATCCGGATGCATATCGCTG AAGAGGGAACGTTCTGT
long	CGTTCCTGACAAAGCAAGCGCTCTGGCTCAGGTATCTCCTGATCCGGATGCATAT CGCTGAAGAGGGAACG
derep	AAGAACTGACAAAGCAAGCGCTCTGGCTCAGGTATCTCCTGATCCGGATGCATA TCGCTGAAGAGGGAACG
His_6-tagged *pqsA*	
pUCP20_RBSmut-fw	GGAATTGTGAGCGGATAACAATTTCACACCCTCTGCAGCTATGACCATGATTAC GAATTCCCCG
pUCP20_RBSmut-rv	CGGGGAATTCGTAATCATGGTCATAGCTGCAGAGGGTGTGAAATTGTTATCCGC TCACAATTCC
*pqsA*_KpnI-fw	TATCGGTACCCCCGTTCCTGACAAAGCAAGCG
*pqsA*_mut_KpnI-fw	TATCGGTACCCCAAGAACTGACAAAGCAAGCGCTCTGGC
*pqsA*_6His-rv	TATCAAGCTTTCATCATCAGTGGTGGTGGTGGTGGTGACATGCCCGTTCCTCCGG AAGGTT
Toeprinting	

T7-*pqsA*200nt-fw	GAAATTAATACGACTCACTATAGGCTCCCCGAAACTTTTTCGTTCGGACTC
T7-*pqsA*132nt-fw	GAAATTAATACGACTCACTATAGGTGACAAAGCAAGCGCTCTGGC
Toe_EcoRV-rv	TAAGATATCGGTATCGGGATCGAAATCGAGGCG
Toe_short-rv	TCGAAATCGAGGCGGAACAGAACC
5'UTR*pqsA*_mut-f	CCAGAGCGCTTGCTTTGTCAGTTCTTGGAAACTAGCGGCGCTGGGC
5'UTR*pqsA*_mut-r	GCCCAGCGCCGCTAGTTTCCAAGAACTGACAAAGCAAGCGCTCTGG

[a]Restriction sites are underlined.

Acknowledgments

We thank Dr Piotr Bielecki (HZI, Braunschweig) for kindly supplying the PA14 *pqsR*⁻ mutant strain. Stephan Brouwer has been supported by the Helmholtz International Graduate School for Infection Research under contract number VH-GS-202.

References

1. **Elkin S, Geddes D**. 2003. Pseudomonal infection in cystic fibrosis: the battle continues. Expert Rev. Anti. Infect. Ther. **1**:609–618.

2. **Latifi A, Foglino M, Tanaka K, Williams P, 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.

3. **Schuster M, Greenberg EP**. 2006. A network of networks: quorum-sensing gene regulation in Pseudomonas aeruginosa. Int. J. Med. Microbiol. **296**:73–81.

4. **Medina G**. 2003. Transcriptional regulation of Pseudomonas aeruginosa rhlR, encoding a quorum-sensing regulatory protein. Microbiology **149**:3073–3081.

5. **Pearson J, Pesci E, Iglewski B**. 1997. Roles of Pseudomonas aeruginosa las and rhl quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. J. Bacteriol. **179**:5756–5767.

6. **Williams P, Cámara M**. 2009. Quorum sensing and environmental adaptation in Pseudomonas aeruginosa: a tale of regulatory networks and multifunctional signal molecules. Curr. Opin. Microbiol. **12**:182–91.

7. **Diggle SP, Matthijs S, Wright VJ, Fletcher MP, Chhabra SR, Lamont IL, Kong X, Hider RC, Cornelis P, Cámara M, Williams P**. 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.

8. **Lépine F, Milot S, Déziel E, He J, Rahme LG**. 2004. Electrospray/mass spectrometric identification and analysis of 4-hydroxy-2-alkylquinolines (HAQs) produced by Pseudomonas aeruginosa. J. Am. Soc. Mass Spectrom. **15**:862–9.

9. **Häussler S, Becker T**. 2008. The pseudomonas quinolone signal (PQS) balances life and death in Pseudomonas aeruginosa populations. PLoS Pathog. **4**:e1000166.

10. **Bredenbruch F, Geffers R, Nimtz M, Buer J, Häussler S**. 2006. The Pseudomonas aeruginosa quinolone signal (PQS) has an iron-chelating activity. Environ. Microbiol. **8**:1318–29.

11. **Diggle SP, Winzer K, Chhabra SR, Worrall KE, Cámara M, Williams P**. 2003. The Pseudomonas aeruginosa quinolone signal molecule overcomes the cell density-dependency of the quorum sensing hierarchy, regulates rhl-dependent genes at the onset of stationary phase and can be produced in the absence of LasR. Mol. Microbiol. **50**:29–43.

