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Gene Islands Integrated into tRNA^{Gly} Genes Confer Genome Diversity on a *Pseudomonas aeruginosa* Clone

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Intraclonal genome diversity of *Pseudomonas aeruginosa* was studied in one of the most diverse mosaic regions of the *P. aeruginosa* chromosome. The ca. 110-kb large hypervariable region located near the *lipH* gene in two members of the predominant *P. aeruginosa* clone C, strain C and strain SG17M, was sequenced. In both strains the region consists of an individual strain-specific gene island of 111 (strain C) or 106 (SG17M) open reading frames (ORFs) and of a 7-kb stretch of clone C-specific sequence of 9 ORFs. The gene islands are integrated into conserved tRNA^{Gly} genes and have a bipartite structure. The first part adjacent to the tRNA gene consists of strain-specific ORFs encoding metabolic functions and transporters, the majority of which have homologs of known function in other eubacteria, such as hemophores, cytochrome *c* biosynthesis, or mercury resistance. The second part is made up mostly of ORFs of yet-unknown function. Forty-seven of these ORFs are mutual homologs with a pairwise amino acid sequence identity of 35 to 88% and are arranged in the same order in the two gene islands. We hypothesize that this novel type of gene island derives from mobile elements which, upon integration, endow the recipient with strain-specific metabolic properties, thus possibly conferring on it a selective advantage in its specific habitat.

Genetic variability within bacterial species can be the result of nucleotide substitutions, intragenomic reshuffling, and acquisition of DNA sequences from another organism (3). The considerable impact of the last strategy, termed horizontal gene transfer, on microbial evolution and its integral role in the diversification and speciation of the bacteria has become apparent from recent analyses based on the growing pool of genomic sequence information (7, 18, 23, 28). Prominent examples are the pathogenicity islands of many obligatory pathogens (14). These chromosomally encoded regions typically contain large clusters of virulence genes not present in closely related nonpathogenic strains and can, upon integration, transform a benign organism into a pathogen. Whereas the molecular mechanism of chromosomal integration has been resolved for some conjugative transposons and bacteriophages and details about the transmissibility of conjugative plasmids are well known, the evolution and mobility of gene islands remain obscure (14). Often these DNA blocks are integrated adjacent to or within tRNA genes, and some contain a phage-related integrase gene near one end, suggesting that gene islands may have been generated by a phage or by a plasmid with integrative functions (14, 42). Nevertheless, the comparative sequence analysis of gene islands so far have not pointed to any common genetic repertoire that confers transmission and acquisition.

The gram-negative bacterium *Pseudomonas aeruginosa* is ubiquitously distributed in aquatic and soil habitats, and it is an opportunistic pathogen for plants, animals, and humans (38). No correlation between certain *P. aeruginosa* clones and disease habitats or environmental niches could be detected (1, 9). Although the genome sequence of the reference strain PAO1 provides insights into the versatility and intrinsic drug resistance of *P. aeruginosa* (48), the genetic origin of the broad range of metabolic capacities and the evolutionary history of chromosome organization have not been determined in sufficient depth for this phenotypically and genetically diverse species. Our previous analyses have shown that the *P. aeruginosa* chromosome possesses three regions with pronounced genomic variability (15, 33). These three so-called hypervariable regions close to the *pilA*, *phnAB*, and *lipH* loci could even be found at the intraclonal level (35). Comparative genome mapping was used to unambiguously identify the chromosomal difference regions of the two related strains C and SG17M, both belonging to the predominant *P. aeruginosa* clone C but recovered from different habitats (40).

In order to resolve the chromosomal structure and the genetic makeup of one of the hypervariable areas of the *P. aeruginosa* genome, we determined the sequence of the region located near the *lipH* gene for strains C and SG17M. The annotation revealed that the hypervariable region resembles a mosaic of species-, clone-, and strain-specific DNA segments in both strains. The two identified strain-specific gene islands have been integrated into tRNA^{Gly} genes and probably originated from mobile circular elements. They are composed of strain-specific open reading frames (ORFs) encoding metabolic functions, of phage- and plasmid-like genes, and of a set

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of previously unknown genes which display a very high degree of homology between the two islands.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The *P. aeruginosa* strains C and SG17M selected for this study both belong to the major *P. aeruginosa* clone C (35). *P. aeruginosa* strain C was isolated from the lung of a cystic fibrosis (CF) patient, while strain SG17M was recovered from the aquatic environment (37). Cloning was done in *E. coli* strain DH5α or XL1-Blue MR (Stratagene) by using the broad-host-range vector pLAFR3 (tetracycline resistance) (47), the cosmid SuperCos-1 (ampicillin resistance) (Stratagene), and the plasmid pTZ19R-Δ*bla-cat* (chloramphenicol resistance) (this study). To construct the plasmid pTZ19R-Δ*bla-cat*, we replaced the *ampR* gene-containing 0.7-kb *Dra*I fragment in pTZ19R (MBI Fermentas) with a chloramphenicol acetyltransferase-encoding *Bss*HII fragment from pHK (22). It was necessary to use chloramphenicol rather than ampicillin resistance because the plasmid vector was used for subcloning of the SuperCos-1 cosmids, which also carry the *ampR* gene. Bacteria were routinely grown at 37°C in Luria-Bertani medium (39). For maintenance of pLAFR3 cosmids in *Escherichia coli* DH5α, the media were supplemented with 20 μg of tetracycline per ml. For *E. coli* XL1-Blue carrying SuperCos-1 cosmids, 2YT medium (17) supplemented with 100 μg of ampicillin per ml was used, and *E. coli* DH5α with pTZ19R-Δ*bla-cat* plasmids was propagated in TB broth (39) containing 25 μg of chloramphenicol per ml.

DNA techniques. DNA manipulations were by standard procedures (5). High-molecular-weight chromosomal DNA of *P. aeruginosa* was prepared by the protocol of Goldberg and Ohman (11). Small-scale isolations of plasmid and cosmid DNAs were performed by using QIAprep spin miniprep kits (Qiagen), while larger amounts of cosmid DNA were purified by using QIAtip100 columns (Qiagen) according to the instructions of the supplier.

Construction of cosmid libraries. A genome-wide cosmid library was constructed for each *P. aeruginosa* strain according to the protocols described previously (52). Chromosomal DNA, partially *Sau*3AI digested and size fractionated by preparative sucrose gradient ultracentrifugation (11), was cloned into the *Bam*HI sites of pLAFR3 for strain SG17M and of SuperCos-1 for strain C. The ligated DNA was packaged into phage λ particles in vitro by using the λ-DNA in vitro packaging module (Amersham). For strain SG17M, *E. coli* DH5α was transfected with the λ particles containing the pLAFR3 cosmid DNA. After selection for tetracycline resistance, 768 recombinant clones were transferred to 96-well plates; the resulting cosmid library was named pKSCS. The packaged SuperCos-1 cosmids with DNA of *P. aeruginosa* C were introduced into *E. coli* XL1-Blue MR. The corresponding cosmid library pKSCC was made by picking 960 recombinant clones resistant to ampicillin into 96-well plates. A further 20,000 colonies were recovered and stored as a pool.

Southern hybridization. For colony blots, cell suspensions were inoculated on Hybond N membranes (Amersham) by using a 96-needle replication device and grown either on Luria-Bertani medium–tetracycline plates or on 2YT-ampicillin plates. Alternatively, colony lifts were performed directly from agar plates onto Hybond N membranes. The cells were lysed, and the DNA was fixed (52). Blotting of chromosomal or cosmid DNA digested with appropriate restriction enzymes to nylon membranes, hybridization, and immunological detection of probe signals were performed by previously described protocols (34).

Probe preparation. The following probes were used for Southern hybridization: strain-specific subtraction clones generated by reciprocal subtractive hybridization (40), cloned gene probes as described previously (35), a selection of *P. aeruginosa* PAO1-derived *Spe*I linking clones (36), and insert DNAs from the cosmids themselves. The probes were prepared from gel-purified restriction fragments of cosmids or plasmids by using a digoxigenin labeling kit (Roche Diagnostics) (34). For the pKSCC library, single-stranded probes specific for the ends of a cosmid insert were obtained by using asymmetric PCR with a T3 (5′-AATTAACCCTCACTAAAGGG) or T7 (5′-CATAATACGACTCACTATAGGG) primer and a digoxigenin PCR labeling mixture (Roche Diagnostics); asymmetric PCR was performed in a volume of 50 μl containing 0.5 μg of cosmid DNA as a template, 1 μM primer, 5 μl of digoxigenin PCR labeling mix, 5% dimethyl sulfoxide, 1.5 mM $MgCl_2$, and 2.5 U of *Taq* polymerase (InViTec) in 1× reaction buffer (InViTec). Extension of the T3 or T7 primer was performed in a Thermo-Cycler (Landgraf) with the following program: 420 s at 95°C and 60 cycles of 120 s at the annealing temperature, 120 s at 72°C, and 120 s at 92°C. The annealing temperatures were 54°C for the T7 primer and 46°C for the T3 primer. After amplification, the reaction mixture was purified as described previously (34).

