Methods

Plant materials

Except where indicated, the Colombia-0 (Col-0) ecotype was used as the wild type. *fkf1* (ref. 2), *cca1-1 lhy-R* (48)[12], *cry1 cry2* (ref. 20), *phot1-5 phot2-1* (ref. 21) and *phyA-201* (ref. 20) have been described. The *fkf1-2* T-DNA insertion line (593_H05) was obtained from the SAIL collection[22]. The *cab2::luc* and *ccr2::luc* reporters and *35S::CO* (ref. 23) were introduced into *fkf1* alleles by crossing. To generate *35S::FKF1–TAP* and *35S::LKP2–TAP*, the coding regions of *FKF1* cDNA and *LKP2* cDNA were cloned into pRTL2–TAP (pRTL2 (ref. 24) containing a TAP[25] fragment) and then subcloned into pBI221 binary vector[26]. To generate the *FKF1* promoter driven FKF1–TAP construct, we replaced the CaMV35S promoter in *p35S::FKF1–TAP* by 1.6 kb of the *FKF1* promoter plus the 5′ untranslated region fragment. Unless otherwise noted, growth and light conditions were the same as described[1,5].

Analysis of gene expressions

In all experiments, plants were harvested on day 10 unless otherwise noted. RNA isolation and *CO*, *FT* and *UBQ* quantification have been described[1,27]. For *FKF1* expression analysis, total RNA (10 μg) was loaded on each lane. We used a 5′ region (0.7 kb) of the *FKF1* cDNA as a probe. All traces of the gene expressions are the means ± s.e.m. of relative values of signals from three independent experiments.

Immunoblot analysis

Soluble proteins were extracted with buffer containing 50 mM HEPES-KOH (pH 7.5), 100 mM KCl, 10% glycerol, 5 mM EDTA, 2 mM dithiothreitol, 5% polyvinylpolypyrrolidone and Complete protease inhibitor cocktail tablets (Roche), and 120 μg of extract was loaded on each lane. We used peroxidase anti-peroxidase (Sigma) and anti-α-tubulin (Sigma) to detect the TAP-tagged proteins and tubulin, respectively. All immunoblot analyses were done twice on independent samples.

Analysis of flowering time

Plants were grown on MS agar plates with 3% sucrose for 2 weeks, and then transferred to soil in LD or SD. Flowering time was measured by counting both rossette and cauline leaves after bolting, when the infloresence was 1-cm high.

Purification and analysis of LOV domain proteins

The LOV domains of FKF1 and the C91A mutant (amino acids 6–205), ZTL (1–190), LKP2 (5–194) and phot1 (449–599) were expressed in *Escherichia coli* as glutathione *S*-transferase (GST) fusion proteins and purified by GST affinity chromatography under safe (dim red) light. Roughly 150 μg of purified protein was used for thin layer chromatography analysis as described[3]. We measured absorbance spectra and difference spectra for the LOV domain fusions as described[28].

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A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance

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Biofilms are surface-attached microbial communities with characteristic architecture and phenotypic and biochemical properties distinct from their free-swimming, planktonic counterparts[1]. One of the best-known of these biofilm-specific properties is the development of antibiotic resistance that can be up to 1,000-fold greater than planktonic cells[2]. We report a genetic determinant of this high-level resistance in the Gram-negative opportunistic pathogen, *Pseudomonas aeruginosa*. We have identified a mutant of *P. aeruginosa* that, while still capable of forming biofilms with the characteristic *P. aeruginosa* architecture, does not develop high-level biofilm-specific resistance to three different classes of antibiotics. The locus identified in our screen, *ndvB*, is required for the synthesis of periplasmic glucans. Our discovery that these periplasmic glucans interact physically with tobramycin suggests that these glucose polymers may prevent antibiotics from reaching their sites of action by seques-

tering these antimicrobial agents in the periplasm. Our results indicate that biofilms themselves are not simply a diffusion barrier to these antibiotics, but rather that bacteria within these microbial communities employ distinct mechanisms to resist the action of antimicrobial agents.