12. **McGrath S, Wade DS, Pesci EC**. 2004. Dueling quorum sensing systems in Pseudomonas aeruginosa control the production of the Pseudomonas quinolone signal (PQS). FEMS Microbiol. Lett. **230**:27–34.

13. **Xiao G, Déziel E, He J, Lépine F, Lesic B, Castonguay M-H, Milot S, Tampakaki AP, Stachel SE, Rahme LG**. 2006. MvfR, a key Pseudomonas aeruginosa pathogenicity LTTR-class regulatory protein, has dual ligands. Mol. Microbiol. **62**:1689–1699.

14. **Wade DS, Calfee MW, Rocha ER, Ling E a, Engstrom E, Coleman JP, Pesci EC**. 2005. Regulation of Pseudomonas quinolone signal synthesis in Pseudomonas aeruginosa. J. Bacteriol. **187**:4372–4380.

15. **Sonnleitner E, Gonzalez N, Sorger-Domenigg T, Heeb S, Richter AS, Backofen R, Williams P, Hüttenhofer A, Haas D, Bläsi U**. 2011. The small RNA PhrS stimulates synthesis of the Pseudomonas aeruginosa quinolone signal. Mol. Microbiol. **80**:868–885.

16. **Xiao G, He J, Rahme LG**. 2006. Mutation analysis of the Pseudomonas aeruginosa mvfR and pqsABCDE gene promoters demonstrates complex quorum-sensing circuitry. Microbiology **152**:1679–1686.

17. **Dötsch A, Eckweiler D, Schniederjans M, Zimmermann A, Jensen V, Scharfe M, Geffers R, Häussler S**. 2012. The Pseudomonas aeruginosa Transcriptome in Planktonic Cultures and Static Biofilms Using RNA Sequencing. PLoS One **7**:e31092.

18. **Kozak M**. 2005. Regulation of translation via mRNA structure in prokaryotes and eukaryotes. Gene **361**:13–37.

19. **Geissmann T, Marzi S, Romby P**. 2009. The role of mRNA structure in translational control in bacteria. RNA Biol. **6**:153–160.

20. **Winkler WC, Breaker RR**. 2005. Regulation of bacterial gene expression by riboswitches. Annu. Rev. Microbiol. **59**:487–517.

21. **De Smit MH, van Duin J**. 1990. Secondary structure of the ribosome binding site determines translational efficiency: a quantitative analysis. Proc. Natl. Acad. Sci. U. S. A. **87**:7668–72.

22. **Kortmann J, Narberhaus F**. 2012. Bacterial RNA thermometers: molecular zippers and switches. Nat. Rev. Microbiol. **10**:255–65.

23. **Skurnik M, Toivanen P**. 1992. the yadA gene of Yersinia enterocolitica and LcrF Is the Temperature-Regulated Activator of the yadA Gene of Yersinia enterocolitica and Yersinia pseudotuberculosis **174**.

24. **Böhme K, Steinmann R, Kortmann J, Seekircher S, Heroven AK, Berger E, Pisano F, Thiermann T, Wolf-Watz H, Narberhaus F, Dersch P**. 2012. Concerted actions of a thermo-labile regulator and a unique intergenic RNA thermosensor control Yersinia virulence. PLoS Pathog. **8**:e1002518.

25. **Krajewski SS, Nagel M, Narberhaus F**. 2013. Short ROSE-like RNA thermometers control IbpA synthesis in Pseudomonas species. PLoS One **8**:e65168.

26. **Pesci EC, Milbank JBJ, Pearson JP, McKnight S, Kende AS, Greenberg EP, Iglewski BH**. 1999. Quinolone signaling in the cell-to-cell communication system of Pseudomonas aeruginosa. Proc. Natl. Acad. Sci. **96**:11229–11234.

27. **D'Argenio DA, Calfee MW, Rainey PB, Pesci EC**. 2002. Autolysis and Autoaggregation in Pseudomonas aeruginosa Colony Morphology Mutants. J. Bacteriol. **184**:6481–6489.

28. **Coleman JP, Hudson LL, McKnight SL, Farrow JM, Calfee MW, Lindsey C a, Pesci EC**. 2008. Pseudomonas aeruginosa PqsA is an anthranilate-coenzyme A ligase. J. Bacteriol. **190**:1247–1255.

29. **Bredenbruch F, Nimtz M, Wray V, Morr M, Müller R, Häussler S**. 2005. Biosynthetic pathway of Pseudomonas aeruginosa 4-hydroxy-2-alkylquinolines. J. Bacteriol. **187**:3630–3635.

30. **Batey RT**. 2006. Structures of regulatory elements in mRNAs. Curr. Opin. Struct. Biol. **16**:299–306.

