SUPPLEMENTARY INFORMATION

The anti-sigma factor MucA is required for viability in Pseudomonas aeruginosa

Melissa C. Schofield, Daniela Rodriguez, Amanda A. Kidman, Erin K Cassin, Lia A. Michaels, Elizabeth A. Campbell, Peter A. Jorth and Boo Shan Tseng

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SUPPLEMENTARY METHODS

Media, antibiotics, and antibodies. Bacteria were grown in liquid LB (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl) and LSLB (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl). Semisolid media was prepared by adding 1.5% Bacto agar to LB or 1.0% Noble agar to VBM (Vogel & Bonner, 1956). Pseudomonas isolation agar (PIA; Hardy Diagnostics), Pseudomonas isolation broth (PIB; US Biological), and synthetic cystic fibrosis medium (SCFM, prepared as previously described (Palmer et al., 2007)) were also used. For experiments involving induction of the araC-P<sub>araBAD</sub> promoter, L-arabinose was used at the indicated concentrations; the rhaSR-P<sub>rhaBAD</sub> promoter, L-rhamnose at 0.05%. Antibiotics were used at the following concentrations: for E. coli, 10 mg/L gentamicin, 50 mg/L kanamycin, 50 mg/L carbenicillin, 100 mg/L ampicillin, 10 mg/L tetracycline; for P. aeruginosa, 60 mg/L gentamicin for chromosomally integrated strains, 50 mg/L gentamicin for plasmid-borne strains, 300 mg/L carbenicillin, and 100 mg/L tetracycline. Antibodies against RpoD (ThermoFisher) were used at 1/1000 and RNAP (ThermoFisher) at 1/10,000.

Calculation of growth rate. Bacteria were grown overnight on LB plates (unless otherwise indicated) at 37˚C. Single colonies were taken from plates to cultures in appropriate liquid media, and grown with shaking at 250rpm at 37˚C until stationary phase. These cultures were then used to seed the cells into a 96 well plate using the indicated medium at OD<sub>600</sub> of 0.001. Using a Synergy Hybrid HTX Microplate Reader (Bio-Tek Instruments), the OD<sub>600</sub> was measured every 5 min for 16 h at 37°C with shaking. To calculate growth rates, the slope of exponential growth phase of the growth curve was determined. For normalized growth rates, the rates were normalized to the positive control of the respective experiment.

Construction of strains. Sequenced strains of P. aeruginosa were obtained from the Pseudomonas Genome Database (www.pseudomonas.com) (Winsor et al., 2016). Bacterial genomic DNA was isolated using the DNeasy Blood & Tissue Kit (Qiagen), and PCR fragments were purified using the Wizard SV Gel and PCR Clean-up System (Promega). To delete genes, allelic exchange was used with the corresponding plasmid containing the deletion allele, as previously described (Hmelo et al., 2015). For miniTn7 and miniCTX, the alleles were chromosomally integrated, as previously described (Choi & Schweizer, 2006, Hoang et al., 2000).

To construct deletion alleles, the regions flanking the gene of interest were produced by PCR of PAO1 gDNA using the respective UpF/UpR and DownF/DownR primer pairs. These fragments were then connected by SOE PCR and inserted into pDONRPEX18Gm using GateWay cloning (Invitrogen) to produce deletion vectors, which were confirmed via Sanger sequencing.

The miniTn7-based plasmid containing the full-length mucA allele under its native promoter (pBT399) was constructed via PCR of PAO1 gDNA using OBT603/604B and OBT605B/606B. These fragments were then connected by SOE PCR and inserted into pDONRPUC18T-miniTn7T2-Gm using GateWay cloning. To create the mucA truncation series, QuikChange site directed mutagenesis (Agilent Technologies) of pBT399 was used to engineer plasmids with alleles of the desired mucA length (pBT406, pBT407, pBT408, pBT410, pBT413, pBT414, pMS009, pMS023, pMS024). To construct mucA single amino acid substitutions, the same methods were used with pBT408.

A miniTn7-based plasmid containing the full-length algU allele under its native promoter (pAAK014) was constructed via PCR of PAO1 gDNA using OAK12/OAK2. To construct algU single amino acid substitutions, PCR of PAO1 gDNA using OAK12 with the respective UpR
domains of E. coli DNA was made by superimposing Construction of AlgU mutations. DNA sequencing reads were aligned to the appropriate reference genomes (Winsor et al., 2016) for PAO1, PA103, and PAK using Breseq (Deatherage & Barrick, 2014). Mutations detected in \( \Delta mucA \) strains were compared to their respective wild-type parent strains to identify suppressor mutations.

### Analysis of whole genome sequencing.

*P. aeruginosa* genomes were sequenced using an Illumina NextSeq platform at the Microbial Genome Sequencing Center (Pittsburgh, PA). Sequencing reads were aligned to the appropriate reference genomes (Winsor et al., 2016) for PAO1, PA103, and PAK using Breseq (Deatherage & Barrick, 2014). Mutations detected in \( \Delta mucA \) strains were compared to their respective wild-type parent strains to identify suppressor mutations.

### Construction of AlgU-holoenzyme model.

The model of AlgU-holoenzyme with promoter DNA was made by superimposing \( \sigma^E \) Region 2 and \( \sigma^E \) Region 4 from the crystal structure of the *E. coli* \( \sigma^E \) with the anti-\( \alpha \) factor RseA (PDB 1OR7)(Campbell et al., 2003) onto the respective domains of \( \sigma^70 \) from an *E. coli* \( \sigma^70 \)-holoenzyme crystal structure (PDB 4LJZ)(Bae et al., 2013).
The promoter DNA is a hybrid of DNAs taken from superimpositions of structures of *E. coli* $\sigma^E$ Region 2 bound to its -10 element (PDB 4LUP)(Campagne *et al.*, 2014) and $\sigma^E$ Region 4 bound to the -35 element (PDB 2H27)(Lane & Darst, 2006). The remaining promoter region was modeled from the *M. tuberculosis* RNAP/open complex structure (PDB 6EDT)(Boyaci *et al.*, 2019).

**Semi-quantitative Western blots.** Western blot quantification was performed using AzureSpot software (Azure BioSystems). All signals were background subtracted. The RpoD signal was then normalized to that of RNAP for the same sample. To determine the relative level, this normalized signal was divided by that of the parental strain within the same replicate.
Table S1. Eliminating alginate biosynthesis does not alleviate *mucA* essentiality.

| PAO1               | Number of isolates resolved to |          |          |
|--------------------|--------------------------------|----------|----------|
|                    | WT                              | ∆*mucA*  |          |
| WT                 | 168                            | 0*       |          |
| ∆*algD*            | 144                            | 0*       |          |
| ∆*algB*            | 158                            | 0*       |          |
| ∆*algR*            | 153                            | 0*       |          |
| ∆*amrZ*            | 131                            | 0*       |          |
| ∆*algB* ∆*algR* ∆*amrZ* | 133                  | 0*       |          |

* p < 0.0001, Fischer’s exact test
Table S2. Description of changes found in \textit{algU} of PAO1 $\Delta$\textit{mucA attTn7::P}_{\textit{rhaBAD-mucA}} revertants that can grow in the absence of \textit{mucA} expression.