Surface-attached bacterial communities, or biofilms, can become 10–1,000 times more resistant to the effects of antimicrobial agents than their planktonic counterparts[2]. This characteristic of biofilms makes them extremely difficult to control in medical and industrial settings[3]. We hypothesized that biofilm resistance to antimicrobial agents is part of a regulated developmental process and thus would require an identifiable set of genetic determinants.

Using a 96-well microtitre plate-based assay for biofilm-specific antibiotic resistance, a library of ~4,000 random *P. aeruginosa* PA14 transposon insertion mutants was screened for the inability to develop the characteristic increase in resistance of wild-type biofilm-grown cells. A mutant was identified, designated 45E7, that had decreased resistance to tobramycin (Tb) when grown in a biofilm, but grew as well as the wild type in planktonic culture, formed a biofilm indistinguishable from a biofilm formed by the wild-type strain in the microtitre plate assay, and displayed a planktonic minimal bactericidal concentration (MBC) for Tb that was indistinguishable from the parent strain.

To assess the generality of biofilm antibiotic sensitivity of the mutant, the MBC of biofilm-grown and planktonically grown wild-type and 45E7 strains was determined for gentamicin (Gm), ciprofloxacin (Cip), nalidixic acid (Nal), chloramphenicol (Cm) and ofloxacin (Ofl). For all of these antibiotics tested, there was no difference in the planktonic MBC between the wild type and the 45E7 mutant. The MBC of biofilm-grown 45E7 was eightfold lower for Gm and Cip, twofold lower for Cm and Ofl, and unchanged for Nal when compared to the wild type (Table 1 and see Supplementary Information). Therefore, the 45E7 locus can account for some, but not all, of the biofilm-mediated resistance observed for most antibiotics.

We used three other assays of biofilm antibiotic resistance to confirm the antibiotic sensitivity phenotype observed in the microtitre plate. The Tb-sensitivity phenotype in a flow cell was demonstrated by treating a 24-h-old, pre-formed biofilm with Tb, then staining the Tb-treated biofilm with the BacLight viability stain (Fig. 1). When viewed by phase-contrast microscopy (Fig. 1a, left panels), no difference in biofilm architecture was discernible between the wild type and the mutant (see also Table 2). The centre and right panels show that there were more live (green, syto-9-stained) than dead (red, stained with propidium iodide) cells in the wild-type biofilm after treatment with Tb. Conversely, there were more dead cells than live ones in the 45E7 biofilm. Quantification of propidium iodide-dependent fluorescence demonstrated 100-fold greater fluorescence intensity for the 45E7 mutant versus the wild type. When 72-h-old biofilms were treated with Tb, the 45E7 mutant biofilm was only threefold more sensitive than the wild-type biofilm (see Supplementary Information), suggesting that the 45E7 locus plays a more important role in younger biofilms. As a control, biofilms were stained with BacLight before exposure to Tb (Fig. 1b) and most cells in both the wild-type and 45E7 biofilms stained green, indicating that the 45E7 mutant does not have a general viability defect.

We used a second, quantitative assay to document the 45E7 mutant phenotype. The colony biofilm assay exploits the observation that bacterial colonies develop some of the properties of biofilms, including increased antibiotic resistance[4,5] (Fig. 2). 45E7 biofilm cells remained as resistant to the effects of Tb as the wild type at the first two time points assayed. However, at 48 h, while the wild-type biofilm cell viability was only reduced by two orders of magnitude, the viable cells detected for the 45E7 mutant decreased by at least five orders of magnitude. There was no difference in the viability of the strains in the absence of antibiotics over this time course. Furthermore, we found that there was no difference in the ability of Tb to diffuse through the colony biofilms formed by either of these strains (data not shown).