31. **Hughes TA**. 2006. Regulation of gene expression by alternative untranslated regions. Trends Genet. **22**:119–122.

32. **Hughes TA, Brady HJM**. 2005. Expression of axin2 is regulated by the alternative 5'-untranslated regions of its mRNA. J. Biol. Chem. **280**:8581–8588.

33. **Hughes TA, Brady HJM**. 2005. E2F1 up-regulates the expression of the tumour suppressor axin2 both by activation of transcription and by mRNA stabilisation. Biochem. Biophys. Res. Commun. **329**:1267–1274.

34. **Sobczak K, Krzyzosiak WJ**. 2002. Structural determinants of BRCA1 translational regulation. J. Biol. Chem. **277**:17349–17358.

35. **Woodcock DM, Crowther PJ, Doherty J, Jefferson S, DeCruz E, Noyer-Weidner M, Smith SS, Michael MZ, Graham MW**. 1989. Quantitative evaluation of Escherichia coli host strains for tolerance to cytosine methylation in plasmid and phage recombinants. Nucleic Acids Res. **17**:3469–3478.

36. **Liberati NT, Urbach JM, Miyata S, Lee DG, Drenkard E, Wu G, Villanueva J, Wei T, Ausubel FM**. 2006. An ordered, nonredundant library of Pseudomonas aeruginosa strain PA14 transposon insertion mutants. Proc. Natl. Acad. Sci. U. S. A. **103**:2833–8.

37. **Hoang TT, Karkhoff-Schweizer RR, Kutchma AJ, Schweizer HP**. 1998. A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked Pseudomonas aeruginosa mutants. Gene **212**:77–86.

38. **Blanka A, Schulz S, Eckweiler D, Franke R, Bielecka A, Nicolai T, Casilag F, Düvel J, Abraham W-R, Kaever V, Häussler S**. 2014. Identification of the alternative sigma factor SigX regulon and its implications for Pseudomonas aeruginosa pathogenicity. J. Bacteriol. **196**:345–56.

39. **Choi K-H, Gaynor JB, White KG, Lopez C, Bosio CM, Karkhoff-Schweizer RR, Schweizer HP**. 2005. A Tn7-based broad-range bacterial cloning and expression system. Nat. Methods **2**:443–8.

40. **Gödeke J, Heun M, Bubendorfer S, Paul K, Thormann KM**. 2011. Roles of two Shewanella oneidensis MR-1 extracellular endonucleases. Appl. Environ. Microbiol. **77**:5342–51.

41. **West SEH, Schweizer HP, Dall C, Sample AK, Runyen-Janecky LJ**. 1994. Construction of improved Escherichia-Pseudomonas shuttle vectors derived from pUC18/19 and sequence of the region required for their replication in Pseudomonas aeruginosa. Gene **148**:81–86.

42. **Choi K-H, Schweizer HP**. 2005. An improved method for rapid generation of unmarked Pseudomonas aeruginosa deletion mutants. BMC Microbiol. **5**:30.

43. **Greenfield NJ**. 2006. Using circular dichroism collected as a function of temperature to determine the thermodynamics of protein unfolding and binding interactions. Nat. Protoc. **1**:2527–35.

Figure legends

Fig.1: Regulatory elements within the 5'-UTR of the *pqsA* gene and constructs used in this study. (A) Promoter region of *pqsA*. The *pqsA* transcriptional start sites are indicated by bent arrows at *pqsA*-339 and *pqsA*-71, and the *pqsA* ATG start codon is underlined. The binding sites of RhlR, PqsR and the ribosome are boxed and labeled. (B) Model of the *pqsA* promoter region. (C) *pqsA* promoter-fusion constructs used in the present study. (-) and (+) indicate position to the start codon of *pqsA*. Lines are not drawn to scale.

Fig.2: Promoter activity of the two alternative transcriptional start sites of *pqsA*. *P. aeruginosa* strains PA14 WT, *pqsR*$^-$ and *rhlR*$^-$ mutants containing plasmids p339 and p71 were cultured as described in Material and Methods and assayed for *lux* expression. Data are presented in relative luminescence and error bars represent one standard deviation of the mean value from three biological replicates (*$p \leq 0.05$ and **$p \leq 0.01$).

Fig.3: Folding dynamics and stability of secondary structures formed by the 5-UTR of *pqsA*. (A) Predicted secondary structures in the 5'-leader sequence of the mRNA expressed by PqsR (short; -14.10 Kcal/mol), RhlR (long; -29.80 Kcal/mol) and a derepressed RNA variant (derep; -14.10 Kcal/mol). Secondary structures were generated using the CONTRAfold method (44). The color-code represents base-pairing probabilities in the structure ensemble, whereby high values (red) close to 1 are the most probable. Numbers indicate nucleotide positions relative to A of the AUG start codon. The Shine-Dalgarno sequence AGGGAA is encircled and mutated nucleotides are emphasized by an asterisk (*). (B) Thermal unfolding behavior of 71–mer single-stranded DNA oligonucleotides equivalent to the RNA sequences shown in (A) monitored by UV-absorption at 255 nm. Markers represent the raw data recalculated to the fraction of unfolded DNA. Continuous lines represent the data fits used to calculate the melting temperatures.