| Isolate | Genomic Change to \textit{algU}$^{\dagger}$ | Effect on AlgU$^{\ddagger}$ |
|---------|---------------------------------------------|-----------------------------|
| **Multi-base pair deletions** | | |
| #1-10 831242-831394 del | No product made |
| #2-9 831298-831491 del | No product made |
| #2-7 831467-831473 del | I56fs X97 |
| #2-11 831469-831502 del | K57fs X88 |
| #2-6 831595-831715 del | V99fs X107 |
| **Single base pair deletions** | | |
| #2-14 831467 del | I56fs X99 |
| #1-1 831510 del | Y72fs X99 |
| #2-10 831538 del | I80fs X99 |
| **Nonsense mutations** | | |
| #2-1 831433 C>T | Q45X |
| #2-8 831521 G>A | W74X |
| **Multi-base pair insertions** | | |
| #1-9 831427-831435 ins GACGCCCAG | D43-A44 ins DAQ |
| #1-8 831430-831438 ins GCCCAGGAA | A44-Q45 ins AQE |
| #1-11 831436-831444 ins GAAGCCCAG | E46-A47 ins EAQ |
| #2-4 831436-831444 ins GAAGCCCAG | E46-A47 ins EAQ |
| #1-5 831445-831456 ins GACGTagCCAG | D49-V50 ins DVAQ |
| #1-6 831451-831459 ins GCCCAGGAA | A51-Q52 ins AQE |
| **Missense mutations** | | |
| #1-3 831353 A>G | D18G |
| #2-12 831362 C>T | A21V |
| #2-3 831386 A>G | Y29C |
| #2-2 831439 G>A | A47T |
| #1-2 831446 A>G | D49G |
| #2-13 831446 A>G | D49G |
| #1-7 831476 A>G | Y59C |
| #2-5 831541 A>G | N81D |
| #1-4 831820 C>G | R174G |

$^{\dagger}$ genomic location based on the Pseudomonas Genome Database (Winsor \textit{et al.}, 2016) for PAO1; bp, base pair; del, deletion; ins, insertion.

$^{\ddagger}$ fs, frameshift; ins, insertion; X, stop codon.
Table S3. Statistical differences in growth rate among groups under conditions of algU induction.

| Medium: LB | Strain | attTn7::P<sub>araBAD</sub>-algU | ΔalgU attTn7::P<sub>araBAD</sub>-algU | ΔalgU ΔmucA attTn7::P<sub>araBAD</sub>-algU |
|------------|--------|---------------------------------|----------------------------------------|------------------------------------------|
| Strain     | Ara    | 0                 | 0.1                        | 0.25                        | 1                                      |
| attTn7::P<sub>araBAD</sub>-algU | 0      | *                 | *                         | *                                        | *                                      |
| ΔalgU attTn7::P<sub>araBAD</sub>-algU | 0      | *                 | *                         | *                                        | *                                      |
| ΔalgU ΔmucA attTn7::P<sub>araBAD</sub>-algU | 0      | *                 | *                         | *                                        | *                                      |

| Medium: PIB | Strain | attTn7::P<sub>araBAD</sub>-algU | ΔalgU attTn7::P<sub>araBAD</sub>-algU | ΔalgU ΔmucA attTn7::P<sub>araBAD</sub>-algU |
|-------------|--------|---------------------------------|----------------------------------------|------------------------------------------|
| Strain      | Ara    | 0                 | 1                                      | 0                                        |
| attTn7::P<sub>araBAD</sub>-algU | 0      | *                 | *                                      | *                                        |
| ΔalgU attTn7::P<sub>araBAD</sub>-algU | 0      | *                 | *                                      | *                                        |
| ΔalgU ΔmucA attTn7::P<sub>araBAD</sub>-algU | 0      | *                 | *                                      | *                                        |

| Medium: SCFM | Strain | attTn7::P<sub>araBAD</sub>-algU | ΔalgU attTn7::P<sub>araBAD</sub>-algU | ΔalgU ΔmucA attTn7::P<sub>araBAD</sub>-algU |
|--------------|--------|---------------------------------|----------------------------------------|------------------------------------------|
| Strain       | Ara    | 0                 | 1                                      | 0                                        |
| attTn7::P<sub>araBAD</sub>-algU | 0      | *                 | *                                      | *                                        |
| ΔalgU attTn7::P<sub>araBAD</sub>-algU | 0      | *                 | *                                      | *                                        |
| ΔalgU ΔmucA attTn7::P<sub>araBAD</sub>-algU | 0      | *                 | *                                      | *                                        |

| Medium: VBMM | Strain | attTn7::P<sub>araBAD</sub>-algU | ΔalgU attTn7::P<sub>araBAD</sub>-algU | ΔalgU ΔmucA attTn7::P<sub>araBAD</sub>-algU |
|--------------|--------|---------------------------------|----------------------------------------|------------------------------------------|
| Strain       | Ara    | 0                 | 1                                      | 0                                        |
| attTn7::P<sub>araBAD</sub>-algU | 0      | *                 | *                                      | *                                        |
| ΔalgU attTn7::P<sub>araBAD</sub>-algU | 0      | *                 | *                                      | *                                        |
| ΔalgU ΔmucA attTn7::P<sub>araBAD</sub>-algU | 0      | *                 | *                                      | *                                        |

Significance was determined via a two-way ANOVA with post-hoc Bonferroni. Ara, concentration of arabinose (%). Asterisk, \( p < 0.05 \). Open box, no statistical difference.
Table S4. Statistical differences in growth rate among groups overexpressing \textit{algU} and/or \textit{rpoD}.

| Strain | Ara | \(\Delta\text{algU}\Delta\text{mucA} P_{\text{BAD-rpoD}}\) | \(\Delta\text{algU}\Delta\text{mucA} P_{\text{BAD-\textit{algU}}^{\text{WT}}}\) | \(\Delta\text{algU}\Delta\text{mucA} P_{\text{BAD-\textit{algU}}^{\text{K57A}}}\) | \(\Delta\text{algU}\Delta\text{mucA} P_{\text{BAD-\textit{algU}}^{\text{K57A}}} P_{\text{BAD-rpoD}}\) |
|--------|-----|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| \(\Delta\text{algU}\Delta\text{mucA}\) | 0   | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     |
|        | 2   | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     |
| \(\Delta\text{algU}\Delta\text{mucA} P_{\text{BAD-rpoD}}\) | 0   | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     |
|        | 2   | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     |
| \(\Delta\text{algU}\Delta\text{mucA} P_{\text{BAD-\textit{algU}}^{\text{WT}}}\) | 0   | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     |
|        | 2   | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     |
| \(\Delta\text{algU}\Delta\text{mucA} P_{\text{BAD-\textit{algU}}^{\text{K57A}}}\) | 0   | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     |
|        | 2   | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     |
| \(\Delta\text{algU}\Delta\text{mucA} P_{\text{BAD-rpoD}}\) | 0   | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     |
|        | 2   | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     | ![Gray square](Gray square)                     |