The rotating disk assay[6] was employed as another quantitative method to assess the phenotype of the 45E7 mutant. After forming on coupons, wild-type and mutant biofilms were exposed to varying concentrations of Tb. A lower concentration of Tb was necessary to kill the cells of the mutant biofilm compared to the wild-type biofilm (see Supplementary Information). These results, taken together with the data presented above, indicate conclusively that the 45E7 mutant, when growing in a biofilm, is less resistant than the wild type to the antimicrobial effects of Tb.

Next, we used flow cells to analyse the architecture of the wild-type and mutant strains. Both strains formed heterogeneous macrocolonies and fluid-filled channels that are characteristic of *P. aeruginosa* biofilms[7] (Fig. 1). To quantitate the architecture of biofilms formed by these strains, we used the COMSTAT software program[7]. COMSTAT analysis showed that there is no discernible difference between these strains for a number of architectural parameters at 24 h (Table 2).

The transposon in the 45E7 strain was mapped to the middle of a

Table 1 **Antibiotic sensitivity of biofilm-grown and planktonically grown strains**

Strain	Tb		Gm		Cip	
	MBC-P	MBC-B	MBC-P	MBC-B	MBC-P	MBC-B
Wild type	8	400	40	500	4	50
45E7	8	25	40	60	4	6
Fold change	1×	16×	1×	8×	1×	8×

MBC-P, MBC of planktonically grown cells; MBC-B, MBC of biofilm-grown cells. All antibiotic concentrations are in $\mu g\,ml^{-1}$. Values are based on results from at least three separate experiments.

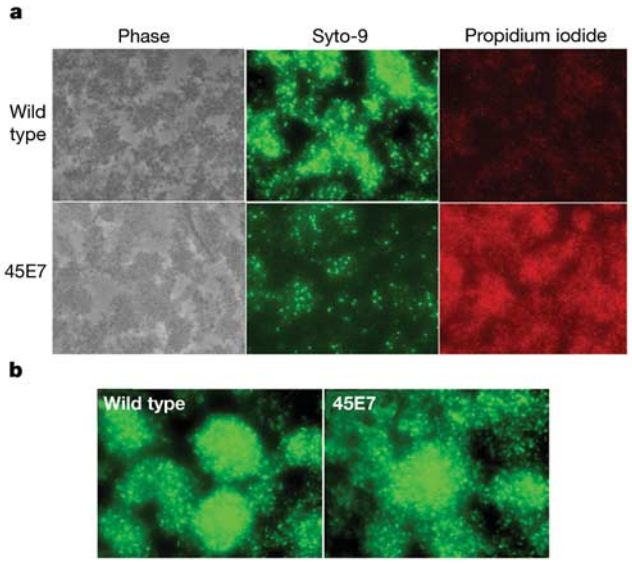

Figure 1 Flow cell assay for antibiotic sensitivity. **a**, The left-most panels (phase-contrast micrographs) show the architecture of 24-h-old biofilms. A flow cell allows a continuous supply of fresh medium to be delivered to a biofilm that is formed on the walls of a small, enclosed chamber. Biofilms were allowed to pre-form for 24 h in the absence of antibiotic treatment. These 24-h-old biofilms were then treated with 20 $\mu g\,ml^{-1}$ of Tb for 24 h, followed by staining with the BacLight viability stain for 1 h, then a 1 h rinse to remove unincorporated stain. The syto-9 panels indicate viable cells (green, intact membranes) and the propidium iodide panels indicate dead cells (red, damaged membranes). Similar results were observed in three separate experiments. **b**, Syto-9 staining of the wild-type and 45E7 biofilm before Tb treatment revealed no difference in viability.

monocistronic open reading frame (ORF), PA1163[8], that is 58% identical across its entire sequence to the *Bradyrhizobium japonicum* gene *ndvB*. The *ndvB* gene codes for a glucosyltransferase that is required for the synthesis of cyclic-β-(1, 3)-glucans[9]. Located in the periplasm and also secreted into the extracellular media, cyclic glucans are circular polymers of glucose that have been shown to play a role in adaptation to low osmotic media, flagella-mediated motility and plant–microbe interactions[10]. Furthermore, a planktonically grown *B. japonicum* strain carrying an *ndvB* mutation is more sensitive to chloramphenicol than the parent strain[11].