Fig.4: Inclusion of the SD sequence in the RhlR-induced *pqsA* mRNA inhibits translation. *P. aeruginosa* strains PA14 WT, *pqsR*$^-$ and *rhlR*$^-$ mutants containing plasmids pshort and plong were cultured as described in Material and Methods and assayed for *lux* expression. Data are presented in relative luminescence and error bars represent one standard deviation of the mean value from three biological replicates (*$p \leq 0.05$ and **$p \leq 0.01$).

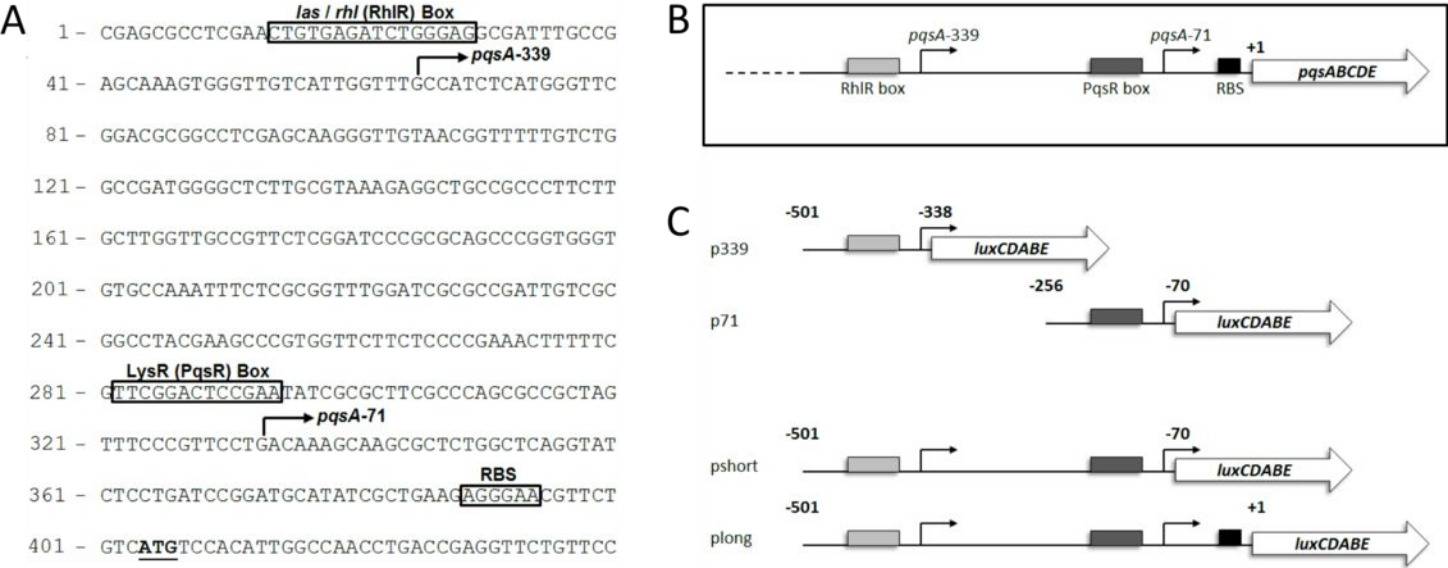
Fig.5: Translational control of *pqsA* expression. (A) Analysis of His6-tagged PqsA expressed under *lac* promoter control but in dependence of the native translation initiation site

518 in *E. coli* BL21 and PA14 WT. *In vivo* levels of PqsA-His were compared between the wild-
519 type 5-UTR comprising -80 nucleotides relative to the start codon and a derepressed 5-UTR
520 causing liberation of the SD sequence. (B) Predicted folding patterns of the *in vitro*
521 transcribed *pqsA* constructs used for the toeprint assay: (1) PqsR-induced *pqsA* transcript (-
522 22.70 Kcal/mol), (2) RhlR-induced *pqsA* transcript (-46.40 Kcal/mol), and (3) a mutated
523 RhlR-induced mRNA exhibiting a destabilized secondary structure (-29.80 Kcal/mol).
524 Folding dynamics of the mRNA constructs were predicted using the CONTRAfold method
525 (44). Secondary structures are color-coded according to base-pairing probabilities in the
526 structure ensemble, whereby high values (red) close to 1 are the most probable. The Shine-
527 Dalgarno sequence AGGGAA is encircled and the AUG start codon is indicated by a black
528 line. Site-specific mutated nucleotides are emphasized by an asterisk (*). (C) Primer extension
529 inhibition assay of the *pqsA* mRNAs drawn in (B), including the 5'-UTR and the first 60
530 nucleotides of the *pqsA* coding sequence. Addition (+) or absence (-) of *E. coli* 30S ribosomal
531 subunits is indicated. The AUG start codon and the SD sequence are marked on the right and
532 full-length products, structure 1 and structure 2 and the toeprint are indicated on the left-hand
533 side.