Construction of ordered cosmid contigs. To identify the cosmids at the borders of the hypervariable genomic region in *P. aeruginosa* strains C and SG17M, the corresponding libraries were both screened with the *lipH* gene probe and a PAO1-derived linking clone covering the *Spe*I junction SpV-SpAK in strain PAO1, SpV-SpX in strain C, and SpAF′-SpX in SG17M (35, 41). To obtain cosmids covering the strain-specific inserts, both libraries were screened with selected subtraction clones (40). The DNA of each cosmid clone identified in this screen was prepared, and probes specific for the whole insert or only for the ends were generated. These probes derived from the insert ends were used for further hybridization experiments in order to identify overlapping cosmids. All cosmids identified in the walk were individually controlled by hybridization to Southern blots of *Spe*I digests of PAO1, C, and SG17M chromosomal DNAs to verify their genomic localization and to exclude chimeric cosmids or false-positive signals associated with repeated regions. Comparison of the *Eco*RI and *Hin*dIII restriction fragment patterns and hybridization with the aforementioned probes were used to order the cosmids and to establish the minimal tilting path for the strain-specific regions. Altogether, 27 pKSCC and 34 pKSCS cosmids were identified for *P. aeruginosa* C and SG17M, respectively, located within the region of interest from the *lipH* gene to the *Spe*I junction SpV-SpX in strain C or SpAF′-SpX in strain SG17M. In strain SG17M the following cosmids were selected for sequence analysis: pKSCS 572, 052, 149, 427, 795, and 282. A remaining gap of about 9 kb between pKSCS 572 and 052 was closed by long-range PCR using the Proofsprinter kit (Hybaid). For strain C it was necessary to use an alternative strategy because extensive cross-hybridization prevented the generation of an unequivocal cosmid contig. In order to obtain unique tags, *Bam*HI, *Hin*dIII, and *Eco*RI sublibraries of the pulsed-field gel electrophoresis gel-eluted *Spe*I fragment SpV were generated. In parallel, the restriction map of the SpV fragment was constructed for the same enzymes by Smith-Birnstiel mapping (16). Thus, the subcloned fragments could be mapped. Subclones carrying unique sequence located within the gap were used as probes for further colony hybridization. More than 3,000 additional pKSCC cosmids had to be screened to gain a contiguous order of cosmids, of which the following five cosmids were selected for sequencing: pKSCC 323, 022, 1064, 1065, and 273.

Sequencing. To determine the DNA sequence of the entire cosmid inserts, separate plasmid libraries were constructed for each cosmid. DNA from each cosmid was sheared by hydrodynamic cleavage (29), size fractionated, and subcloned into the *Sma*I site of pTZ19R-Δ*bla-cat*. DNA sequencing of the resulting plasmid libraries was performed on a LICOR 4200 sequencer (MWG Biotech) or on an ABI 377 sequencer (Applied Biosystems). For each cosmid, the individual reads were assembled into contigs by using the base-caller program Phred (8) and the Staden package (46) with the Phrap algorithm integrated (12). Sequencing gaps were closed by primer walking, while combinatorial PCR was used to span physical gaps. The sequence of the 9.8-kb long-range PCR product was determined by primer walking. Finally, the sequences of the individual cosmids and the PCR product were assembled into one contig for each *P. aeruginosa* strain.

Annotation. Putative ORFs were identified by using GeneMark.HMM and GeneMark (6, 26). Public databases were searched for similar sequences with the BlastN, BlastX, and BlastP algorithms (2). Predicted ORFs were reviewed individually for start codon assignment based on additional contextual information such as the proximity of ribosome binding sequence motifs. tRNA genes were identified by the program tRNA-scan-SE (25). Pairwise sequence comparisons and multiple alignments were generated using Clustal W (50). Long-range restriction maps were constructed with the in-house program MasterMap (51). Codon usage patterns were analyzed using the in-house programs and the program CodonW (written by John Peden and available at ftp://molbiol.ox.ac.uk/cu). The relative synonymous codon usage (RSCU) was determined for each gene; the RSCU is the observed frequency of a particular codon divided by its expected frequency under the assumption of equal usage of the synonymous codons for an amino acid (43). The genomic codon index (GCI) (21) is a quantitative measure for the synonymous codon bias of a particular gene compared to the average codon usage in the genome. It is defined as the geometric mean of the RSCU values corresponding to each of the codons used in that gene, divided by the maximum possible GCI for a gene of the same amino acid composition:

$$\mathrm{GCI} = \frac{\mathrm{GCI}_{\mathrm{obs}}}{\mathrm{GCI}_{\mathrm{max}}}$$

$$\mathrm{GCI}_{\mathrm{obs}} = \left(\prod_{k=1}^{L} \mathrm{RSCU}_k \right)^{1/L}$$

$$\mathrm{GCI}_{\mathrm{max}} = \left(\prod_{k=1}^{L} \mathrm{RSCU}_{k\mathrm{genome}} \right)^{1/L}$$

TABLE 1. Comparison of general features of the sequenced gene islands and the PAO1 genome

Genomic region	Size (bp)	% G+C	% Coding regions	No. of ORFs		Mean GCI
				Total	Per 10 kb	
PAGI-2(C)	104,955	64.7	90.4	113	10.7	0.537
PAGI-3(SG)	103,304	59.2	82.7	105	10.2	0.448
C-specific DNA	6,872	66.1	83.8	9	13.1	0.65
PAO genome[a]	6,264,403	66.6	89.4	5,570	8.9	0.678

[a] From references 21 and 48.

where $RSCU_k$ is the RSCU value for the kth codon in the gene, $RSCU_{kgenome}$ is the maximal genomic RSCU value for the amino acid encoded by the kth codon in the gene, and L is the number of codons in the gene. The GCI was defined in analogy to the codon adaptation index (43).

For comparison with the *P. aeruginosa* PAO1 genome sequence, the information at http://www.pseudomonas.com was used (48). Preliminary sequence data were obtained from the Department of Energy Joint Genome Institute at http://www.jgi.doe.gov/tempweb/JGI_microbial/html/index.html.

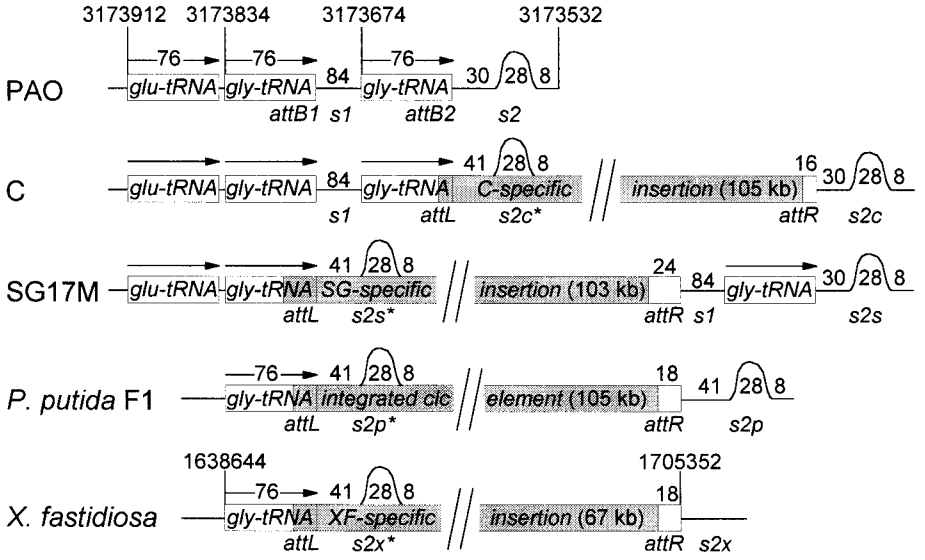
Nucleotide sequence accession numbers. The nucleotide sequences reported in this paper have been deposited in the GenBank database (accession no. AF440523 for *P. aeruginosa* C and AF440524 for SG17M).

RESULTS AND DISCUSSION

A mosaic of species-, clone-, and strain-specific DNA makes up one of the most diverse regions of the *P. aeruginosa* chromosome. Among the three hypervariable regions in the *P. aeruginosa* clone C genome (35, 41), the most diverse region near the *lipH* gene was selected for comparative sequencing of the two *P. aeruginosa* strains C and SG17M. Both strains belong to clone C, but they were recovered from different habitats. An ordered cosmid contig covering this hypervariable region was constructed for each strain. A contiguous set of cosmids was selected for each strain and sequenced by a shotgun approach. The final contig was 158,230 bp in size for strain C and 128,136 bp for strain SG17M. Sequence comparison revealed that each strain contains an individual large, novel gene cluster flanked by species-specific DNA known from the *P. aeruginosa* PAO1 genome sequencing project (48). Both insertions are composed of a minor portion of 6,872 bp of DNA, identical in both clone C strains, and a major portion of strain-specific DNA sequence [104,955 bp for strain C, designated PAGI-2(C), and 103,304 bp for SG17M, designated PAGI-3(SG)] (Table 1). (PAGI stands for *P. aeruginosa* genomic island, in accordance with the nomenclature introduced by Liang et al. [24]). Instead of the 6,872-bp clone C-specific DNA, the genome of *P. aeruginosa* PAO1 carries a 2,001-bp individual sequence from bp 3173531 to 3171531 at this chromosomal position (Fig. 2). The alignment of the strain C and PAO1 sequences revealed that the analyzed portion of 46.4-kb species-specific DNA shows a very high degree of conservation characterized by identical gene order and a very low nucleotide substitution rate of 0.39%, in agreement with published data of 0.3% sequence diversity in housekeeping genes of *P. aeruginosa* (20). In total, 184 nucleotide substitutions without any frameshifts or nonsense mutations were identified in this 46.4 kb of DNA. Fewer than 20% of these are nonsynonymous substitutions, resulting in a protein with an altered amino acid composition. Furthermore, no nucleotide alterations could be detected between strains C and SG17M in the analyzed portion of 24.8 kb of shared DNA sequence.