To confirm that mutation in the *ndvB* gene was causative for the observed phenotypes, we disrupted the gene in *P. aeruginosa* strains PA14, PAO1 and PAK, and tested the antibiotic sensitivity of these mutants in the 96-well microtitre plate assay. In all cases, the mutant was more sensitive than the wild-type strain when growing in a biofilm, but no differences were observed between the strains grown planktonically (see Supplementary Information). However, in the two additional strains, the influence of *ndvB* was not as great as for PA14. Most notably, only a twofold difference was observed for PA01. Although the data support an involvement for *ndvB* in biofilm antibiotic resistance, it may be that some strains of *Pseudomonas* have compensatory mechanisms for *ndvB* mutations.

To determine whether a difference in periplasmic glucan production existed between the wild type and the *ndvB* mutant, we examined ethanol-extracted periplasmic material[9] purified from both strains by gel filtration over a Sephadex G-75 column (detected using the anthrone-sulphuric acid method[12]). The most striking difference between wild-type and mutant chromatograms was observed between fractions 60 and 68 (Fig. 3a). Whereas a significant amount of anthrone-positive material was present in these fractions in the wild type, the equivalent fractions from the *ndvB* mutant contained relatively little anthrone-positive material. Because periplasmic cyclic glucan material from *Sinorhizobium meliloti* eluted from the same column in the same area (the main peak at fraction 62; data not shown), we reasoned that the missing material from the mutant 45E7 might be periplasmic glucans. Monosaccharide composition analyses using Dionex high-performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD)[13] indicated that the material present in fractions 60–68 from the wild type, and missing from the mutant, was composed exclusively of glucose (see Supplementary Information). Taken together, our genetic and carbohydrate analyses strongly suggest that the *ndvB* mutant is deficient in production of periplasmic glucans.

On the basis of the current understanding of the roles of periplasmic glucans in *B. japonicum*, *S. meliloti* and *Agrobacterium tumefaciens*, we envisioned several possible models to account for the role of these molecules in development of resistance in biofilm populations. Unlike the effects of *ndvB* mutations in Rhizobacteria[10], the *P. aeruginosa ndvB* mutant had no apparent defects in adaptation to low osmotic stress conditions or flagella-mediated motility. From the observation that related cyclic sugar polymers called cyclodextrins could sequester "guest molecules" in a non-covalent fashion[14], we considered the possibility that one role of periplasmic glucans may be to sequester antimicrobial agents in biofilm-grown cells[10]. If periplasmic glucans were indeed sequestering Tb, we predicted that these molecules should interact *in vitro*. To test this, we examined interactions of crude and purified glucans with Tb by hydrophobic interaction (HI) chromatography. Tb alone was loaded onto a HI interaction resin (C18) column, and was detected in the column flow-through and first water wash of the

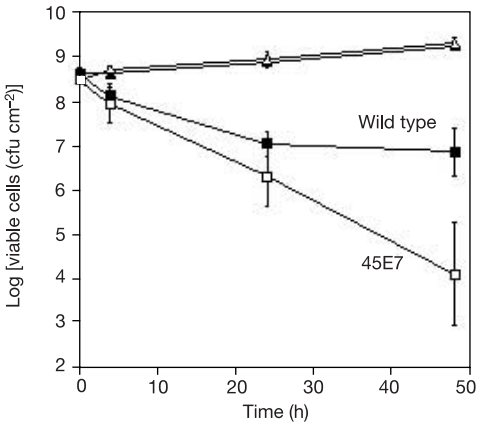

Figure 2 Colony biofilm assay for antibiotic sensitivity. 48-h-old colonies of the wild-type (filled symbols) and the 45E7 mutant (open symbols) were transferred to solid medium without (triangles) or with 10 μg ml^{-1} Tb (squares). The viability of the wild type decreased 100-fold with Tb treatment relative to the untreated control, while the 45E7 mutant decreased 10^5-fold. In the absence of antibiotic, there was no difference in viability between the two strains.