534 **Fig.6: Model of transcriptional and translational control of *pqsA*.** *P. aeruginosa* releases
535 the signal molecules N-butanoyl-L-homoserine lactone (C4-HSL) and the *Pseudomonas*
536 quinolone signal (PQS) in a cell-dependent manner. Upon binding to their cognate ligand, the
537 transcriptional regulators RhlR-C4-HSL and PqsR-PQS induce transcription of the *pqsA-E*
538 operon from the transcription start sites *pqsA*-339 and *pqsA*-71, respectively. In the *pqsA*-339-
539 induced mRNA the formation of a hairpin at the translation initiation site of *pqsA* blocks
540 access of the ribosome to the SD-sequence. As a consequence, production of the anthranilate-
541 coenzyme A ligase PqsA is hindered rendering *P. aeruginosa* unable to generate HHQ / PQS.

542

543 44. **Do CB, Woods D a, Batzoglou S**. 2006. CONTRAfold: RNA secondary structure
544 prediction without physics-based models. Bioinformatics **22**:e90–8.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las / rhl (RhlR) Box
pqsA-339
1 - CGAGCGCCTCGAACTGTGAGATCTGGGAGGCGATTTGCCG
41 - AGCAAAGTGGGTTGTCATTGGTTTGCCATCTCATGGGTTC
81 - GGACGCGGCCTCGAGCAAGGGTTGTAACGGTTTTTGTCTG
121 - GCCGATGGGGCTCTTGCGTAAAGAGGCTGCCGCCCTTCTT
161 - GCTTGGTTGCCGTTCTCGGATCCCGCGCAGCCCGGTGGGT
201 - GTGCCAAATTTCTCGCGGTTTGGATCGCGCCGATTGTCGC
241 - GGCCTACGAAGCCCGTGGTTCTTCTCCCCGAAACTTTTTC
LysR (PqsR) Box
281 - GTTCGGACTCCGAATATCGCGCTTCGCCCAGCGCCGCTAG
pqsA-71
321 - TTTCCCGTTCCTGACAAAGCAAGCGCTCTGGCTCAGGTAT
RBS
361 - CTCCTGATCCGGATGCATATCGCTGAAGAGGGAACGTTCT
401 - GTCATGTCCACATTGGCCAACCTGACCGAGGTTCTGTTCC
B
pqsA-339
pqsA-71
+1
RhlR box
PqsR box
RBS
pqsABCDE
C
-501
-338
p339
luxCDABE
-256
-70
p71
luxCDABE
-501
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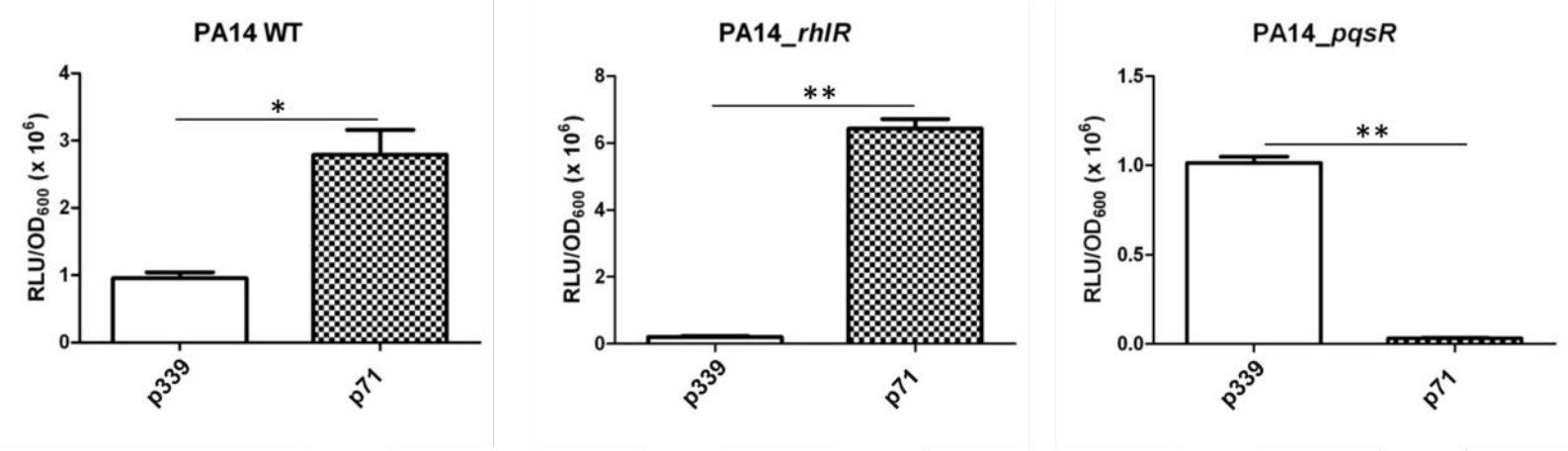
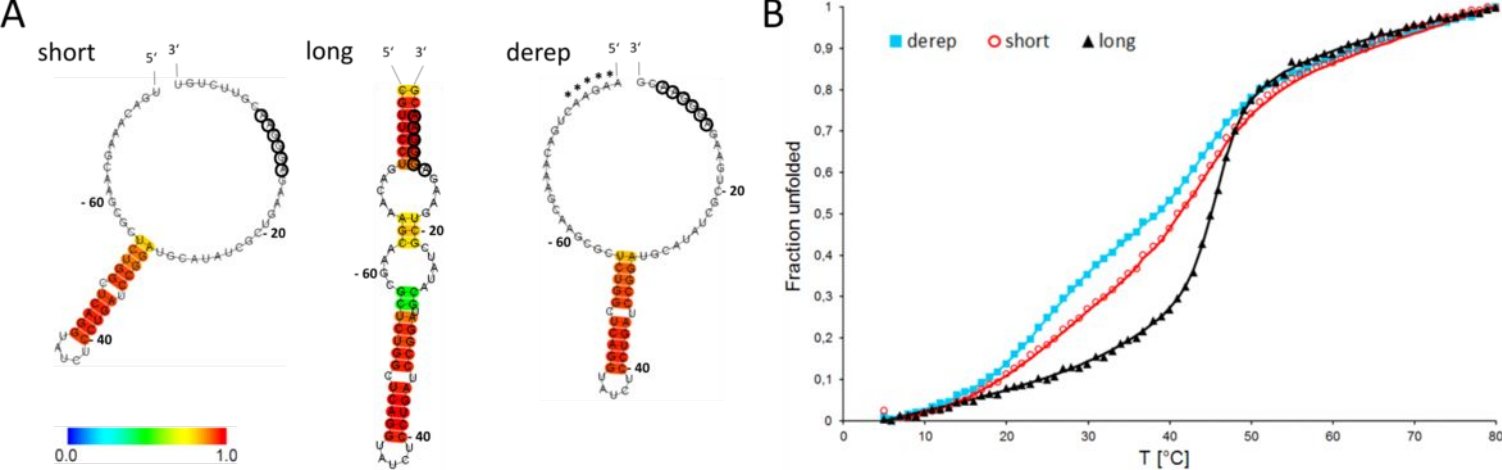
pshort
luxCDABE
-501
+1
plong
luxCDABE