Significance was determined via a two-way ANOVA with post-hoc Bonferroni. Ara, concentration of arabinose (%). Asterisk, p < 0.05. Open box, no statistical difference.
| Strains | Relevant characteristics | Source |
|---------|--------------------------|--------|
| **Escherichia coli** | | |
| NEB5α | For cloning; *fuA2 (argF-lacZ)U169 phoA glnV44 Φ80lacZ (lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17 | NEB |
| *ccdB* Survival2 T1R | F^- mcrA (mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 araΔ139 (ara-1eu)7697 galU galK rpsL endA1 nupG *fuA::IS2*, Sm’ | Invitrogen |
| S17.1 (λpir) | For conjugation; F^- RP4-2-Mu aphA::Tn7 recA λpir lysogen, Sm’, Tc’ | (Kristensen *et al.*, 1995) |
| **Pseudomonas aeruginosa** | | |
| PAO1 | Wild-type PAO1 | (Holloway, 1955) |
| PAO1 Δ*mucA* | PAO1 containing an unmarked deletion of *mucA* | (Pritchett *et al.*, 2015) |
| PA103 | Wild-type PA103 | (Frank *et al.*, 1994) |
| PA103 Δ*mucA* | PA103 containing nonpolar deletion of *mucA* | (Intile *et al.*, 2014) |
| PAK | Wild-type PAK | (Takeya & Amako, 1966) |
| PAK | *attCTXlacP1Δlac1-lac2* | (Fulcher *et al.*, 2010) |
| PAK Δ*mucA* | Δ*mucA attCTX::lacP1 Δlac1-lac2* | (Jones *et al.*, 2010) |
| PA14 | Wild-type PA14 | (Rahme *et al.*, 1995) |
| CF127 | Mucoid CF isolate | (Wolfgang *et al.*, 2003) |
| CF18 | Non-mucoid CF isolate | (Wolfgang *et al.*, 2003) |
| CF27 | Rugose CF isolate | (Wolfgang *et al.*, 2003) |
| X13273 | Blood isolate | (Wolfgang *et al.*, 2003) |
| X24509 | UTI isolate | (Wolfgang *et al.*, 2003) |
| MSH10 | Water isolate | (Wolfgang *et al.*, 2003) |
| E2 | Tomato plant isolate | (Wolfgang *et al.*, 2003) |
| BTPa156 | PAO1 containing an in-frame deletion of *algD* | (Tseng *et al.*, 2013) |
| BTPa669 | PAO1 containing an in-frame deletion of *algB* | This study |
| BTPa671 | PAO1 containing an in-frame deletion of *amrZ* | This study |
| BTPa675 | PAO1 containing an in-frame deletion of *algR* | This study |
| BTPa685 | PAO1 containing an in-frame deletions of *algB, amrZ*, and *algR* | This study |
| BTPa391 | PAO1 Δ*algU ΔmucA* | This study |
| BTPa355 | PAO1 Δ*mucA attTn7::P*algU*-mucA(1-194)-10His | This study |
| BTPa628 | PAO1 Δ*algD attTn7::P*algU*-mucA(1-194)-10His | This study |
| BTPa715 | PAO1 Δ*algD attTn7::P*algU*-mucA(1-143)-10His | This study |
| BTPa620 | PAO1 Δ*algD attTn7::P*algU*-mucA(1-110)-10His | This study |
| BTPa622 | PAO1 Δ*algD attTn7::P*algU*-mucA(1-75)-10His | This study |
| BTPa601 | PAO1 ∆algD attTn7::P<sub>algU</sub>-mucA(1-62)-10His | This study |
|---------|-------------------------------------------------|------------|
| BTPa603 | PAO1 ∆algD attTn7::P<sub>algU</sub>-mucA(1-50)-10His | This study |
| BTPa605 | PAO1 ∆algD attTn7::P<sub>algU</sub>-mucA(1-40)-10His | This study |
| BTPa606 | PAO1 ∆algD attTn7::P<sub>algU</sub>-mucA(1-24)-10His | This study |
| BTPa673 | PAO1 ∆algD attTn7::P<sub>algU</sub>-mucA(51-194)-10His | This study |
| BTPa832 | PAO1 ∆algD attTn7::P<sub>algU</sub>-mucA(1-75) D15A-10His | This study |
| BTPa844 | PAO1 ∆algD attTn7::P<sub>algU</sub>-mucA(1-75) E22A-10His | This study |
| BTPa835 | PAO1 ∆algD attTn7::P<sub>algU</sub>-mucA(1-75) R42A-10His | This study |
| BTPa643 | PAO1 ∆mucA ∆algD attTn7::P<sub>algU</sub>-mucA(1-194)-10His | This study |
| BTPa635 | PAO1 ∆mucA ∆algD attTn7::P<sub>algU</sub>-mucA(1-110)-10His | This study |
| BTPa762 | PAO1 ∆mucA ∆algD attTn7::P<sub>algU</sub>-mucA(1-143)-10His | This study |
| BTPa637 | PAO1 ∆mucA ∆algD attTn7::P<sub>algU</sub>-mucA(1-75)-10His | This study |
| BTPa639 | PAO1 ∆mucA ∆algD attTn7::P<sub>algU</sub>-mucA(1-62)-10His | This study |
| BTPa641 | PAO1 ∆mucA ∆algD attTn7::P<sub>algU</sub>-mucA(1-50)-10His | This study |
| BTPa548 | PAO1 ∆mucA ∆algD attTn7::P<sub>algU</sub>-mucA(1-75) E22A-10His | This study |
| BTPa812 | PAO1 attTn7::P<sub>algU</sub>-mucA(1-155)-10His | This study |
| BTPa816 | PAO1 ∆mucA attTn7::P<sub>algU</sub>-mucA(1-155)-10His | This study |
| BTPa382 | PAO1 ∆algU | This study |
| BTPa740 | PAO1 ∆algU attTn7::P<sub>algU</sub>-algU | This study |
| BTPa748 | PAO1 ∆algU attTn7::P<sub>algU</sub>-algU K57A | This study |
| BTPa754 | PAO1 ∆algU attTn7::P<sub>algU</sub>-algU N81A | This study |
| BTPa846 | PAO1 ∆algU attTn7::P<sub>algU</sub>-algU E46G | This study |
| BTPa757 | PAO1 ∆algU attTn7::P<sub>algU</sub>-algU A58T | This study |
| BTPa763 | PAO1 ∆algU ∆mucA attTn7::P<sub>algU</sub>-algU K57A | This study |
| BTPa764 | PAO1 ∆algU ∆mucA attTn7::P<sub>algU</sub>-algU N81A | This study |
| BTPa849 | PAO1 ∆algU ∆mucA attTn7::P<sub>algU</sub>-algU E46G | This study |
| BTPa837 | PAO1 ∆algU ∆mucA attTn7::P<sub>algU</sub>-algU A58T | This study |
| BTPa616 | PAO1 attTn7::rhaSR-P<sub>araBAD</sub>-mucA-10His | This study |
| BTPa624 | PAO1 ∆mucA attTn7::rhaSR-P<sub>araBAD</sub>-mucA-10His | This study |
| BTPa646 | PAO1 attTn7::P<sub>araBAD</sub>-algU-10His::Gm | This study |
| BTPa527 | PAO1 ∆algU attTn7::P<sub>araBAD</sub>-algU::Gm | This study |
| BTPa529 | PAO1 ∆algU ∆mucA attTn7::P<sub>araBAD</sub>-algU::Gm | This study |
| BTPa853 | PAO1 ∆algU ∆mucA attCTX2::P<sub>araBAD</sub>-rpoD | This study |
| BTPa855 | PAO1 ∆algU ∆mucA attCTX2::P<sub>araBAD</sub>-rpoD attTn7::P<sub>BADara</sub>-algU::Gm | This study |
| BTPa859 | PAO1 ∆algU ∆mucA attTn7::P<sub>araBAD</sub>-algU K57A::Gm | This study |
| BTPa861 | PAO1 ∆algU ∆mucA attCTX2::P<sub>araBAD</sub>-rpoD attTn7::P<sub>BADara</sub>-algU K57A::Gm | This study |
| BTPa149 | PAO1 pBT336 (vector control) | This study |
| BTPa550 | PAO1 pBT435 | This study |
| BTPa559 | PAO1 ∆algU pBT435 | This study |
| BTPa552 | PAO1 ∆algU attTn7::P<sub>algU</sub>-algU pBT435 | This study |
| BTPa553 | PAO1 ∆algU attTn7::P<sub>algU</sub>-algU K57A pBT435 | This study |
| BTPa554 | PAO1 ∆algU attTn7::P<sub>algU</sub>-algU N81A pBT435 | This study |
| BTPa555 | PAO1 ∆algU attTn7::P<sub>algU</sub>-algU E46G pBT435 | This study |
| BTPa556 | PAO1 ∆algU attTn7::P<sub>algU</sub>-algU A58T pBT435 | This study |
| BTPa557 | PAO1 ∆mucA attTn7::rhaSR-P<sub>araBAD</sub>-mucA-10His pBT435 | This study |
| MSPa82 | PAO1 ∆mucA attTn7::rhaSR-P<sub>araBAD</sub>-mucA-10His revertant; #1-10 | This study |
| MSPa83 | PAO1 ∆mucA attTn7::rhaSR-P<sub>araBAD</sub>-mucA-10His revertant; #1-2 | This study |
This study