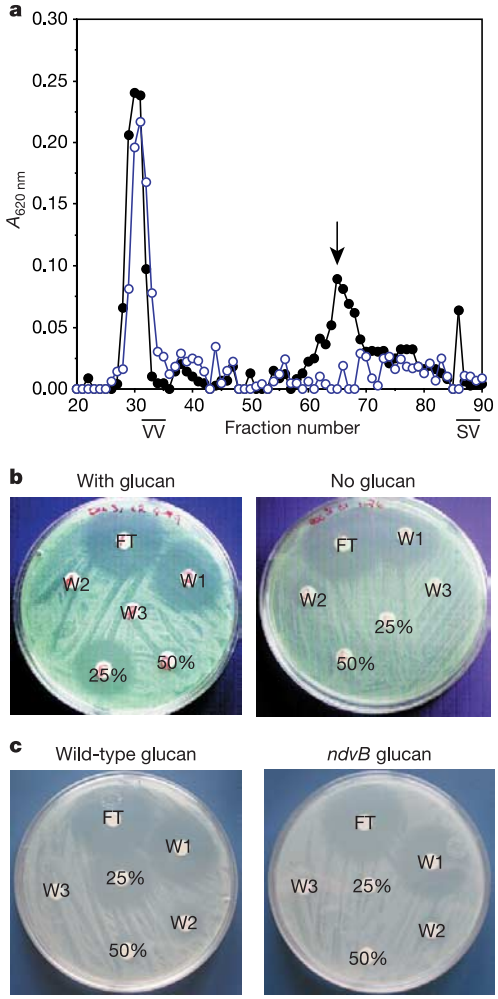

Figure 3 Periplasmic glucans interact with Tb. **a**, Gel filtration chromatography profiles of the periplasmic extracts of wild type (filled circles) and *ndvB* mutant (open circles). The arrow indicates the periplasmic glucan peak. vv, void volume; sv, salt volume. **b**, Disk diffusion assays with and without pre-loaded crude wild-type periplasmic glucans. Eluates from C18 columns were spotted on a lawn of Tb-sensitive *E. coli*. FT, column flow-through; W1–W3, water washes; 25%, 25% acetonitrile fractions; 50%, 50% acetonitrile fractions. The 75% and 100% acetonitrile fractions did not contain any Tb (data not shown). **c**, Disk diffusion assay with pre-loaded wild-type-derived (left) and *ndvB*-derived (right) fractions 60–68. Disks are labelled as above.

Table 2 **Quantifying biofilm architecture**

Strain	Total biomass ($\mu m^3\,\mu m^{-2}$)	Average thickness (μm)	Roughness coefficient	Surface area covered (μm^2)	Surface/volume ($\mu m^2\,\mu m^{-3}$)	Max thickness (μm)
Wild type	4.98 (3.18)	5.08 (3.24)	1.05 (0.31)	1.2×10^7 (4.9×10^6)	0.30 (0.10)	17.67 (2.47)
45E7	7.05 (3.64)	7.33 (3.80)	0.79 (0.38)	1.8×10^7 (7.4×10^6)	0.33 (0.09)	18.46 (2.01)

Note. Standard deviation (s.d.) for each data set is shown in parentheses. These results are an average of 12 data sets and all differences are within error. Roughness coefficient is a measure of biofilm heterogeneity.

column, but not in any subsequent fractions (Fig. 3b, right). In contrast, when a crude periplasmic carbohydrate extract from the wild-type strain was pre-loaded onto the column, some of the Tb was retained on the column and eluted with 25% acetonitrile (Fig. 3b, left). Conversely, when crude extract from the *ndvB* mutant strain was pre-loaded onto the column, very little Tb was retained on the column (see Supplementary Information).