PA14 WT
RLU/OD600 (x 10^6)
4
3
2
1
0
*
p339
p71
PA14_rhlR
RLU/OD600 (x 10^6)
8
6
4
2
0
**
p339
p71
PA14_pqsR
RLU/OD600 (x 10^6)
1.5
1.0
0.5
0.0
**
p339
p71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short
long
derep
5′
3′
- 60
- 20
- 40
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1.0
B
derep
short
long
Fraction unfolded
T [°C]
0
0,1
0,2
0,3
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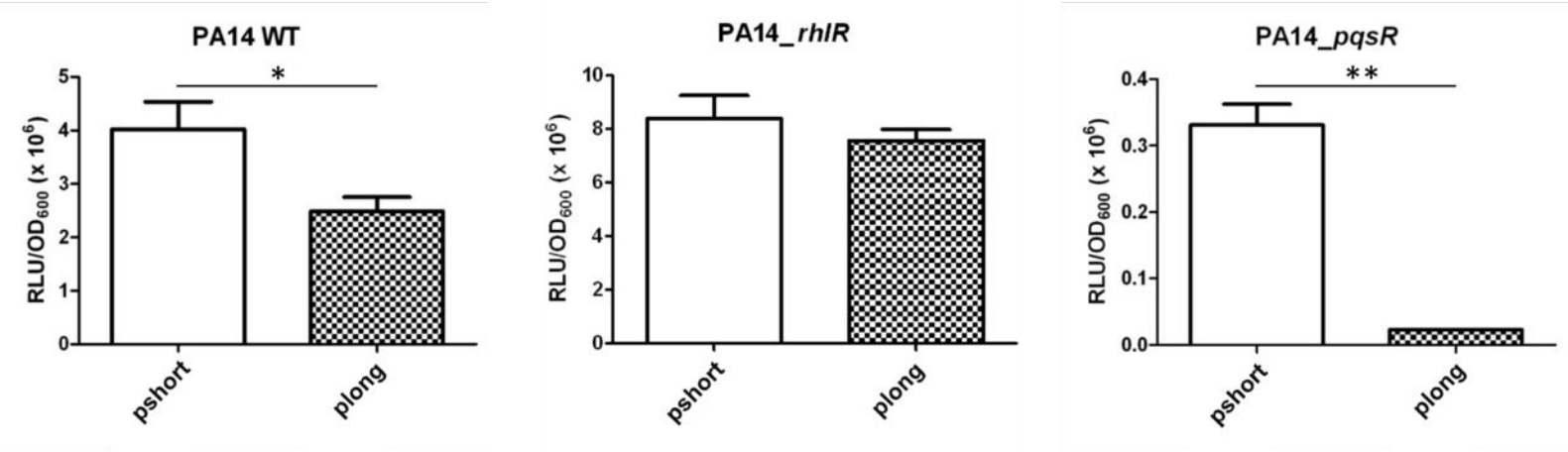
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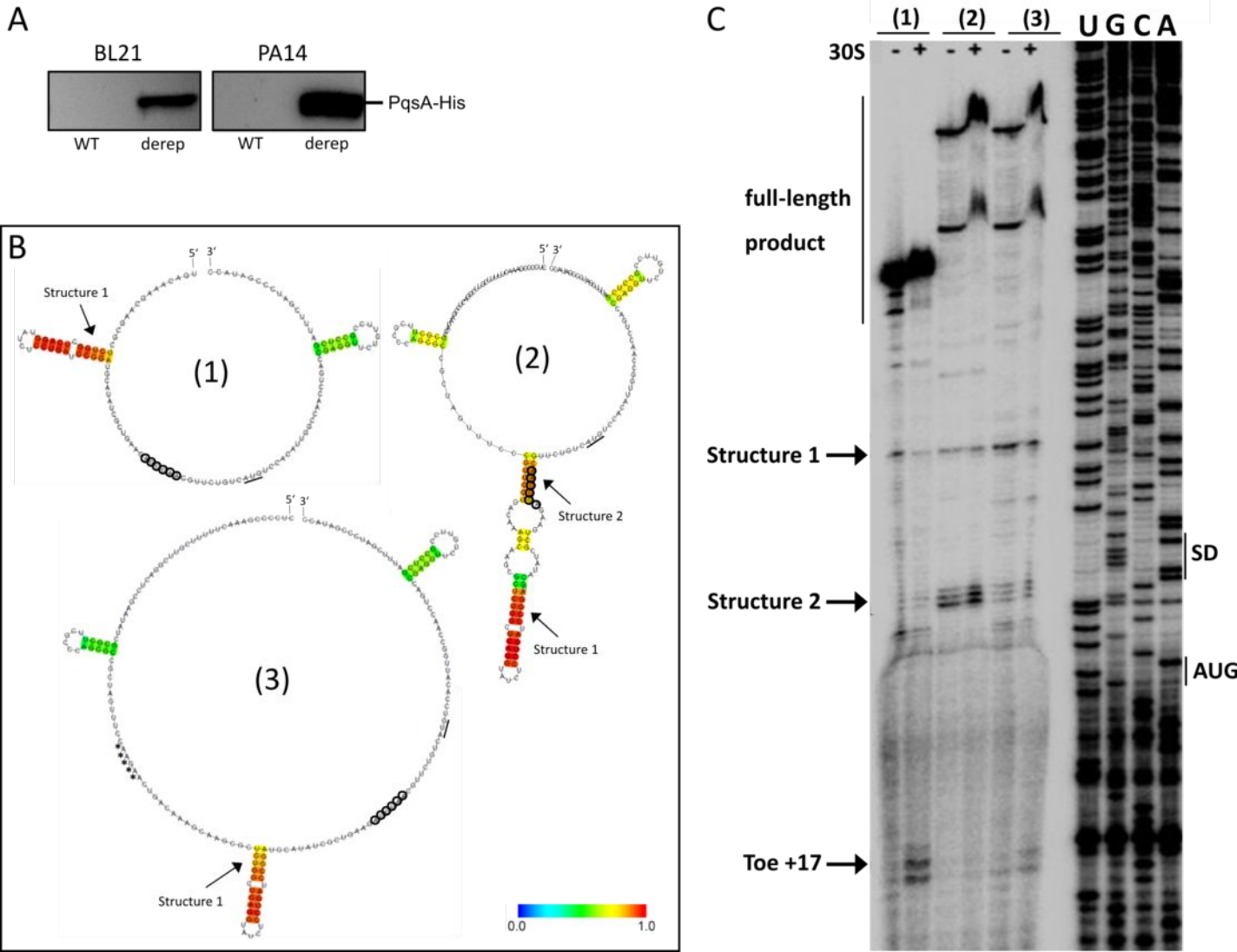