MSPa84  PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #1-3
MSPa85  PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #1-4
MSPa86  PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #1-5
MSPa87  PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #1-6
MSPa88  PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #1-7
MSPa90  PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #1-8
MSPa91  PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #1-9
MSPa92  PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #1-10
MSPa93  PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #1-11
MSPa94  PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #2-1
MSPa95  PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #2-2
MSPa96  PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #2-3
MSPa98  PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #2-4
MSPa100 PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #2-5
MSPa101 PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #2-6
MSPa102 PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #2-7
MSPa103 PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #2-8
MSPa106 PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #2-9
MSPa107 PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #2-10
MSPa109 PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #2-11
MSPa111 PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #2-12
MSPa113 PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #2-13
MSPa114 PAO1 ΔmucA attTn7::rhaSR-P\text{rhaBAD-mucA}-10His revertant; #2-14
MSPa130 MSPa82 pBT435
MSPa131 MSPa83 pBT435
MSPa132 MSPa84 pBT435
MSPa133 MSPa85 pBT435
MSPa134 MSPa86 pBT435
MSPa135 MSPa87 pBT435
MSPa136 MSPa88 pBT435
MSPa137 MSPa90 pBT435
MSPa138 MSPa91 pBT435
MSPa139 MSPa92 pBT435
MSPa140 MSPa93 pBT435
MSPa141 MSPa94 pBT435
MSPa142 MSPa95 pBT435
MSPa143 MSPa96 pBT435
MSPa144 MSPa98 pBT435
MSPa145 MSPa100 pBT435
MSPa146 MSPa101 pBT435
MSPa147 MSPa102 pBT435
MSPa148 MSPa103 pBT435
MSPa149 MSPa106 pBT435
MSPa150 MSPa107 pBT435
MSPa151 MSPa109 pBT435
MSPa152 MSPa111 pBT435
MSPa153 MSPa113 pBT435
| MSPa154 | MSPa114 pBT435 | This study |
|---------|----------------|------------|
| Saccharomyces cerevisiae | | |
| MaV203 | MATa, leu2-3,112, trp1-901, his3Δ200, ade2-101, gal4Δ, gal80Δ, SPAL10::URA3, GAL1::lacZ, HIS3::HIS3@LYS2, can1R, cyh2R | Invitrogen |
| BTSc1-4 | MaV203 containing pEXP32-Krev1 and pEXP22-RalGDS-WT | This study |
| BTSc9-12 | MaV203 containing pEXP32-Krev1 and pEXP22-RalGDS-m2 | This study |
| BTSc13-16 | MaV203 containing pDEST32 and pDEST22 | This study |
| BTSc20,41-44 | MaV203 containing pAAK004 and pAAK001 | This study |
| BTSc21-24 | MaV203 containing pAAK002 and pAAK003 | This study |
| BTSc25-28 | MaV203 containing pAAK002 and pDEST22 | This study |
| BTSc29-32 | MaV203 containing pAAK004 and pDEST22 | This study |
| BTSc33-36 | MaV203 containing pDEST32 and pAAK003 | This study |
| BTSc37-40 | MaV203 containing pDEST32 and pAAK001 | This study |
| BTSc68-71 | MaV203 containing pAAK033 and pAAK001 | This study |
| BTSc76-79 | MaV203 containing pAAK034 and pAAK001 | This study |
| BTSc131-134 | MaV203 containing pAAK042 and pAAK001 | This study |
| Plasmids                      | Relevant characteristics                                      | Source                       |
|------------------------------|--------------------------------------------------------------|------------------------------|
| pDONR221 P5-P2               | GateWay-compatible vector with attR5 and attR2 recombination sites and ccdB; Kn', Cm' | Invitrogen                  |
| pDONRPEX18Gm                 | pEX18-based, GateWay-compatible suicide vector (Accession No. KM880128) with attP1 and attP2 recombination sites and ccdB; Ap', Gm', Cm' | (Hmoel et al., 2015)        |
| pUC18-miniTn7T2-Gm-GW        | GateWay-compatible miniTn7 vector with attR1 and attR2 recombination sites and ccdB; Ap', Gm', Cm' | (Zhao et al., 2013)         |
| pDONRPUC18T-miniTn7T2-Gm     | GateWay-compatible miniTn7 vector with attP1 and attP2 recombination sites and ccdB; Ap', Gm', Cm' | (Joe J. Harrison            |
| pUCP22T2-GW                  | GateWay-compatible pUCP22 vector with attR1 and attR2 recombination sites and ccdB; Ap', Gm', Cm' | (Almblad et al., 2015)      |
| pTNS2                        | T7 transposase expression vector; Ap'                       | (Choi & Schweizer, 2006)    |
| pFLP2                        | Plasmid that expresses Flp recombinase; Ap'                 | (Choi & Schweizer, 2006)    |
| pJJH187                      | pDONR221P5r-based Gateway-compatible vector encoding the araC repressor protein and the P_{araBAD} promoter; Kn' | (Khamkova et al., 2013)     |
| pJM220                       | pUC18T-miniTn7T based vector encoding rhaSR and the P_{rhaBAD} promoter; Gm' | (Meisner & Goldberg, 2016)  |
| pBX39                        | pEX18Ap with an in-frame deletion of amrZ; Ap'             | (Xu & Wozniak, 2015)        |
| pDEST22                      | Gal4 activation domain-based prey vector for Yeast Two-Hybrid system; Ap', Cm' | Invitrogen                  |
| pDEST32                      | Gal4 DNA binding domain-based bait vector for Yeast Two-Hybrid system; Gm', Cm' | Invitrogen                  |
| pDEST22-Krev1                | Two-hybrid control plasmid containing full-length rat Krev1; Ap' | Invitrogen                  |
| pDEST32-RalGDS-WT            | Two-hybrid control plasmid containing wild-type RalGDS domain; Gm' | Invitrogen                  |
| pDEST32-RalGDS-m2            | Two-hybrid control plasmid containing mutated RalGDS domain; Gm' | Invitrogen                  |
| pBT212                       | GateWay compatible plasmid containing gfpmut3 flanked by attR5 and attL1 recombination sites; Kn' | (Armbruster et al., 2019)   |
| pBT244                       | GateWay-compatible plasmid containing a null promoter flanked by attL2 and attL5 recombination sites; Kn' | (Tseng et al., 2013)        |
| pBT336                       | pUCP22T2.1 containing gfpmut3 driven by a null promoter; Ap', Gm' | This study                  |
| pBT342                       | pDONRPEX18Gm with an in-frame deletion allele of algD; Gm' | (Tseng et al., 2013)        |
| pBT396                       | pDONRPEX18Gm with an in-frame deletion allele of mucA; Gm' | This study                  |
| pBT399                       | pUC18-miniTn7T2 with an allele encoding all 194 aa of mucA, driven by its native promoter; Ap', Gm' | This study                  |
| pBT406                       | pUC18-miniTn7T2 with an allele encoding the first 50 aa of mucA, driven by its native promoter; Ap', Gm' | This study                  |
| pBT407                       | pUC18-miniTn7T2 with an allele encoding the first 110 aa of mucA, driven by its native promoter; Ap', Gm' | This study                  |
| pBT408                       | pUC18-miniTn7T2 with an allele encoding the first 75 aa of mucA, driven by its native promoter; Ap', Gm' | This study                  |
| Plasmid Code | Description | References |
|--------------|-------------|------------|
| pBT410       | pUC18-miniTn7T2 with an allele encoding the first 62 aa of mucA, driven by its native promoter; Ap', Gm' | This study |
| pBT411       | pDONRPEX18Gm with an in-frame deletion allele of algU; Gm' | This study |
| pBT412       | pDONRPEX18Gm with an in-frame deletion allele of mucA, for use in a ∆algU background; Gm' | This study |
| pBT413       | pUC18-miniTn7T2 with an allele encoding the first 40 aa of mucA, driven by its native promoter; Ap', Gm' | This study |
| pBT414       | pUC18-miniTn7T2 with an allele encoding the first 24 aa of mucA, for use in a ∆algU background; Gm' | This study |
| pBT435       | pUCP22T2.1 containing gfpmut3 driven by the algD promoter; Ap', Gm' | This study |
| pMS009       | pUC18-miniTn7T2 with an allele encoding the aa 51-195 of mucA, driven by its native promoter; Ap', Gm' | This study |
| pMS014       | pDONRPEX18Gm with an in-frame deletion allele of algB; Gm' | This study |
| pMS015       | pDONRPEX18Gm with an in-frame deletion allele of algR; Gm' | This study |
| pMS023       | pUC18-miniTn7T2 with an allele encoding the first 143 aa of mucA, driven by its native promoter; Ap', Gm' | This study |
| pMS024       | pUC18-miniTn7T2 with an allele encoding the first 155 aa of mucA, driven by its native promoter; Ap', Gm' | This study |
| pMS034       | pUC18-miniTn7T2 with an allele encoding an R42A substitution in mucA, driven by its native promoter; Ap', Gm' | This study |
| pMS035       | pUC18-miniTn7T2 with an allele encoding a D15A substitution in mucA, driven by its native promoter; Ap', Gm' | This study |
| pMS037       | miniCTX2T2.1-GW with an allele encoding rpoD driven by an arabinose-inducible promoter; Tc' | This study |
| pMS039       | pUC18-miniTn7T2 with an allele encoding an E22A substitution in mucA, driven by its native promoter; Ap', Gm' | This study |
| pMS041       | pUC18-miniTn7T2 with an allele encoding an E46G substitution in algU, driven by its native promoter; Ap', Gm' | This study |
| pMS050       | pUC18-miniTn7T2 with an allele encoding an K57A substitution in algU, driven by an arabinose-inducible promoter; Ap', Gm' | This study |
| pAAK001      | pDEST22 based expression plasmid encoding AlgU fused to the Gal4 activating domain | This study |
| pAAK002      | pDEST32 based expression plasmid encoding AlgU fused to the Gal4 DNA binding domain | This study |
| pAAK003      | pDEST22 based expression plasmid encoding the first 75 aa of MucA fused to the Gal4 activating domain | This study |
| pAAK004      | pDEST32 based expression plasmid encoding the first 75 aa of MucA fused to the Gal4 activating domain | This study |
| pAAK014      | pUC18-miniTn7T2 with an allele encoding full length algU, driven by its native promoter; Ap', Gm' | This study |
| pAAK018      | pUC18-miniTn7T2 with an allele encoding an N81A substitution in algU, driven by its native promoter; Ap', Gm' | This study |
| pAAK020      | pUC18-miniTn7T2 with an allele encoding an K57A substitution in algU, driven by its native promoter; Ap', Gm' | This study |
| pAAK021      | pUC18-miniTn7T2 with an allele encoding an A58T substitution in algU, driven by its native promoter; Ap', Gm' | This study |
| pAAK033      | pDEST32 based expression plasmid encoding the first 75 aa of MucA with an R42A substitution fused to the Gal4 activating domain | This study |
| Plasmid | Description                                                                 | Source       |
|---------|-----------------------------------------------------------------------------|--------------|
| pAAK034 | pDEST32 based expression plasmid encoding the first 75 aa of MucA with an D15A substitution fused to the Gal4 activating domain | This study   |
| pAAK042 | pDEST32 based expression plasmid encoding the first 75 aa of MucA with an E22A substitution fused to the Gal4 activating domain | This study   |
| pCT2    | pUC18-miniTn7T2 with an allele encoding AlgU, driven by an arabinose-inducible promoter; Ap', Gm' | This study   |
| pLM7    | pUC18T-miniTn7T with an allele encoding MucA, driven by a rhamnose-inducible promoter; Gm' | This study   |
Table S7. Oligonucleotides used in this study.