To determine which components of the crude extract were responsible for the Tb retention on the C18 column, we performed the same experiment as above except the C-18 column was pre-loaded with partially pure material from fractions 60–68 from G-75 columns (Fig. 3c). Tb was retained on the column that was pre-loaded with material from the wild-type extract and eluted from the column with 25% acetonitrile. In contrast, no Tb activity was present in the 25% acetonitrile fraction from the column that was pre-loaded with the corresponding fractions isolated from the *P. aeruginosa ndvB* mutant. These data support the hypothesis that periplasmic glucans may sequester antibiotics, thereby slowing or stopping the diffusion of these molecules to their site of action.

On the basis of the biofilm-specific role of *ndvB* in resistance, we predicted that this gene is expressed only in biofilm-grown cells. RNA was prepared from biofilm-grown and planktonically grown cells from a continuous culture system[15]. Semi-quantitative RT–PCR using primers to *ndvB* revealed that there was preferential expression of *ndvB* in the biofilm mode of growth (Fig. 4).

We propose a mechanism for biofilm-specific resistance wherein periplasmic glucans bind to and sequester antibiotics. This extracytoplasmic mode of antibiotic sequestration would be expected to interfere with the passage of antibiotics through the periplasmic space to their site of action in the cytoplasm. High levels of the antibiotic needed to eliminate biofilm bacteria may be required to overwhelm the ability of the glucans to bind antimicrobial agents effectively. Alternatively, periplasmic glucans may not entirely account mechanistically for all of the antibiotic resistance of biofilm cells but may contribute to it by slowing the diffusion of antibiotics into the cell, thereby allowing the bacteria additional time to adapt to the antibiotic insult, an idea consistent with the model proposed previously[15].

We also showed that the *P. aeruginosa ndvB* mutant had increased biofilm-sensitivity to five antibiotics from three different structural families. This observation is consistent with previous reports that cyclic glucans can bind a range of chemically distinct molecules[10] and the reported ability of biofilms to develop broad resistance to antimicrobial agents[16]. It may now be possible to identify new strategies to block the development of biofilm resistance. For example, a co-therapeutic approach where traditional antibiotics are combined with a drug that interferes with biofilm-specific resistance may render biofilms more susceptible to treatment. □

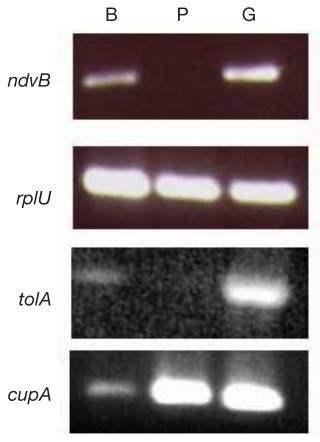

Figure 4 *ndvB* is preferentially expressed in biofilms. RT–PCR analysis of *ndvB* expression. PCR products derived from RNA isolated from planktonic (P) and biofilm (B) cells were analysed by agarose gel electrophoresis. A control with added genomic DNA (G) shows the expected size of the band with the primer set used. The constitutively expressed *rplU* gene was used as a control in the experiment. The similar bands from P and B samples indicate that an equal amount of RNA was present in each sample. *tolA* and *cupA1* transcripts were used as biofilm and planktonic controls[15], respectively. When RNA, but not cDNA, was used in the reaction, no bands were detected (data not shown).