Strain-specific gene islands integrated into $tRNA^{Gly}$ genes. Comparison of the *P. aeruginosa* C, SG17M, and PAO1 sequences showed that the two large strain-specific gene islands are inserted into one $tRNA^{Gly}$ gene within a cluster comprising one $tRNA^{Glu}$ gene followed by two identical $tRNA^{Gly}$ genes (Fig. 1). Within the PAO sequence these tRNA genes are located from bp 3173912 to 3173599. In strain SG17M, the first $tRNA^{Gly}$ gene was used for integration of PAGI-3(SG), whereas in strain C, the PAGI-2(C) DNA was incorporated into the second $tRNA^{Gly}$ gene. Upon integration, the entire $tRNA^{Gly}$ gene was reconstructed at the left end of the gene island, designated *attL*, whereas in strain C the terminal 16 nucleotides and in strain SG17M the terminal 24 nucleotides of the 3′ end of the $tRNA^{Gly}$ gene were present as direct repeat at the right end, designated *attR* (Fig. 1). Alignment of the attachment sites *attL* and *attR* showed a high degree of sequence homology at both junctions (data not shown). The *attL* sites of both integrated gene islands and the *attB2* chromosomal target sites following the second $tRNA^{Gly}$ gene share similar AT-rich inverted repeat sequences. Interestingly, similar genomic structures were found by analyzing the chromosomal insertions of the 105-kb *clc* element in *Pseudomonas putida* (30, 31) and of a 67-kb gene island in the plant pathogen *Xylella fastidiosa* (reference 44 and this study). In both cases, the complete $tRNA^{Gly}$ gene was reconstructed at the left border, whereas the 18-bp 3′ end of the $tRNA^{Gly}$ gene was repeated at the right border of the integrated element (Fig. 1). All four gene islands possess similarly structured attachment sites and surrounding sequences including the conserved inverted repeats (Fig. 1). Only the length of *attR* varies between the different gene islands (Fig. 1). At the left junction the four gene islands share not only the *attL* sites but also a highly homologous intergenic spacer (228 bp in strain C, 225 bp in strain SG17M, 226 bp in the *P. putida clc* element, and 226 bp in *X. fastidiosa*) and the first ORF, encoding very similar site-specific integrases of the bacteriophage P4 integrase subfamily (the sequence alignment is at our website, http://www.mh-hannover.de/kliniken/kinderheilkunde/kfg/index.htm). The three highly related integrases of strain C, *P. putida*, and *X. fastidiosa* are of considerably higher molecular weight than the typical phage P4-related integrases and possess an unusual C terminus showing homology to a putative transposase of *Pseudomonas* sp. strain B4 (accession no. emb/CAB93963).

The integrase int-B13 of *P. putida* has been shown to be responsible for site-specific integrative recombination between the *clc* element's attachment site (*attP*) and chromosomal attachment (*attB*) genes (30, 31, 45). The 105-kb self-transmissible *clc* element, encoding the degradation of 3-chlorobenzoate, is capable of integrating site and sequence specifically into



Sequence alignments

FIG. 1. Organization of the boundaries of the gene islands. The structure of the genomic region around a cluster of three tRNA genes is shown for *P. aeruginosa* strains PAO1, C, and SG17M. In *P. putida* F1 (structure adapted from references 30 and 31) and *X. fastidiosa* (sequence taken from reference 44), the gene islands integrated into a single $tRNA^{Gly}$ gene. Map positions in the genome sequence are indicated for *P. aeruginosa* PAO1 and *X. fastidiosa*. Large inverted repeats (IRs) are shown as loop structures. Numbers above the maps indicate the lengths (in base pairs) of the corresponding sequences. The 84-bp spacer *s1* separating the two $tRNA^{Gly}$ genes differs by only two nucleotide substitutions between *P. aeruginosa* PAO1 and the two clone C strains. The localization of attachment sites *attB*, *attL*, and *attR* (see text for explanation) is indicated. All sequences flanking inverted repeats were named (*s2*, *s2c*, and *s2c*∗, etc.) and aligned to visualize the high degree of homology among the different gene islands and strains. Additionally, the sequences of the depicted $tRNA^{Gly}$ genes, highlighted in black, are shown for the three species.

a $tRNA^{Gly}$ gene of its host. The *clc* element is transferred in plate matings with a frequency of about 10^{-7} per recipient cell (27). Despite these low frequencies, transfer of the *clc* element to endogeneous bacteria seems to readily occur in complex microbial communities, such as sludges from soil or wastewater treatment plants (49, 53). When the *clc*-carrying *P. putida* strain BN210 was inoculated into a bacterial population in 3-chlorobenzoate-contaminated wastewater, the *clc* element was taken up by *P. aeruginosa* strains or by strains belonging to the genus *Ralstonia* or related β-proteobacteria such as *Comamonas* (45). Although PAGI-2(C) and PAGI-3(SG) have been stably kept by strains C and SG17M in vitro and in the lungs of the affected CF patient for more than 17 years now with no evidence for loss of the island, these data on the *clc* element suggest that PAGI-2(C) and PAGI-3(SG) could potentially be mobilized and transferred to other strains, even across species barriers. Hence, gene islands of this type may be widely distributed in terms of species, geographical region, and habitat. This hypothesis is supported by the fact that a copy of PAGI-2(C) with 99.972% nucleotide sequence identity was identified in the *Ralstonia metallidurans* CH34 chromosome (preliminary sequence data were obtained at http://www.jgi-.doe.gov/tempweb/JGI_microbial/html/index.html). *P. aeruginosa* strain C was isolated in 1986 from a patient in northern Germany, whereas the sequenced *R. metallidurans* strain was isolated 1976 from the sludge of a zinc decantation tank in Belgium that was polluted with high concentrations of several heavy metals.

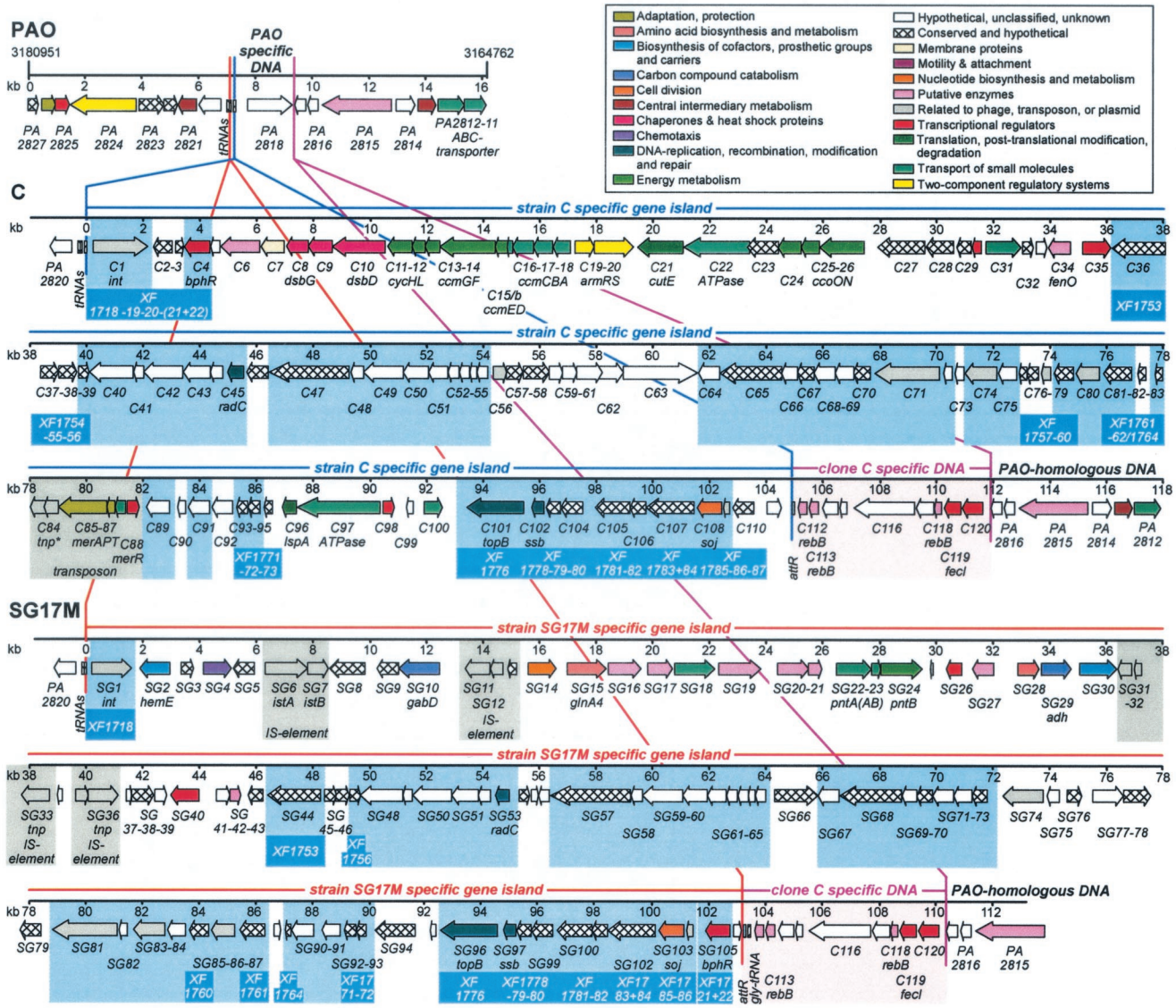


FIG. 2. Gene maps of the *P. aeruginosa* strain PAO1, C, and SG17M hypervariable genome regions. Predicted coding regions are shown by arrows indicating the direction of transcription. The tRNA genes and attachment sites are depicted by rectangles. Vertical lines and their connections represent the borders of the gene islands and their sites of integration in comparison to the PAO1 genome. Genes are color coded according to their functional category (adapted from http://www.pseudomonas.com). All genes carry identification numbers (C1 to C111 and SG1 to SG105 in the two strain-specific gene islands and C112 to C120 in the clone C specific region [highlighted in pink]), but some have been omitted because of space limitations. In cases of a high degree of homology to already-characterized proteins, three-letter designations are provided for individual genes. ORFs with mutual homologs in both gene islands are shown with a light-blue background. Additionally, ORFs with equivalents in the detected gene island of *X. fastidiosa* are marked with blue boxes and the corresponding gene identification numbers of the sequencing project (44). IS elements and transposons are shaded in gray.