| Primer | Sequence |
|--------|----------|
| OBT401 (algD SeqF) | AGCCCTTGTGCCGGAATAG |
| OBT402 (algD SeqR) | GCTTGTCGACCCTCCTC |
| OMS103 (algR SeqF) | TCGAGGGCTGGCGTAGTG |
| OMS104 (algR UpF) | ggggacatgtaaagctggaagcagcag |
| OMS105 (algR UpR) | AGGCCTGATGAGCATACCTC |
| OMS106 (algR DownF) | ggcgtagctgatgagctgag |
| OMS107 (algR DownR) | GGGACCACTTGTACCTGTAC |
| OMS108 (algR SeqR) | GCTGGACCTGTTCGACCTGTC |
| OMS109 (algB SeqF) | GGAGGCGAACCGGCCTGTC |
| OMS110 (algB UpF) | ggggacatgtaaagctggaagcagcag |
| OMS111 (algB UpR) | CAGCAGGATGCGCCCCTG |
| OMS112 (algB DownF) | cagaggtgcagtaaagctggaagcagcag |
| OMS113 (algB DownR) | GGGACCACTTGTACCTGTAC |
| OBT597 (mucA UpF) | ggggacatgtaaagctggaagcagcag |
| OBT598 (mucA UpR) | CTCCTCGGTGCTTCTAGAAG |
| OBT599 (mucA DownF) | ggtctcaggtgatgagctgag |
| OBT600 (mucA DownR) | GGGACCACTTGTACCTGTAC |
| OBT601 (mucA SeqF) | GCCATCAGCCGAGAC |
| OBT602 (mucA SeqR) | TCGGTCAGCCGCAAGCTG |
| OBT603 (algU promoter UpF) | ggggacatgtaaagctggaagcagcag |
| OBT604 (algU promoter UpR) | GGGACCACTTGTACCTGTAC |
| OBT605B (mucA DownF) | ggtctcaggtgatgagctgag |
| OBT606B (mucA DownR) | GGGACCACTTGTACCTGTAC |
| OBT620 (MucA 1-194 aa) | CAGGCTTGGAAAACCGCCGAGCAGCCATCAC |
| OBT621 (MucA 1-194 aa) | GGTAGTGCTGTCGCGGTGGTATAGC |
| OBT622 (MucA 1-50 aa) | GGGACCACTTGTACCTGTAC |
| OBT623 (MucA 1-50 aa) | GGTAGTGCTGTCGCGGTGGTATAGC |
| OBT624 (MucA 1-110 aa) | GGTAGTGCTGTCGCGGTGGTATAGC |
| OBT625 (MucA 1-110 aa) | GGTAGTGCTGTCGCGGTGGTATAGC |
| OBT673 (MucA 1-75 aa) | GGGACCACTTGTACCTGTAC |
| OBT674 (MucA 1-75 aa) | GGTAGTGCTGTCGCGGTGGTATAGC |
| OBT679 (MucA 1-62 aa) | GGGACCACTTGTACCTGTAC |
| OBT680 (MucA 1-62 aa) | GGTAGTGCTGTCGCGGTGGTATAGC |
| OBT681 (algU UpF) | ggggacatgtaaagctggaagcagcag |
| OBT682 (algU UpR) | GGTAGTGCTGTCGCGGTGGTATAGC |
| OBT683 (algU DownF) | GGTAGTGCTGTCGCGGTGGTATAGC |
| OBT684 (algU DownR) | GGTAGTGCTGTCGCGGTGGTATAGC |
| OBT685 (algU SeqF) | ATGAGCAGGCGGGCGCTGTAC |
| OBT686 (algU SeqR) | GGGACCACTTGTACCTGTAC |
| Sequence ID | Description | Oligo Sequence |
|-------------|-------------|----------------|
| OBT687 | (mucA UpF for  \Delta algU) | `ggggacaaggttgtataaaaaaagcaggctcaGTTCCAAAGCAGGATGCCTGAAGAC` |
| OBT690 | (MucA 1-40 aa) | `CTTGCGTTCCACCTGGGGCAGCAGCCATCAC` |
| OBT691 | (MucA 1-40 aa) | `CTGCGTTCCACCTGGGGCAGCAGCCATCAC` |
| OBT692 | (MucA 1-40 aa) | `GTGATGGCTGCTGCCCCAGGTGGAACGCAG` |
| OBT695 | (MucA 1-24 aa) | `GAACTCGAGTTGCGGGGCAGCAGCCATCAC` |
| OMS077 | (MucA 51-195 aa) | `OBT690 (MucA 1-40 aa)` |
| OMS078 | (MucA 51-195 aa) | `OMS077 (MucA 51-195 aa)` |
| OMS118 | (mucA UpF) | `OMS118 (mucA UpF)` |
| OMS119 | (MucA DownR) | `OMS119 (MucA DownR)` |
| OMS132 | (MucA 1-143 aa) | `OMS132 (MucA 1-143 aa)` |
| OMS133 | (MucA 1-143 aa) | `OMS133 (MucA 1-143 aa)` |
| OMS158 | (MucA 1-155 aa) | `OMS158 (MucA 1-155 aa)` |
| OMS173 | (MucA R42A) | `OMS173 (MucA R42A)` |
| OMS174 | (MucA R42A) | `OMS174 (MucA R42A)` |
| OMS175 | (MucA D15A) | `OMS175 (MucA D15A)` |
| OMS176 | (MucA D15A) | `OMS176 (MucA D15A)` |
| OMS214 | (MucA E22A) | `OMS214 (MucA E22A)` |
| OMS215 | (MucA E22A) | `OMS215 (MucA E22A)` |
| OMS218 | (AlgU E46G) | `OMS218 (AlgU E46G)` |
| OMS219 | (rpoD DownR) | `OMS219 (rpoD DownR)` |
| OAK20 | (AlgU N81A UpR) | `OAK20 (AlgU N81A UpR)` |
| OAK21 | (AlgU N81A DownF) | `OAK21 (AlgU N81A DownF)` |
| OAK22 | (AlgU K57A UpR) | `OAK22 (AlgU K57A UpR)` |
| OAK23 | (AlgU K57A DownF) | `OAK23 (AlgU K57A DownF)` |
| OAK26 | (AlgU A58T UpR) | `OAK26 (AlgU A58T UpR)` |
| OAK27 | (AlgU A58T DownF) | `OAK27 (AlgU A58T DownF)` |
| OAK29 | (AlgU E46G DownF) | `OAK29 (AlgU E46G DownF)` |
| OAK30 | (AlgU E46G UpR) | `OAK30 (AlgU E46G UpR)` |
| OCT1 | (AlgU UpF) | `OCT1 (AlgU UpF)` |
| OCT2 | (AlgU DownR) | `OCT2 (AlgU DownR)` |
| OLM24 | (MucA Fwd) | `OMS077 (MucA 51-195 aa)` |
| OLM25 | (MucA Rev) | `OMS077 (MucA 51-195 aa)` |
| OBT432 | (GFP DownR) | `OBT432 (GFP DownR)` |
| OBT846 | (algD promoter UpF) | `OBT846 (algD promoter UpF)` |
| OBT847 | (algD promoter UpR) | `OBT847 (algD promoter UpR)` |
| OBT848 | (GFP DownF) | `OBT848 (GFP DownF)` |
Figure S1. Allelic exchange assay.