Methods

Bacterial strains, plasmids, media and chemicals

P. aeruginosa PA14 was grown on rich medium (Luria Bertani, LB) or minimal medium at 37 °C. The minimal medium used was minimal M63 salts[17] supplemented with arginine (0.4%) and $MgSO_4$ (1 mM). The pSMC21 (green fluorescent protein, GFP) plasmid used to mark cells for flow cell studies is a kanamycin-resistant derivative of the reported pSMC2 plasmid[18]. We generated pTFM1 by ligating a 2-kilobase *ndvB* fragment into the *Bam*H1 and *Eco*R1 sites of pEX18Gm. The *ndvB* fragment contained 650 nucleotides of 5′ *ndvB* sequence, generated using primers p242: 5′-GGCGGGATCCCAACCACCTCTG AAACCGAATCGCGGATTG-3′ and p243: 5′-GGCGGCGCATATGGGCTTCGATGACG AAGTAGCTGTAGCCTTC-3′ in a standard polymerase chain reaction (PCR) reaction, and 650 nucleotides of 3′ *ndvB* sequence, generated using the primers p244: 5′-GGCGG CGCATATGCTGTTCGTCTT CAAGGTCGGCAAGATCCTC-3′ and p245: 5′-GGCGG AATTCGAGTTCAGGTTG GAAACCTGGAAGCTGGTC-3′, in a standard PCR reaction. The BacLight Live/Dead viability stain was purchased from Molecular Probes.

Genetic and molecular techniques

Transposon mutants were generated with Tn*5*-B30[19] as described[20]. To isolate strains defective in biofilm antibiotic resistance, individual transposon mutants were inoculated from cultures grown overnight in LB into sterile 96-well microtitre plates containing minimal arginine medium. Biofilms were allowed to form for 24 h, after which time the medium was replaced with fresh minimal arginine medium supplemented with 50 $\mu g\,ml^{-1}$ Tb (Research Products International Corporation). After 24 h of exposure to Tb, biofilms were allowed to recover in fresh media lacking Tb for an additional 24 h; their viability was then assessed by plating a small volume of each culture on LB plates. The minimal bactericidal concentration for biofilm-grown cells (MBC-B) was determined by exposing 24-h-old biofilms to various concentrations of antibiotic for 24 h, followed by a 24 h recovery period and plating for viability. The MBC of planktonic bacteria (MBC-P) was determined by adding antibiotic to bacteria at the time of inoculation, incubating for 24 h, followed by plating for viability. In these experiments, the number of bacteria used in planktonic cultures and microtitre plate-grown biofilms was roughly equivalent ($\sim 10^7$ c.f.u. per well). DNA sequence flanking the transposon mutant 45E7 was determined as described[20,21]. The original screen of ~4,000 transposon mutants yielded two mutants with the desired phenotype, one of which is presented in this report (see text). The *ndvB* deletion strains of PA14, PA01 and PAK were generated using pTFM1 as reported[22].

Antibiotic resistance assays

Flow cells were prepared, sterilized and analysed as described[23]. Image acquisition and quantification of fluorescence intensity were performed with OpenLab software using the Morphology Module (Improvision). Quantification of biofilm architecture was performed with the COMSTAT software package as described[7,24]. Colony biofilms[4,5] and rotating disk experiments[6] were performed as reported.

Periplasmic glucan purification

Periplasmic glucans were purified as described[9]. Briefly, 750 ml cultures of wild-type and

ndvB strains were grown for 24 h at 37 °C in arginine minimal medium. The cells were pelleted and the glucans were extracted with 70% EtOH at 70 °C for 30 min. Cell debris was pelleted and glucans were precipitated with ten volumes of 100% EtOH and 100 mM NaCl. Glucans were pelleted and resuspended in water. Gel filtration chromatography of the crude periplasmic extract was performed with Sephadex G–75 resin as described[25].