Sequence analysis and annotation of PAGI-2(C) and PAGI-3(SG). The organization of predicted ORFs within the hypervariable region is displayed in Fig. 2. The G+C content and the proportion of coding sequence of PAGI-2(C) are closer to those of the PAO genome than are those of PAGI-3(SG) (Table 1). The mean GCI is significantly lower in PAGI-2(C) and PAGI-3(SG) than in the *P. aeruginosa* PAO1 genome, indicating that in these islands codon usage is different from that of a typical *P. aeruginosa* gene. The 6,872-bp region of clone C-specific DNA, however, exhibits a G+C content and GCI values characteristic of *P. aeruginosa*.

The annotation revealed 111 ORFs in PAGI-2(C) (Table 2) and 106 ORFs in PAGI-3(SG) (Table 3). Tables 2 and 3 show for each ORF its coordinates within the gene island, direction of transcription, size of the gene product, G+C content, and GCI value. Furthermore, the accession number and the name of the homolog that was chosen to assign the function of the gene product are given, together with the corresponding E

TABLE 2. Annotation of all ORFs located within the gene island PAGI-2(C) in *P. aeruginosa* strain C

Gene identification	Coordinates		Direction	Length (amino acids)	G+C (%)	GCI	Gene name	Homolog product	GenBank accession no.	E value (Blast search)
	Left	Right								
C1[a]	229	2160	→	644	63.8	0.571	*int*	Phage-related integrase XF1718 (*X. fastidiosa*)	AAF84527	0E + 00
C2[a]	2360	3016	←	219	59.1	0.341		Hypothetical protein XF1719 (*X. fastidiosa*)	AAF84528	1E − 71
C3[a]	3136	3429	→	98	58.8	0.280		Hypothetical protein XF1720 (*X. fastidiosa*)	AAF84529	9E − 30
C4[a]	3451	4341	←	297	61.6	0.427	*bphR*	BphR regulatory protein (*R. eutropha*)	CAB72138	9E − 83
C5	4379	4702	←	108	63.0	0.396		No significant similarity		
C6	4734	6110	←	459	67.4	0.537		Pyridine nucleotide-disulfide oxidoreductase, class I, VC2638 (*Vibrio cholerae*)	AAF95779	1E − 73
C7	6153	6959	←	269	65.6	0.508		Conserved hypothetical protein str1262 (*Synechocystis* sp. strain PCC 6803)	BAA17856	5E − 25
C8	7050	7823	←	258	64.0	0.541	*dsbG*	Thiol:disulfide interchange protein DsbG (PA2476) (*P. aeruginosa*)	AAG05864	2E − 51
C9	7826	8662	←	279	63.2	0.437		Probable thiol:disulfide interchange protein (PA2477) (*P. aeruginosa*)	AAG05865	6E − 48
C10	8662	10515	←	618	64.9	0.464	*dsbD*	Probable thiol:disulfide interchange protein (PA2478) (*P. aeruginosa*)	AAG05866	1E − 135
C11	10598	11479	←	294	61.8	0.359	*cycH*	Cytochrome *c*-type biogenesis protein CycH (*Sinorhizobium meliloti*)	P45400	5E − 17
C12	11476	11931	←	152	57.5	0.392	*cycL*	Cytochrome *c*-type biogenesis protein CycL precursor (*S. meliloti*)	P45406	3E − 25
C13	11928	12452	←	175	60.4	0.473	*ccmG*	Cytochrome *c* biogenesis protein CcmG (PA1481) (*P. aeruginosa*)	AAG04870	3E − 37
C14	12449	14410	←	654	62.3	0.431	*ccmF*	Cytochrome *c*-type biogenesis protein CcmF (PA1480) (*P. aeruginosa*)	AAG04869	0E + 00
C15	14414	14860	←	149	60.6	0.427	*cycJI ccmE*	Cytochrome *c*-type biogenesis protein CycJ (*P. fluorescens*)	AAC44225	6E − 35
C15b	14844	15035	←	64	62.5	0.430	*ccmD*	Heme exporter protein D (cytochrome *c*-type biogenesis protein CcmD) (*V. cholerae*)	AAF95200	2E − 03
C16	15032	15769	←	246	62.3	0.519	*ccmC*	Heme exporter protein C (cytochrome *c*-type biogenesis protein CcmC) (*V. cholerae*)	AAF95201	9E − 67
C17	15782	16468	←	229	63.2	0.415	*ccmB*	Cytochrome *c* maturation protein B (*Shewanella putrefaciens*)	AAC02694	6E − 65
C18	16465	17076	←	204	59.6	0.354	*ccmA*	Heme exporter protein A (cytochrome *c*-type biogenesis ATP/binding protein CcmA) (*V. cholerae*)	AAF95203	7E − 39
C19	17257	17925	→	223	65.0	0.525	*armR*	Response regulator ArmR (two-component transcriptional regulator) (*Pseudomonas* sp. strain JR1)	AAF80268	4E − 59
C20	17922	19307	→	462	63.1	0.424	*armS*	Sensor kinase ArmS (two-component sensor protein) (*Pseudomonas* sp. strain JR1)	AAF80269	5E − 77
C21	19461	21059	←	532	64.5	0.459	*cutE*	Apolipoprotein *N*-acyltransferase (copper homeostasis protein CutE homolog) (*P. aeruginosa*)	AAC97167	3E − 67
C22	21084	23399	←	772	65.5	0.459		Putative metal transporter ATPase [*Streptomyces coelicolor* A3(2)]	CAB96031	9E − 87
C23	23323	24420	←	366	61.7	0.441		Hypothetical protein PA2481 (*P. aeruginosa*) (probable cytochrome *c*)	AAG05869	4E − 70
C24	24404	25222	←	273	64.3	0.476		ORF21 (*Moritella marina*) (probable cytochrome c_4)	BAA89395	4E − 23
C25	25219	25860	←	214	64.0	0.490	*fixO*/*ccoO*	ORF20 (*M. marina*) (cytochrome *c* oxidase, monoheme subunit, membrane-bound)	BAA89394	3E − 17
C26	25857	27413	←	519	60.4	0.498	*fixN*/*ccoN*	ORF20 (*M. marina*) (cytochrome *c* oxidase, heme *b*- and copper-binding subunit, membrane bound)	BAA89393	6E − 68
C27	27932	29602	←	557	67.6	0.651		Conserved hypothetical protein PA2345 (*P. aeruginosa*)	AAG05733	1E − 125
C28	29651	30610	←	320	64.8	0.564		Hypothetical protein PA2915 (*P. aeruginosa*)	AAG06303	2E − 99
C29	30717	31244	←	176	68.2	0.544		Hypothetical protein (*E. coli*)	AAC75715	1E − 47
C30	31267	31578	←	104	61.9	0.611		Transcriptional activator HlyU (*V. cholerae*)	AAF93843	6E − 16
C31	31728	32954	→	409	70.2	0.646		Similar to metabolite transport protein (*Bacillus subtilis*)	CAB12326	2E − 34
C32	33031	33408	→	126	65.9	0.535		Hypothetical protein Rv1767 (*Mycobacterium tuberculosis*)	CAB09310	3E − 22
C33	33519	33890	→	124	58.1	0.491		No significant similarity		
C34	33950	34744	←	265	64.9	0.619	*fenO*	Hydroxybutyryl dehydratase (*B. subtilis*) (probable enoyl coenzyme A hydratase/isomerase)	AAF32340	2E − 24