(A) Schematic of assay. To delete the endogenous mucA (short red box with mucA) from the genome, the deletion vector pBT396, which contains an allele of mucA that is missing >95% of the coding region (tall red box with delta), was introduced into \textit{P. aeruginosa} via conjugation. Regions of homology (tall blue boxes) to the genome (short blue boxes) approximately 400 base pairs in length flank the deletion allele and allow for recombination into the genome (X) to create a merodiploid. This first recombination event (1\textsuperscript{st}) can be selected for using antibiotics due to integration of the vector backbone, which contains a gentamicin resistance marker. At least six merodiploids were confirmed via PCR to ensure the genome contained both the deletion and endogenous mucA allele. Merodiploids can then undergo a second recombination event (2\textsuperscript{nd}) that will lead to the loss of one of the two alleles, resolving to either the endogenous or deletion allele, which results in a cell that is either wild-type (top) or a deletion mutant (bottom), respectively. For each of the six confirmed merodiploids, we counter-selected for the second recombination event via the loss of sacB, a vector backbone marker. Using PCR, eight isolates per merodiploid were tested to determine which allele each isolate resolved to. If a gene is non-essential, we expect to observe both wild type or deletion mutants. However, if a gene is essential, we expect to isolate only those cells that resolved to wild type, as the cells cannot survive with the deletion allele. If we are unable to delete mucA from the first replicate of this experiment, we perform two additional biological replicates, for a minimum total of 125 colonies screened. If all isolates resolve to wild-type, we deem mucA to be essential in that strain background (p < 0.0001, Fisher's exact test). (B) Representative image of merodiploid confirmation. The PCR products corresponding to the endogenous allele and the deletion allele are indicated with an arrow labelled “WT” and “Δ,” respectively. Positive (WT ctrl; PAO1) and negative (ΔmucA ctrl; BTPa355) controls are included. PCR products from six representative merodiploids are shown. (C) Representative image of PCR products from isolates after the second recombination. Image is labeled as in (B). Top, the PCR products of 8 isolates from a strain in which mucA was deemed essential. Bottom, the PCR products of 8 isolates from a strain in which mucA was not essential.
Figure S2. Published mutations in mucA.
Top, schematic of MucA with the regions indicated encoding for the AlgU binding domain (green, AlgU BD), the transmembrane domain (blue, TM), and the MucB binding domain (purple, MucB BD). Hash marks on top indicate the residue number in MucA. Bottom, sites of mucA mutations that lead to the production of a truncated protein in published isolates. Each line represents one or more individual isolates described in the references on the right. The mutations for the upper five references (Boucher et al., 1997, Candido Cacador et al., 2018, Ciofu et al., 2008, Martin et al., 1993, Pulcrano et al., 2012) are in cystic fibrosis clinical isolates of P. aeruginosa, while the mutations in the last reference (Turner et al., 2015) are random transposon insertions in the laboratory PAO1 and PA14 strains.
Figure S3. MucA aa 1-75 and AlgU interact via yeast two-hybrid assay.
The indicated proteins were fused to the Gal4 DNA binding domain (DBD; "bait") or the Gal4
activation domain (AD; "prey"). Interaction of the bait and prey proteins drive the expression of
lacZ. Beta-galactosidase activity (in Miller units) was used as a proxy for the protein interaction
strength. The average (Avg) and standard error of the mean (SEM) of each bar are indicated
(N=3). As positive and negative controls, Krev1 is known to interact with wild-type RalGDS
(RalWT), but not the mutant RalGDS (RalMut) via yeast two-hybrid. AlgU, construct encoding full-
length wild-type AlgU; MucA1-75, construct encoding only the first 75 residues of wild-type MucA;
–, no fusion protein included; error bars, SEM (N=3); letters, statistical groups with the different
letters representing statistically different groups (p < 0.01; biological triplicate with technical
quadruplicates; ANOVA with post-hoc Tukey HSD).