Tobramycin–glucan interaction assays

C18 Sep-Pak columns (Waters) were pre-washed with two column volumes of 50% methanol, followed by two column volumes of water. Washed columns were pre-loaded with 275 μg of resuspended glucans, or an equal volume (250 μl or 2.5% of the extract) of the crude wild-type or *ndvB* periplasmic extracts, washed with two column volumes of water then loaded with 1 mg of Tb. Following water washes (three column volumes), the column was eluted step-wise with one column volume each of 25%, 50%, 75% and 100% acetonitrile. Fractions were evaporated to dryness and resuspended in water. 12.5% of each sample was spotted on a sterile Whatman no. 1 paper disk on a lawn of *Escherichia coli* DH5α spread onto a LB plate. The lawn of *E. coli* was prepared by diluting an overnight culture 1:100, then spreading the dilution on a LB plate. Plates were incubated at 37 °C overnight before photographing. Tb-containing fractions were detected by the zone of inhibition surrounding the paper disk.

RT–PCR

Biofilm and planktonic RNA used in reverse transcriptase (RT)–PCR assays was purified with the RNeasy kit (Qiagen) from bacteria grown in continuous flow reactors as described[15]. Complementary DNA was synthesized using the first strand cDNA synthesis kit (Pharmacia) and the resulting RNA/cDNA hybrid was used as a template in PCR reactions using the primer sets designed from the *P. aeruginosa* PAO1 genome sequence[8]: *ndvB*: 5′-CGAACAGATGCGCACCGACC-3′ and 5′-CGCAGGTAGATGGCCTGGTC-3′; *rplU*: 5′-CGCAGTGATTGTTACCGGTG-3′ and 5′-AGGCCTGAATGCCGGTGATC-3′; *tolA*: 5′-CACGTTCTGATCTTCGCCATGCTG-3′ and 5′-GTCGGTCGTATCCGAAAGG AACTC-3′; *cupA1*: 5′-CATGCGCAGTGGTATTGGCCTTTG-3′ and 5′-GAACAGGGT GGTGAAATGCTCGTC-3′. The PCR was performed by using Taq polymerase (Qiagen) for 20 cycles with an annealing temperature of 64 °C (*ndvB*) or 56 °C (*rplU*, *tolA* and *cupA1*) and an extension temperature of 72 °C. The resulting products were analysed on a 1.2% agarose gel.

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Transposon silencing in the *Caenorhabditis elegans* germ line by natural RNAi

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Transposable elements are stretches of DNA that can move and multiply within the genome of an organism. The *Caenorhabditis elegans* genome contains multiple Tc*1* transposons that jump in somatic cells, but are silenced in the germ line[1–3]. Many mutants that have lost this silencing have also lost the ability to execute RNA interference (RNAi)[2,3], a process whereby genes are suppressed by exposure to homologous double-stranded RNA (dsRNA). Here we show how RNAi causes transposon silencing in the nematode germ line. We find evidence for transposon-derived dsRNAs, in particular to the terminal inverted repeats, and show that these RNAs may derive from read-through transcription of entire transposable elements. Small interfering RNAs of Tc*1* were detected. When a germline-expressed reporter gene is fused to a stretch of Tc*1* sequence, this transgene is silenced in a manner dependent on functional mutator genes (*mut-7*, *mut-16* and *pk732*). These results indicate that RNAi surveillance is triggered by fortuitous read-through transcription of dispersed Tc*1* copies, which can form dsRNA as a result of 'snap-back' of the terminal inverted repeats. RNAi mediated by this dsRNA silences transposase gene expression.

Genome-sequencing projects have shown that genomes contain large numbers of transposable elements; for example, 45% of the human genome consists of remnants of transposon sequences, whereas 2% encodes protein. Transposon sequences comprise 12% of the *C. elegans* genome; of the several DNA transposons, Tc*1* is the most abundant element, with 31 intact copies[4]. Transposition (through a cut-and-paste mechanism) is mediated by a Tc*1*-encoded transposase that specifically recognizes the Tc*1* terminal inverted repeats (TIRs)[5]. Transposition only occurs in somatic cells and not in the germ line[1]. The identification of mutator mutants, which do show germline transposition, indicated that an active transposon-silencing process exists in the germ line. Several