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TABLE 2—*Continued*

Gene identification	Coordinates		Direction	Length (amino acids)	G+C (%)	GCI	Gene name	Homolog product	GenBank accession no.	E value (Blast search)
	Left	Right								
C35	35156	36151	→	332	63.6	0.476		Probable transcriptional regulator (PA1182) (*P. aeruginosa*)	AAG04571	2E − 42
C36[a]	36199	38091	←	631	65.0	0.489		Hypothetical protein XF1753 (*X. fastidiosa*)	AAF84562	0E − 00
C37[a]	38407	39033	→	209	65.6	0.603		Conserved hypothetical protein XF1754 (*X. fastidiosa*)	AAF84563	1E − 105
C38[a]	39046	39678	→	211	64.0	0.588		Conserved hypothetical protein XF1755 (*X. fastidiosa*)	AAF84564	1E − 112
C39[a]	39752	40111	→	120	65.6	0.466		Hypothetical protein XF1756 (*X. fastidiosa*)	AAF84565	1E − 15
C40[a]	40127	41674	←	516	62.2	0.536		No significant similarity		
C41[a]	41690	42046	←	119	70.0	0.611		No significant similarity		
C42[a]	42043	43437	←	465	67.5	0.611		No significant similarity		
C43[a]	43447	44397	←	317	66.7	0.689		No significant similarity		
C44[a]	44394	44840	←	149	68.0	0.512		No significant similarity		
C45[a]	45005	45499	←	165	63.0	0.516	*radC*	DNA repair protein (XF0148) (*X. fastidiosa*)	AAF82961	9E − 34
C46[a]	45675	46439	←	255	67.5	0.508		Hypothetical protein PA0982 (*P. aeruginosa*)	AAG04371	4E − 28
C47[a]	46464	49295	←	944	66.6	0.606		Low homology at the N terminus to sex pilus assembly and synthesis protein (*Sphingomonas aromaticivorans*); origin of replication binding domain	AAD03958	1E − 07
C48[a]	49356	49796	←	147	68.9	0.704		No significant similarity		
C49[a]	49777	51195	←	473	68.7	0.672		No significant similarity		
C50[a]	51185	52096	←	304	71.5	0.631		No significant similarity		
C51[a]	52093	52785	←	231	68.1	0.659		No significant similarity		
C52[a]	52782	53180	←	133	69.9	0.618		No significant similarity		
C53[a]	53193	53552	←	120	66.7	0.733		No significant similarity		
C54[a]	53569	53802	←	78	64.5	0.628		No significant similarity		
C55[a]	53799	54182	←	128	72.4	0.599		No significant similarity		
C56	54386	54856	→	157	59.7	0.442		Putative excisionase ORF277 (*S. aromaticivorans* plasmid pNL1)	AAD03880	2E − 16
C57	54853	55428	→	192	61.1	0.467		Hypothetical protein ORF271 (*S. aromaticivorans* plasmid pNL1)	AAD03879	2E − 23
C58	55446	56360	→	305	59.1	0.405		CG11743 gene product (*Drosophila melanogaster*)	AAF54250	7E − 26
C59	56357	56827	→	157	61.4	0.407		No significant similarity		
C60	56824	57324	→	167	56.7	0.449		No significant similarity		
C61	57324	58226	→	301	58.7	0.536		No significant similarity		
C62	58031	58990	→	320	60.4	0.430		No significant similarity		
C63	59000	61624	→	875	66.7	0.534		No significant similarity		
C64[a]	61665	62414	←	250	66.0	0.649		No significant similarity		
C65[a]	62411	64600	←	730	65.6	0.612		Hypothetical protein (*Salmonella enterica serovar* Typhi)	AAF69957	7E − 30
C66[a]	64605	65153	←	183	72.9	0.583		No significant similarity		
C67[a]	65150	65740	←	197	73.1	0.622		Hypothetical protein RP457 (*Rickettsia prowazekii*)	CAA14913	4E − 12
C68[a]	65722	66459	←	246	70.6	0.684		No significant similarity		
C69[a]	66472	67116	←	215	71.0	0.616		No significant similarity		
C70[a]	67113	67712	←	200	68.8	0.514		PilL (type IV pili) (*Salmonella* serovar Typhi)	AAF14812	3E − 19
C71[a]	67851	70130	←	760	64.9	0.615		Hypothetical protein pXO1-08 (*Bacillus anthracis* virulence plasmid pXO1) (with helicase domain)	AAD32312	9E − 43
C72[a]	70267	70572	←	102	64.1	0.576		No significant similarity		
C73[a]	70662	70982	←	107	61.7	0.556		No significant similarity		
C74[a]	71033	72142	←	370	66.2	0.597		Hypothetical protein pXO1-10 (*B. anthracis* virulence plasmid pXO1)	AAD32314	5E − 11
C75[a]	72207	72854	←	216	67.3	0.646		No significant similarity		
C76[a]	72931	73191	←	87	60.5	0.573		Hypothetical protein XF1757 (*X. fastidiosa*)	AAF84566	1E − 39
C77[a]	73208	73615	←	136	65.7	0.577		Hypothetical protein XF1758 (*X. fastidiosa*)	AAF84567	6E − 68
C78[a]	73720	74061	←	114	61.7	0.460		Conserved plasmid protein XF1759 (*X. fastidiosa*)	AAF84568	3E − 50
C79[a]	74156	74845	←	230	67.2	0.566		Hypothetical protein XF1760 (*X. fastidiosa*)	AAF84569	1E − 106
C80[a]	74940	75767	←	276	63.4	0.567		Hypothetical protein ORF273 (*oriT* 5′ region) (*E. coli* plasmid F)	AAA99218	2E − 88
C81[a]	75913	76911	←	333	64.9	0.570		Hypothetical protein XF1761 (*X. fastidiosa*)	AAF84570	1E − 156
C82[a]	77129	77413	←	95	75.1	0.638		Conserved hypothetical protein XF1762 (*X. fastidiosa*)	AAF84571	2E − 41
C83[a]	77721	77981	←	87	69.0	0.570		Hypothetical protein XF1764 (*X. fastidiosa*)	AAF84573	7E − 36

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TABLE 2—*Continued*

Gene identification	Coordinates		Direction	Length (amino acids)	G+C (%)	GCI	Gene name	Homolog product	GenBank accession no.	E value (Blast search)
	Left	Right								
C84	78051	78692	←	214	65.3	0.652	*tnp**	Transposase (*P. fluorescens*)	CAA70408	2E − 90
C84b	78533	79048	←	172	66.3	0.458	*tnp**	TnpA transposase (Tn*21*) (*E. coli*)	AAC33926	6E − 51
C85	79067	80755	←	563	69.0	0.620	*merA*	Mercuric [Hg(II)] reductase (*Thiobacillus* sp.)	CAA72398	0E + 00
C86	80766	81053	←	96	66.0	0.671	*merP*	Periplasmic mercuric ion binding protein (*Sphingomonas paucimobilis*)	AAD23805	5E − 32
C87	81066	81416	→	117	67.2	0.626	*merT*	MerT protein (mercuric transport protein) (*E. coli* plasmid pDU1358)	AAA98222	2E − 55
C88	81488	81895	→	136	63.5	0.517	*merR*	Organomercurial resistance regulatory protein (*P. stutzen*)	AAC38229	9E − 52
C89[a]	82157	82981	←	275	64.7	0.611		No significant similarity		
C90[a]	83270	83548	←	93	60.2	0.655		No significant similarity		
C91[a]	83646	84383	←	246	64.8	0.585		No significant similarity		
C92[a]	84467	85204	←	246	63.7	0.594		No significant similarity		
C93[a]	85336	85728	←	131	62.1	0.629		Hypothetical protein XF1771 (*X. fastidiosa*)	AAF84580	2E − 66
C94[a]	85750	86163	←	138	63.5	0.442		Hypothetical protein XF1772 (*X. fastidiosa*)	AAF84581	2E − 35
C95[a]	86300	86611	←	104	62.2	0.423		Hypothetical protein XF1773 (*X. fastidiosa*)	AAF84582	1E − 17
C96	86948	87448	←	167	61.5	0.444	*lspA*	Lipoprotein signal peptidase LspA (*Serratia marcescens*)	AAC82524	1E − 32
C97	87452	90364	←	971	65.7	0.584		Probable metal-transporting P-type ATPase (PA3690) (*P. aeruginosa*)	AAG07078	0E +00
C98	90456	90854	→	133	60.2	0.476		Probable transcriptional regulator (PA3689) (*P. aeruginosa*)	AAG07077	5E − 37
C99	91309	91545	←	79	61.2	0.496		No significant similarity		
C100	91930	92565	→	212	61.5	0.417		Putative integral membrane protein/transporter (*Neisseria meningitidis*)	AAF42077	8E − 26
C101[a]	93289	95319	←	677	66.4	0.580	*topB*	DNA topoisomerase III (XF1776) (*X. fastidiosa*)	AAF84584	0E + 00
C102[a]	95603	96043	←	147	65.5	0.644	*ssb*	Single-stranded-DNA binding protein (XF1778) (*X. fastidiosa*)	AAF84586	1E − 71
C103[a]	96117	96644	←	176	64.2	0.533		Hypothetical protein XF1779 (*X. fastidiosa*)	AAF84587	9E − 78
C104[a]	96641	97432	←	264	66.4	0.601		Hypothetical protein XF1780 (*X. fastidiosa*)	AAF84588	1E − 123
C105[a]	97862	99100	←	413	67.7	0.513		Hypothetical protein XF1781 (*X. fastidiosa*)	AAF84589	0E + 00
C106[a]	99104	99664	←	187	65.6	0.661		Conserved hypothetical protein (XF1782) (*X. fastidiosa*)	AAF84590	1E − 96
C107[a]	99679	101358	←	560	69.9	0.590		Protein fused from two hypothetical proteins (XF1783 and XF1784) (*X. fastidiosa*)	AAF84591, AAF84592	1E − 111, 1E − 117
C108[a]	101604	102479	←	292	68.7	0.551	*soj*	Chromosome partitioning-related protein (XF1785) (*X. fastidiosa*)	AAF84593	1E − 150
C109[a]	102522	102743	←	74	64.0	0.703		Phage-related protein (XF1786) (*X. fastidiosa*)	AAF84594	9E − 35
C110[a]	102853	103599	←	249	57.6	0.402		Hypothetical protein XF1787 (*X. fastidiosa*)	AAF84595	1E − 101
C111[a]	104050	104550	→	167	55.7	0.399		No significant similarity		

[a] ORF defined as noncargo in the text (including the homologs).

value from the Blast search. More than 60% of the genes are either conserved hypothetical genes of unknown function or genes with no apparent homology to any reported sequences (Fig. 2; Table 4). Interestingly, these hypothetical ORFs are clustered in the gene islands.

In both strains the gene islands are partitioned into two blocks (Fig. 2). The cluster adjacent to the *attL* site consists of genes that are specific for each strain. The encoded function could be attributed to most of these so-called strain-specific genes (termed cargo ORFs in Table 4). The other cluster predominantly contains hypothetical ORFs, of which 47 are mutual homologs in both gene islands. Of these 47 ORFs, 28 ORFs in strain C and 18 ORFs in strain SG17M have homologs in the $tRNA^{Gly}$-associated island of *X. fastidiosa* mentioned above (Table 4; Fig. 2). The putative function could be recognized for a few homologs (Tables 2 to 4). Three genes encode elements of DNA recombination or repair (*ssb* [single-strand binding protein], C102 and SG97 [accession number XF1778]; *topB* [topoisomerase B], C101 and SG96 [XF1776]; and *radC* [DNA repair protein], C45 and SG53). One gene product is associated with the partitioning of chromosomal or extrachromosomal elements in the cell (*soj*, C108 and SG103 [XF1785]), and another gene product is associated with site-specific integration into the chromosome (*int* [phage-type P4 integrase], C1 and SG1 [XF1718]) (see above). Additionally, a few conserved hypothetical genes show strong homology to already identified plasmid (C71 and SG81, C74 and SG83, C78, and C80 and SG86) or phage (C109 and SG104) genes.

The cargo ORFs, of which 51 each were found in PAGI-2(C) and PAGI-3(SG), build up the individual part of the gene island. Of these 102 ORFs, the closest homolog identified from BLAST searches was frequently found in other *P. aeruginosa* strains [12 in PAGI-2(C) and 10 in PAGI-3(SG)]; in other type I pseudomonads, such as *P. fluorescens*, *P. syringae*, *P. putida*, or *P. stutzeri* [3 in PAGI-2(C) and 6 in PAGI-3(SG)]; or in "honorary" pseudomonads that had been removed from the *Pseudomonas* genus after introduction of the ribosomal DNA-based phylogeny [3 in PAGI-2(C) and 1 in PAGI-3(SG)].