|       | DBD | Krev1 | Krev1 | MucA1-75 | MucA1-75 | – | AlgU | – | AlgU | – |
|-------|-----|-------|-------|----------|----------|---|------|---|------|---|
| AD    | RalWT | RalMut | AlgU  | –        | AlgU     | MucA1-75 | MucA1-75 | – |
| Avg   | 2.832 | 0.017 | 17.142 | 0.008    | 0.010    | 6.833    | 0.017    | 0.009 |
| SEM   | 0.402 | 0.010 | 0.221  | 0.001    | 0.004    | 0.355    | 0.006    | 0.003 |

Beta-galactosidase activity (Miller units)
Figure S4. Location of affected AlgU residues in revertants that can grow in the absence of MucA.

Model of the RNAP holoenzyme containing $\sigma^E$ bound to the promoter. Gray, RNAP core; cyan, $\beta$ flap; pink, $\beta'$ coiled coil; green, $\sigma^E$ Region 2; yellow, $\sigma^E$ Region 4; blue/light blue, promoter element. Insets, location of residues that are substituted in the revertants with missense algU mutations. The side chain of the affected residue is in white. Substitution of A21 and A47 (V in model) likely affect protein packing and folding of AlgU. Substitution of D18, Y29, and Y59 would reduce predicted intra- and inter-molecular hydrogen bonding and likely affect AlgU folding. Substitution of D49, N81, and R174 would likely affect the interaction of AlgU with the RNAP core or the promoter. Dashed yellow lines, hydrogen bonds; red atoms, oxygen; blue atoms, nitrogen; orange, phosphorus.
Figure S5. Overexpression of algU in the absence of mucA causes a growth defect in various media.
Growth rate of indicated strains grown in (A) LB, (B) PIB, (C) SCFM, and (D) VBMM with (+) or without (−) 1% arabinose (relative to PAO1 attTn7::P_{araBAD}−algU grown in the absence of arabinose). Error bars, SEM (N=3). Asterisk, statistically different from the same strain grown in the absence of arabinose (p < 0.01, N = 3, two-way ANOVA with post-hoc Bonferroni). See Table S3 for full statistical comparisons. LB contains tryptone and yeast extract, both of which provide protein hydrolates, as the main carbon source. The main carbon source of PIB is also protein hydrolates, but from pancreatic gelatin digest. While PIB also contains glycerol, which can serve as a carbon source, it is not a preferred carbon source for Pseudomonas aeruginosa. SCFM is a defined medium that contains amino acids as the primary carbon source, and mimics the nutrients found in the CF lung environment. VBMM is a minimal medium that contains citrate as the sole carbon source.
Figure S6. RpoD production in strains with an ectopic inducible rpoD allele.
Expression of rpoD was inferred via semi-quantitative Western blot in the indicated strains (top). RNAP was used as a loading control (bottom). Strains were grown in LB with (+) or without (−) 2% arabinose. The relative amount of RpoD is indicated, where 1.00x represents RpoD levels in the parental strain without arabinose (± SD; N=3). While the strains produce significantly more RpoD in the presence of arabinose relative to the same strain in the absence of arabinose (p < 0.01), there is no statistical difference in the amount of RpoD produced from the induced strains containing an ectopic rpoD allele (two-way ANOVA with post-hoc Bonferroni).
SUPPLEMENTARY REFERENCES
Almblad, H., Harrison, J.J., Rybtke, M., Groizeleau, J., Givskov, M., Parsek, M.R., and Tolker-Nielsen, T. (2015) The Cyclic AMP-Vfr Signaling Pathway in Pseudomonas aeruginosa Is Inhibited by Cyclic Di-GMP. J Bacteriol 197: 2190-2200.
Armbruster, C.R., Lee, C.K., Parker-Gilham, J., de Anda, J., Xia, A., Zhao, K., Murakami, K., Tseng, B.S., Hoffman, L.R., Jin, F., Harwood, C.S., Wong, G.C., and Parsek, M.R. (2019) Heterogeneity in surface sensing suggests a division of labor in Pseudomonas aeruginosa populations. Elife 8.
Bae, B., Davis, E., Brown, D., Campbell, E.A., Wigneshweraraj, S., and Darst, S.A. (2013) Phage T7 Gp2 inhibition of Escherichia coli RNA polymerase involves misappropriation of 70 domain 1.1. Proceedings of the National Academy of Sciences 110: 19772-19777.
Boucher, J.C., Yu, H., Mudd, M.H., and Deretic, V. (1997) Mucoid Pseudomonas aeruginosa in cystic fibrosis: characterization of muc mutations in clinical isolates and analysis of clearance in a mouse model of respiratory infection. Infect Immun 65: 3838-3846.
Boyaci, H., Chen, J., Jansen, R., Darst, S.A., and Campbell, E.A. (2019) Structures of an RNA polymerase promoter melting intermediate elucidate DNA unwinding. Nature 565: 382-385.
Campagne, S., Marsh, M.E., Capitani, G., Vorholt, J.A., and Allain, F.H. (2014) Structural basis for -10 promoter element melting by environmentally induced sigma factors. Nat Struct Mol Biol 21: 269-276.
Campbell, E.A., Tupy, J.L., Gruber, T.M., Wang, S., Sharp, M.M., Gross, C.A., and Darst, S.A. (2003) Crystal structure of Escherichia coli sigmaE with the cytoplasmic domain of its anti-sigma RseA. Mol Cell 11: 1067-1078.
Candido Cacador, N., Paulino da Costa Capizzani, C., Gomes Monteiro Marin Torres, L.A., Galetti, R., Ciofu, O., da Costa Darini, A.L., and Hoiby, N. (2018) Adaptation of Pseudomonas aeruginosa to the chronic phenotype by mutations in the algTmucABD operon in isolates from Brazilian cystic fibrosis patients. PLoS One 13: e0208013.
Choi, K.H., Kumar, A., and Schweizer, H.P. (2006) A 10-min method for preparation of highly electrocompetent Pseudomonas aeruginosa cells: application for DNA fragment transfer between chromosomes and plasmid transformation. J Microbiol Methods 64: 391-397.
Choi, K.H., and Schweizer, H.P. (2006) mini-Tn7 insertion in bacteria with single attTn7 sites: example Pseudomonas aeruginosa. Nat Protoc 1: 153-161.
Ciofu, O., Lee, B., Johannesson, M., Hermansen, N.O., Meyer, P., and Hoiby, N. (2008) Investigation of the algT operon sequence in mucoid and non-mucoid Pseudomonas aeruginosa isolates from 115 Scandinavian patients with cystic fibrosis and in 88 in vitro non-mucoid revertants. Microbiology 154: 103-113.
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.
Deatherage, D.E., and Barrick, J.E. (2014) Identification of mutations in laboratory-evolved microbes from next-generation sequencing data using breseq. Methods Mol Biol 1151: 165-188.
Frank, D.W., Nair, G., and Schweizer, H.P. (1994) Construction and characterization of chromosomal insertional mutations of the Pseudomonas aeruginosa exoenzyme S trans-regulatory locus. Infect Immun 62: 554-563.
Fulcher, N.B., Holliday, P.M., Klem, E., Cann, M.J., and Wolfgang, M.C. (2010) The Pseudomonas aeruginosa Chp chemosensory system regulates intracellular cAMP levels by modulating adenylate cyclase activity. Mol Microbiol 76: 889-904.
Hmelo, L.R., Borlee, B.R., Almblad, H., Love, M.E., Randall, T.E., Tseng, B.S., Lin, C., Irie, Y., Storek, K.M., Yang, J.J., Siehnel, R.J., Howell, P.L., Singh, P.K., Tolker-Nielsen, T., Parsek, M.R., Schweizer, H.P., and Harrison, J.J. (2015) Precision-engineering the
Pseudomonas aeruginosa genome with two-step allelic exchange. *Nat Protoc* **10**: 1820-1841.