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derep
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Structure 1
(1)
(2)
Structure 2
Structure 1
(3)
Structure 1
0.0
1.0
C
(1)
(2)
(3)
U G C A
30S
- + - + - +
full-length
product
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Structure 2
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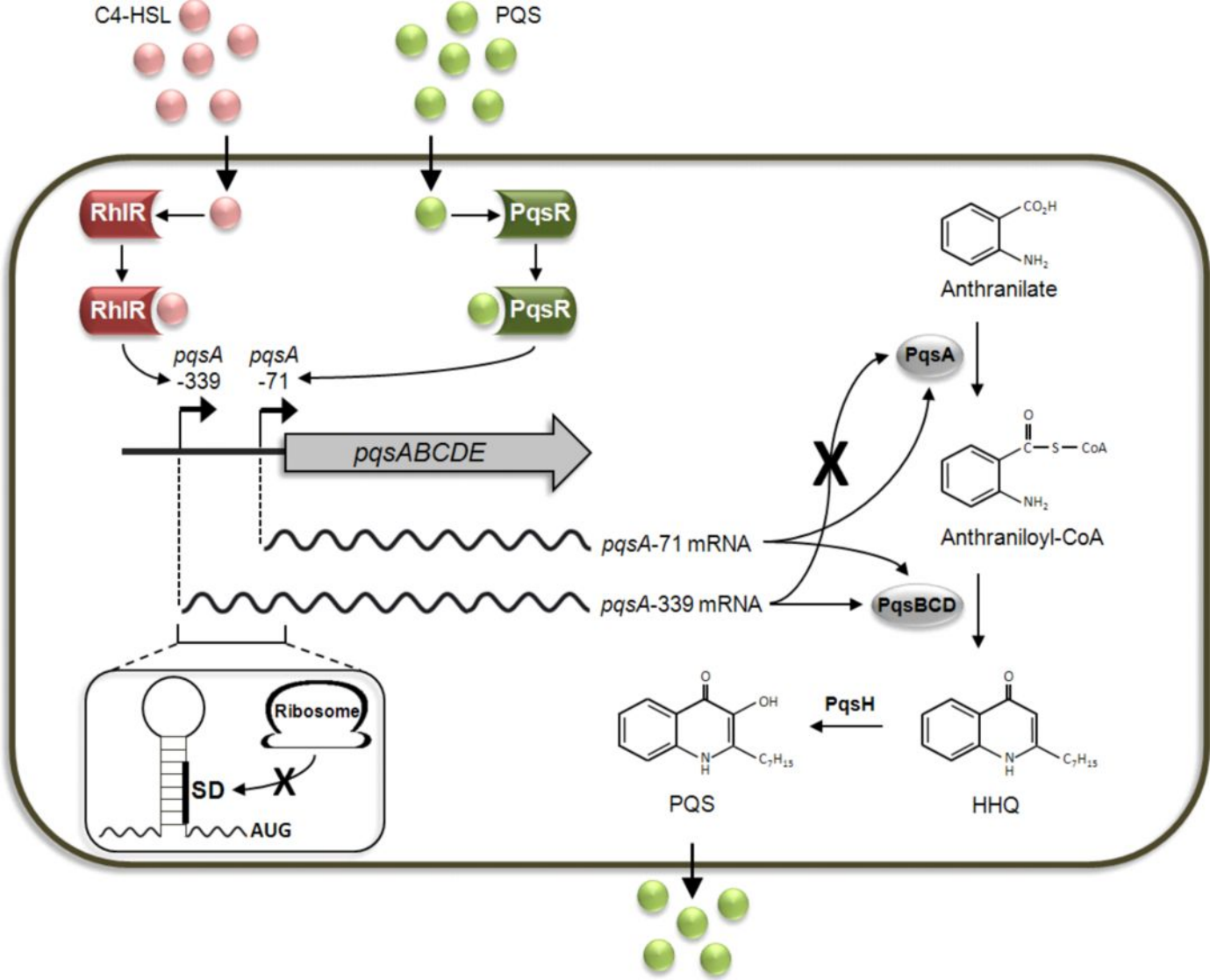
SD
AUG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Ribosome
SD
AUG
Anthranilate
PqsA
Anthraniloyl-CoA
PqsBCD
PqsH
PQS
HHQ