TABLE 3. Annotation of all ORFs located within the gene island PAGI-3(SG) in *P. aeruginosa* strain SG17M

Gene identification	Coordinates		Direction	Length (amino acids)	G+C (%)	GCI	Gene name	Homolog product	GenBank accession no.	E value (Blast search)
	Left	Right								
SG1[a]	226	1635	→	470	61.1	0.458	*int*	Phage-related integrase (XF1718) (*X. fastidiosa*)	AAF84527	1E − 178
SG2	1909	2970	←	354	56.0	0.352	*hemE*	Uroporphyrinogen decarboxylase (*E. coli* K-12)	AAC76971	1E − 149
SG3	3360	3815	→	152	55.7	0.350		Conserved hypothetical protein (*Paracoccus denitrificans*)	AAC44549	2E − 14
SG4	4145	5131	→	329	46.1	0.215		Methyl-accepting domain of probable chemotaxis transducer PA4844 (*P. aeruginosa*)	AAG08229	1E − 17
SG5	5201	5953	←	251	46.1	0.205		Domain of conserved hypothetical protein PA4601 (*P. aeruginosa*)	AAG07989	1E − 51
IS element	6212	8612	→	[2,401 bp]				IS with inverted repeats and two ORFs (transposase and nucleoside triphosphate-binding protein); upon insertion into ORF (5 + 8), a sequence of 7 bp (CCTTAGT) was repeated	No homology at the nucleotide level	
SG6	6318	7823	→	502	56.4	0.339	*istA*	Transposase IstA (IS*1326*)	AAA79725	1E − 109
SG7	7813	8556	→	248	55.4	0.370	*istB*	Nucleoside triphosphate-binding protein IstB (IS*1326*) (*Ralstonia eutropha*)	AAA79726	9E − 72
SG8	8596	9831	←	412	53.2	0.255		Conserved hypothetical protein ORF1 (*Rhizobium etli*)	AAC64871	9E − 30
SG5 + SG8	5201	9831	←	740	50.6	0.232		Conserved hypothetical protein PA4601 (*P. aeruginosa*) (after deletion of the I*S* element)	AAG07989	6E − 88
SG9	10249	11025	←	259	53.9	0.363		Conserved hypothetical protein Orf3 (*Methylobacterium extorquens*)	AAB66495	5E − 23
SG10	11025	12479	←	485	55.3	0.296	*gabD*	Succinate semialdehyde dehydrogenase (*Pseudonocardia* sp. strain K1)	CAC10505	5E − 56
IS element	13380	15209	←	[1,830 bp]				IS containing three ORFs (two fragments of a putative transposase and a hypothetical protein); no flanking repeats could be detected	No homology at the nucleotide level	
SG11	13380	14258	←	293	58.4	0.382		Similar to domain of conserved hypothetical protein (*Wolbachia* sp. strain wKue) (putative transposase)	BAA89629	8E − 49
SG12	14280	14723	←	148	59.2	0.436		Similar to domain of conserved hypothetical protein (*Wolbachia* sp. strain wKue) (putative transposase)	BAA89629	5E − 31
SG11 + SG12	13380	14723	←	448	58.9	0.402		Fusion of ORFs SG11 and SG12 (change of the stop codon TAG to TCG); full-length similarity to conserved hypothetical protein (*Wolbachia* sp. strain wKue) (putative transposase)	BAA89629	7E − 90
SG13	14892	15209	←	106	58.8	0.353		Conserved hypothetical protein PA0979 (*P. aeruginosa*); in other species often associated with IS elements	AAG05325	8E − 13
SG14	15612	16592	→	327	56.0	0.371	*yumC*	Thioredoxin reductase (*Bacillus halodurans*)	BAB07127	2.E − 71
SG15	16993	18375	→	461	51.4	0.326	*glnA4*	Putative glutamine-synthetase GlnA4 (*Mycobacterium tuberculosis*)	CAA15522	2E − 73
SG16	18447	19607	→	387	51.8	0.320		Cytochrome P450 (monooxygenase) (*Rhizobium* sp. strain NGR234)	AAB91895	2E − 45

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TABLE 3—*Continued*

Gene identification	Coordinates		Direction	Length (amino acids)	G+C (%)	GCI	Gene name	Homolog product	GenBank accession no.	E value (Blast search)
	Left	Right								
SG17	19840	20724	→	295	50.3	0.251		Vng2501c (*Halobacterium* sp. strain NRC-1) putative glutamine amidotransferase	AAG20565	1E − 11
SG18	20789	22219	→	477	48.6	0.241		Putative amino acid permease [*Streptomyces coelicolor* A3(2)]	CAB46781	3E − 68
SG19	22330	23838	→	503	54.5	0.285		Aldehyde dehydrogenase PA5312 (*P. aeruginosa*)	AAG08697	1E − 162
SG20	24412	25527	→	372	57.9	0.391		Enoyl coenzyme A hydratase (*P. putida*)	AAB62303	1E − 120
SG21	25509	25970	→	154	58.2	0.391		Acyl coenzyme A dehydrogenase (*Bacillus subtilis*)	CAB14346	1E − 12
SG22	26463	27677	→	405	67.7	0.656	*pntAA*	Proton-translocating NAD(P) transhydrogenase, alpha subunit, PntAA (*Rhodospirillum rubrum*)	AAA62493	4E − 93
SG23	27689	28006	→	106	62.6	0.756	*pntAB*	Proton-translocating NAD(P) transhydrogenase, alpha2 subunit, PntAB (*R. rubrum*)	AAA62494	1E − 23
SG24	28006	29469	→	488	64.9	0.744	*pntB*	Pyridine nucleotide transhydrogenase, beta subunit, PA0196 (*P. aeruginosa*)	AAG03585	0E + 00
SG25	29816	29914	←	33	58.6	0.398		Only fragment of transposase (*Agrobacterium tumefaciens*)	CAA79150	0.033
SG26	30368	30913	←	182	50.4	0.274		Transcriptional regulator, HTH_3 family (*Vibrio cholerae*)	AAF96189	1E − 16
SG27	31278	32030	←	251	57.8	0.462		Putative short-chain type dehydrogenase/reductase [*S. coelicolor* A3(2)]	CAA20822	9E − 38
SG28	32892	33644	→	251	57.2	0.353		Probable glutamine amidotransferase PA0297 (*P. aeruginosa*)	AAG03686	1E − 51
SG29	33730	34770	→	347	57.9	0.383	*adh*	Alcohol dehydrogenase PA5427 (*P. aeruginosa*)	AAG08812	7E − 45
SG30	35076	36383	→	436	56.6	0.335		Aminotransferase class III (adenosylmethionine-8-amino-7-oxononanoate) (*B. halodurans*)	BAB05979	2E − 94
SG31	36446	36931	←	162	60.5	0.349		Fragment of transposase-like protein TnpA1 (*P. stutzeri*)	AAD02143	3E − 28
SG32	37018	37281	←	88	53.4	0.345		Fragment of transposase-like protein TnpA1 (*P. stutzeri*)	AAD02143	9E − 08
SG33	37736	38716	←	327	58.9	0.565	*tnp*	Transposase (*P. putida*)	AAC98743	0E + 00
SG34	39004	39186	←	61	42.6	0.182		No significant similarity		
IS element	39545	41180	→	[1,636 bp]				IS with inverted repeats and two ORFs (transposase and hypothetical protein)	No homology at the nucleotide level	
SG35	39645	40109	→	155	63.9	0.545		ORF within IS*1240* (*P. syringae*)	AAB81643	7E − 35
SG36	40106	41155	→	350	67.0	0.642	*tnp*	Transposase within IS*1240* (*P. syringae*)	AAB81642	1E − 100
SG37	41450	41629	→	60	38.3	0.181		No significant similarity		
SG38	41634	42404	→	257	49.8	0.318		Conserved hypothetical protein (*B. subtilis*)	BAA19344	1E − 64
SG39	42455	42865	→	137	45.3	0.261		No significant similarity		
SG40	43008	44006	←	333	50.4	0.258		Probable transcriptional regulator (AraC family) PA3782 (*P. aeruginosa*)	AAG07169	2E − 87
SG41	44594	45082	→	163	57.5	0.476		No significant similarity		
SG42	45079	45471	→	131	60.3	0.404		Monophosphatase (*Synechocystis* sp.)	BAA18648	2E − 08
SG43	45732	46247	←	172	61.6	0.550		Hypothetical protein jhp0584 (*Helicobacter pylori* strain J99)	AAD06175	1E − 34
SG44[a]	46405	48207	←	601	62.3	0.437		Hypothetical protein XF1753 (*X. fastidiosa*)	AAF84562	0E + 00
SG45[a]	48517	48834	→	106	61.7	0.416		HtaR suppressor protein slr0724 (*Synechocystis* sp. strain PCC 6803)	BAA16671	4E − 06