Hoang, T.T., Kutchma, A.J., Becher, A., and Schweizer, H.P. (2000) Integration-proficient plasmids for Pseudomonas aeruginosa: site-specific integration and use for engineering of reporter and expression strains. *Plasmid* **43**: 59-72.

Holloway, B.W. (1955) Genetic recombination in Pseudomonas aeruginosa. *J Gen Microbiol* **13**: 572-581.

Intile, P.J., Diaz, M.R., Urbanowksi, M.L., Wolfgang, M.C., and Yahr, T.L. (2014) The AlgZR two-component system recalibrates the RsmAYZ posttranscriptional regulatory system to inhibit expression of the Pseudomonas aeruginosa type III secretion system. *J Bacteriol* **196**: 357-366.

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.

Khakimova, M., Ahlgren, H.G., Harrison, J.J., English, A.M., and Nguyen, D. (2013) The stringent response controls catalases in Pseudomonas aeruginosa and is required for hydrogen peroxide and antibiotic tolerance. *J Bacteriol* **195**: 2011-2020.

Kristensen, C.S., Eberl, L., Sanchez-Romero, J.M., Givskov, M., Molin, S., and De Lorenzo, V. (1995) Site-specific deletions of chromosomally located DNA segments with the multimer resolution system of broad-host-range plasmid RP4. *J Bacteriol* **177**: 52-58.

Lane, W.J., and Darst, S.A. (2006) The structural basis for promoter -35 element recognition by the group IV sigma factors. *PLoS Biol* **4**: e269.

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 U S A* **90**: 8377-8381.

Meisner, J., and Goldberg, J.B. (2016) The Escherichia coli rhaSR-PrhaBAD Inducible Promoter System Allows Tightly Controlled Gene Expression over a Wide Range in Pseudomonas aeruginosa. *Appl Environ Microbiol* **82**: 6715-6727.

Palmer, K.L., Aye, L.M., and Whiteley, M. (2007) Nutritional cues control Pseudomonas aeruginosa multicellular behavior in cystic fibrosis sputum. *J Bacteriol* **189**: 8079-8087.

Pritchett, C.L., Little, A.S., Okkotsu, Y., Frisk, A., Cody, W.L., Covey, C.R., and Schurr, M.J. (2015) Expression analysis of the Pseudomonas aeruginosa AlgZR two-component regulatory system. *J Bacteriol* **197**: 736-748.

Pulcrano, G., Iula, D.V., Raia, V., Rossano, F., and Catania, M.R. (2012) Different mutations in mucA gene of Pseudomonas aeruginosa mucoid strains in cystic fibrosis patients and their effect on algU gene expression. *New Microbiol* **35**: 295-305.

Rahme, L.G., Stevens, E.J., Wolford, S.F., Shao, J., Tompkins, R.G., and Ausubel, F.M. (1995) Common virulence factors for bacterial pathogenicity in plants and animals. *Science* **268**: 1899-1902.

Takeya, K., and Amako, K. (1966) A rod-shaped Pseudomonas phage. *Virology* **28**: 163-165.

Tseng, B.S., Reichhardt, C., Merrihew, G.E., Araujo-Hernandez, S.A., Harrison, J.J., MacCoss, M.J., and Parsek, M.R. (2018) A Biofilm Matrix-Associated Protease Inhibitor Protects Pseudomonas aeruginosa from Proteolytic Attack. *MBio* **9**.

Tseng, B.S., Zhang, W., Harrison, J.J., Quach, T.P., Song, J.L., Penterman, J., Singh, P.K., Chopp, D.L., Packman, A.I., and Parsek, M.R. (2013) The extracellular matrix protects Pseudomonas aeruginosina biofilms by limiting the penetration of tobramycin. *Environ Microbiol* **15**: 2865-2878.

Turner, K.H., Wessel, A.K., Palmer, G.C., Murray, J.L., and Whiteley, M. (2015) Essential genome of Pseudomonas aeruginosa in cystic fibrosis sputum. *Proc Natl Acad Sci U S A* **112**: 4110-4115.
Vogel, H.J., and Bonner, D.M. (1956) Acetylornithinase of Escherichia coli: partial purification and some properties. *J Biol Chem* **218**: 97-106.

Winsor, G.L., Griffiths, E.J., Lo, R., Dhillon, B.K., Shay, J.A., and Brinkman, F.S. (2016) Enhanced annotations and features for comparing thousands of Pseudomonas genomes in the Pseudomonas genome database. *Nucleic Acids Res* **44**: D646-653.

Wolfgang, M.C., Kulasekara, B.R., Liang, X., Boyd, D., Wu, K., Yang, Q., Miyada, C.G., and Lory, S. (2003) Conservation of genome content and virulence determinants among clinical and environmental isolates of Pseudomonas aeruginosa. *Proc Natl Acad Sci U S A* **100**: 8484-8489.

Xu, B., and Wozniak, D.J. (2015) Development of a Novel Method for Analyzing Pseudomonas aeruginosa Twitching Motility and Its Application to Define the AmrZ Regulon. *PLoS One* **10**: e0136426.

Zhao, K., Tseng, B.S., Beckerman, B., Jin, F., Gibiansky, M.L., Harrison, J.J., Luijten, E., Parsek, M.R., and Wong, G.C.L. (2013) Psl trails guide exploration and microcolony formation in Pseudomonas aeruginosa biofilms. *Nature* **497**: 388-391.