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TABLE 3—*Continued*

Gene identification	Coordinates		Direction	Length (amino acids)	G+C (%)	GCI	Gene name	Homolog product	GenBank accession no.	E value (Blast search)
	Left	Right								
SG46[a]	48834	49292	→	153	61.7	0.502		Conserved hypothetical protein slr0725 (*Synechocystis* sp. strain PCC 6803)	BAA16672	2E − 28
SG47[a]	49322	49675	→	118	61.2	0.401		Hypothetical protein XF1756 (*X. fastidiosa*)	AAF84565	6E − 03
SG48[a]	49672	51174	←	501	62.4	0.588		No significant similarity		
SG49[a]	51187	51516	←	110	70.3	0.700		No significant similarity		
SG50[a]	51513	52916	←	468	66.1	0.609		No significant similarity		
SG51[a]	52925	53860	←	312	66.1	0.638		No significant similarity		
SG52[a]	53857	54294	←	146	65.8	0.442		No significant similarity		
SG53[a]	54459	54959	←	167	60.7	0.496	*radC*	Probable DNA repair protein RadC VC1786 (*V. cholerae*)	AAF94935	3E − 30
SG54[a]	55313	55684	→	124	61.8	0.451		Hypothetical protein (similar to *spdB3* gene in pSG5) (*A. rhizogenes*)	BAB16262	4E − 20
SG55[a]	55748	55993	→	82	71.1	0.637		No significant similarity		
SG56[a]	56002	56388	←	129	62.8	0.484		No significant similarity		
SG57[a]	56401	59268	←	956	67.4	0.670		Low homology at the N terminus to sex pilus assembly and synthesis protein (*Sphingomonas aromaticivorans*); origin of replication binding domain	AAD03958	4E − 10
SG58[a]	59268	59687	←	140	67.6	0.654		No significant similarity		
SG59[a]	59668	61089	←	474	66.5	0.608		No significant similarity		
SG60[a]	61079	61954	←	292	71.1	0.649		No significant similarity		
SG61[a]	61951	62625	←	225	67.3	0.658		No significant similarity		
SG62[a]	62622	63017	←	132	67.4	0.535		No significant similarity		
SG63[a]	63034	63396	←	121	67.2	0.666		No significant similarity		
SG64[a]	63409	63642	←	78	63.2	0.630		No significant similarity		
SG65[a]	63639	64010	←	124	67.7	0.443		No significant similarity		
SG66	64314	65819	→	502	62.0	0.512		Domain of hypothetical protein ORF261 [*S. aromaticivorans* plasmid pNL1]	AAD03878	6E − 08
SG67[a]	65838	66587	←	250	64.7	0.641		No significant similarity		
SG68[a]	66584	68758	←	725	65.8	0.622		Hypothetical protein (*Salmonella enterica serovar* Typhi)	AAF69957	1E − 31
SG69[a]	68769	69296	←	176	69.3	0.552		No significant similarity		
SG70[a]	69293	69856	←	188	70.9	0.574		Hypothetical protein RP457 (*Rickettsia prowazekii*)	CAA14913	1E − 12
SG71[a]	69856	70581	←	242	70.0	0.571		No significant similarity		
SG72[a]	70591	71244	←	218	68.0	0.567		No significant similarity		
SG73[a]	71241	71768	←	176	68.8	0.471		PilL (type IV pili) (*Salmonella* serovar Typhi)	AAF14812	7E − 20
SG74	72311	73741	←	477	59.6	0.502		Conserved hypothetical protein PA1368 (*P. aeruginosa*), putative transposase	AAG04757	0E + 00
SG75	73871	74323	←	151	53.2	0.270		No significant similarity		
SG76	74592	75101	→	170	56.3	0.345		Conserved hypothetical protein PA2582 (*P. aeruginosa*)	AAG05970	4E − 45
SG77	75509	76585	→	359	43.8	0.217		No significant similarity		
SG78	76585	77550	→	322	44.0	0.209		Domain of conserved hypothetical protein (*Deinococcus radiodurans*)	AAF11191	3E − 12
SG79	77705	78451	←	249	49.9	0.197		Domain of hypothetical protein Y4jT (*Rhizobium* sp. strain NGR234) plasmid pNGR234a	AAB91732	2E − 23
SG80	78232	78501	←	90				Hypothetical ORF, no significant similarity		
SG81[a]	78843	81116	←	758	62.7	0.556		Hypothetical protein pXO1-08 (*Bacillus anthracis* virulence plasmid pXO1) (with helicase domain)	AAD32312	3E − 42
SG82[a]	81203	81499	←	99	63.0	0.504		No significant similarity		
SG83[a]	81718	82827	←	370	64.2	0.557		Hypothetical protein pXO1-10 (*B. anthracis* virulence plasmid pXO1)	AAD32314	2E − 09

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TABLE 3—*Continued*

Gene identification	Coordinates		Direction	Length (amino acids)	G+C (%)	GCI	Gene name	Homolog product	GenBank accession no.	E value (Blast search)
	Left	Right								
SG84[a]	82892	83548	←	219	63.2	0.465		No significant similarity		
SG85[a]	83683	84354	←	224	64.3	0.466		Hypothetical protein XF1760 (*X. fastidiosa*)	AAF84569	1E − 87
SG86[a]	84444	85271	←	276	62.8	0.590		Hypothetical protein, ORF273 plasmid protein (*E. coli* K-12)	AAC75681	1E − 83
SG87[a]	85460	86344	←	295	61.8	0.510		Hypothetical protein XF1761 (*X. fastidiosa*)	AAF84570	4E − 97
SG88[a]	86670	86894	→	75	54.7	0.252		No significant similarity		
SG89[a]	87095	87262	←	56	61.3	0.398		Hypothetical protein XF1764 (*X. fastidiosa*)	AAF84573	2E − 06
SG90[a]	87280	88077	←	266	60.3	0.481		No significant similarity		
SG91[a]	88389	89102	←	238	60.4	0.491		No significant similarity		
SG92[a]	89199	89591	←	131	60.3	0.443		Hypothetical protein XF1771 (*X. fastidiosa*)	AAF84580	4E − 51
SG93[a]	89615	90022	←	136	66.7	0.408		Hypothetical protein XF1772 (*X. fastidiosa*)	AAF84581	3E − 19
SG94	90168	91730	←	521	60.1	0.452		Domain of hypothetical protein ORF299 (*Sphingomonas aromaticivorans* plasmid pNL1)	AAD03882	3E − 08
SG95	92316	92435	←	40	59.2	0.297		No significant similarity		
SG96[a]	92619	94637	←	673	66.5	0.573	*topB*	DNA topoisomerase III (XF1776) (*X. fastidiosa*)	AAF84584	0E +00
SG97[a]	94881	95276	←	132	62.6	0.569	*ssb*	Single-stranded-DNA binding protein (XF1778) (*X. fastidiosa*)	AAF84586	3E − 50
SG98[a]	95273	95824	←	184	63.4	0.460		Hypothetical protein XF1779 (*X. fastidiosa*)	AAF84587	4E − 56
SG99[a]	95821	96612	←	264	66.3	0.617		Hypothetical protein XF1780 (*X. fastidiosa*)	AAF84588	1E − 102
SG100[a]	96782	97945	←	388	64.1	0.483		Hypothetical protein XF1781 (*X. fastidiosa*)	AAF84589	1E − 127
SG101[a]	97950	98510	←	187	61.0	0.537		Conserved hypothetical protein (XF1782) (*X. fastidiosa*)	AAF84590	6E − 73
SG102[a]	98529	100196	←	556	62.5	0.471		Protein fused from two hypothetical proteins (XF1783 and XF1784) (*X. fastidiosa*)	AAF84591 + AAF84592	3E − 62 + 1E − 100
SG103[a]	100376	101239	←	288	64.2	0.501	*soj*	Chromosome partitioning-related protein (XF1785) (*X. fastidiosa*)	AAF84593	1E − 109
SG104[a]	101270	101488	←	73	56.2	0.399		Phage-related protein (XF1786) (*X. fastidiosa*)	AAF84594	3E −20
SG105[a]	101939	102838	←	300	57.6	0.384	*bphR*	LysR-type regulatory protein BphR (*Pseudomonas* sp. strain KKS102)	BAA07613	1E −56
SG106[a]	102979	103197	→	73	57.5	0.269		No significant similarity		

[a] ORF defined as noncargo in the text (including the homologs).

Hence, a substantial portion of the genes have homologs in other pseudomonads.

The cargo genes endow the strains with some extra metabolic features and transport and resistance capacities (Tables 2 and 3). PAGI-3(SG) of the environmental isolate SG17M is a metabolic island of complex architecture that encodes a broad variety of enzymes, the majority of which are encoded by single genes. The strain-specific portion of PAGI-3(SG) contains genes related to the metabolism and transport of amino acids (SG15, SG17, SG18, and SG28), coenzymes (SG22 to SG24), and porphyrins (SG2), and other putative enzymes (SG10, SG14, SG16,. SG19, SG20, SG21, SG27, SG29, SG30, and SG42). Various small transposable elements such as insertion sequences (ISs) are integrated into this part of the gene island, sometimes disrupting the encoded genes (e.g., ORFs SG5 and SG8 in Table 3). Future functional studies will determine to what extent this set of enzymes strengthens the metabolic versatility of strain SG17M.

The cargo genes of PAGI-2(C) encode proteins for the complexation and transport of heavy metal ions. Gene clusters encoding all nine essential proteins for the cytochrome *c* biogenesis system I (C11 to C18) and related thiol-disulfide exchange proteins (C8 to C10) could be identified. Additionally, proteins associated with the transport of cations (C22 and C97), a two-component regulatory system (C19 and C20), several transcriptional regulators (C30, C35, and C98), a transposon conferring mercuric resistance (C84 to C88), and several other transporters are located on PAGI-2(C). Strain C is a disease isolate from the airways of a patient with CF. The expression of the genes for cytochrome *c* biogenesis encoded by PAGI-2(C) could facilitate iron uptake and inactivation of peroxides (10) and thus may confer an advantage for the bac-

TABLE 4. Features of coding sequences within the strain-specific gene islands

Category[a]	No. of ORFs in:					
	PAGI-2(C)			PAGI-3(SG)		
	All	Cargo[b]	Noncargo[c] (all/XF/SG17M)	All	Cargo[d]	Noncargo[c] (all/XF/C)
Strong homologs of genes with demonstrated function	30	24	6/5/6	18	12	6/5/6
Genes with proposed function based on motif searches or limited homology	14	12	2/2/1	19	18	1/1/1
Homologs of reported genes of unknown function	36	7	29/21/19	36	13	22/12/19
No homology to any reported sequences	33	8	25/0/21	32	7	25/0/21
Total	113	51	62/28/47	105	51	54/18/47

[a] Definitions are as for the PAO1 genome (48).
[b] Cargo ORFs in strain C are C5 to C35, C56 to C63, C84 to C88, and C96 to C100.
[c] All ORFs of the gene island except the cargo ORF. Subgroup XF, ORFs with homologs in the *X. fastidiosa* gene island, subgroup SG17M or C, ORFs with mutual homologs in SG17M and C, respectively. Compare with Fig. 2 for the exact gene identifications within the subgroups.
[d] Cargo ORFs in strain SG17M are SG2 to SG43, SG66, SG74 to SG79, SG94 and SG95.

teria to persist in the CF lung, where they are exposed to iron limitation and oxidative stress (13, 32). However, it is not obvious why the presence of a copper homeostasis protein (C21) or a mercuric resistance operon (C84 to C88) could be of advantage for survival in the CF host. These genes should be highly relevant in an environment with high concentration of heavy metal ions. A copy of PAGI-2(C) was identified in the unfinished sequence of the *R. metallidurans* CH34 genome. The *R. metallidurans* island is also integrated into a $tRNA^{Gly}$ gene and differs from PAGI-2(C) by only 29 nucleotide substitutions in a stretch of 105,049 bp (PAO coordinates 3173676 to 3173597) (Fig. 2). *R. metallidurans* flourishes in millimolar concentrations of toxic heavy metals, and all cargo genes of PAGI-2(C) can add to the bacterial fitness against heavy metal stress.

Comparison of gene islands. Table 5 displays the distribution of G+C contents and GCI values in PAGI-2(C), PAGI-3(SG), and the small clone C-specific segment compared to those in the PAO1 genome. Whereas the G+C content of most noncargo genes with their many mutual homologs comes quite close to typical values of the GC-rich *P. aeruginosa,* the strain-specific cargo genes are less GC rich, which is more pronounced in PAGI-3(SG) than in PAGI-2(C). The plot of the GC content in Fig. 3, with its broad range and numerous shifts, visually shows this mosaicism between cargo and noncargo genes. As indicated by their low GCI values, the codon usages of the majority of PAGI-3(SG) and PAGI-2(C) genes are significantly different from those in the PAO1 genome. The *P. aeruginosa* PAO1 genes are characterized by consistently high GCI values, which do not vary with the chromosomal localization of the respective gene (21). The only exceptions are 15 islands that carry five or more consecutive genes with low GCI values (21). Hence, we conclude that PAGI-2(C) and PAGI-3(SG), with their more than 100 genes, represent a very large island with atypical codon usage in *P. aeruginosa* C, where the cargo genes are more atypical in their codon usage than the noncargo genes and PAGI-3(SG) is more atypical than PAGI-2(C).

The homologous proteins in the gene islands of strain C, strain SG17M, *R. metallidurans*, and *X. fastidiosa* exhibit high levels of amino acid identity and similarity. The pairwise comparison revealed the highest values between the corresponding genes of strain C, *R. metallidurans*, and *X. fastidiosa*. The average amino acid identity between C and *R. metallidurans* was 100%, that between C and *X. fastidiosa* was 79.8%, that between C and SG17M was 64.8%, and that between SG17M and *X. fastidiosa* was 62.6%. In other words, the homologs of strain C are more related to those in the gene islands of phylogenetically unrelated species than to those found in a member of the same *P. aeruginosa* clone. This statement is corroborated by the finding that the *X. fastidiosa* gene island shares 28 ho-

TABLE 5. Distribution of G+C contents and GCI values of PAGI-2(C) and PAGI-3(SG) compared to those in the PAO1 genome

Genomic region	ORFs[a] (*n*)	G+C content (%)		GCI	
		Avg	Median (inner quartiles; range)	Avg	Median (inner quartiles; range)
PAGI-2(C)	All (113)	64.6	64.8 (61.9–66.7; 55.7–75.1)	0.537	0.541 (0.460–0.611; 0.280–0.733)
	Cargo (51)	63.2	63.2 (61.1–65.4; 56.7–70.2)	0.495	0.476 (0.434–0.537; 0.354–0.671)
	Noncargo (62)	65.8	65.6 (63.7–68.1; 55.7–75.1)	0.573	0.589 (0.534–0.627; 0.280–0.733)
PAGI-3(SG)	All (105)	59.8	61.0 (56.2–64.3; 38.3–71.1)	0.448	0.452 (0.349–0.557; 0.181–0.756)
	Cargo (51)	55.2	56.3 (50.9–59.0; 38.3–67.6)	0.371	0.350 (0.272–0.420; 0.181–0.756)
	Noncargo (54)	64.2	63.8 (61.7–66.7; 54.7–71.1)	0.521	0.507 (0.459–0.590; 0.252–0.700)
Clone C DNA	All (9)	65.4	65.4 (63.3–66.5; 62.6–70.0)	0.645	0.639 (0.629–0.667; 0.539–0.724)
PAO genome	All (5,570)	66.7	67.3 (64.9–69.3; 29.9–76.2)	0.678	0.697 (0.638–0.741; 0.139–0.896)

[a] For definitions of subgroups, see Table 2, footnote *a*.

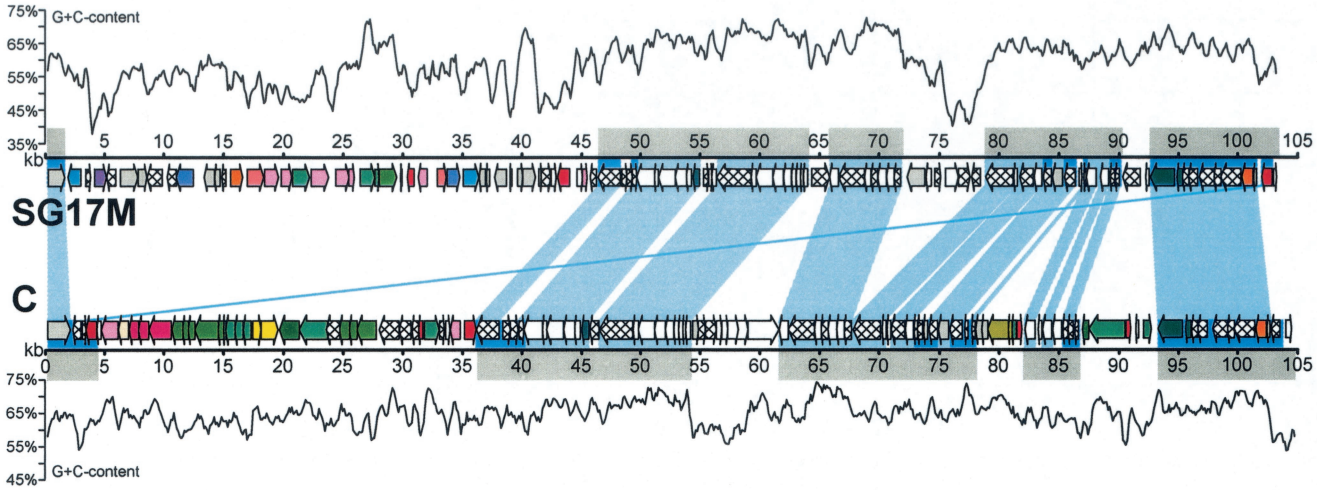

FIG. 3. Comparison of the strain-specific gene islands in *P. aeruginosa* SG17M (upper line) and C (lower line). Genes are represented by arrows as in Fig. 2. Homologous ORFs are linked by light blue bars. A slightly darker blue line connects the corresponding *bphR* genes located at the right border of the SG17M gene island and at the left border of the C-specific insertion. Genes with homologs in the *X. fastidiosa* gene island are highlighted with a dark blue background. Gray boxes above and below the gene maps mark all ORFs that are presumably associated with the mobilization and transfer of the gene islands (called noncargo ORFs in the text; compare with Tables 2 and 3 for the corresponding gene identification numbers). Additionally, a 500-bp sliding window plot of G+C content is displayed for each gene island.

mologs with PAGI-2(G) but only 18 homologs with PAGI-3(SG) (Table 4).

The order of the homologs is conserved in PAGI-2(C) and PAGI-3(SG) for 46 of the 47 genes. The exception encodes the transcriptional regulator BphR (C4 and SG105). The gene contig, however, is disrupted several times by the insertion of strain-specific ORFs (Fig. 3).

PAGI-2(C) and PAGI-3(SG) are not the only gene islands that are known in *P. aeruginosa.* We have previously described 100-kb large gene islands that were derived from episomal plasmids and reversibly recombined with either of the two $tRNA^{Lys}$ genes of clone C and K chromosomes (19). The $tRNA^{Lys}$- and $tRNA^{Gly}$-associated gene islands share P4-type *int* and homologous *soj* genes adjacent to the recombination breakpoints, but otherwise their genetic contents are different (unpublished data). Gene islands, however, are not necessarily inserted into tRNA genes. So far, two islands that are not integrated into a tRNA gene have been identified in *P. aeruginosa*. The first example is the 48.9-kb PAGI-1, which has been found in 85% of tested *P. aeruginosa* clinical isolates from sepsis and urinary tract infections and hence has been suggested to confer virulence traits (24). The other example is a ca. 16-kb large DNA segment in strain PAK that carries genes for the glycosylation of a-flagellin, among others (4).

PAGI-2(C) and PAGI-3(SG) have a bipartite structure: a set of strain-specific ORFs encoding metabolic functions and transporters and a set of conserved hypothetical genes and unknown genes, of which most genes are homologs with high sequence similarity. The conserved order of the homologs (many of which are also found in a $tRNA^{Gly}$-associated island in *X. fastidiosa*), the similar global structures of PAGI-2(C) and PAGI-3(SG), and the role of the few homologs with a recognized function in DNA recombination or repair (*ssb*, *topB*, and *radC*) are three striking features that point to important and conserved roles of the large cassette of homologous genes. We hypothesize that besides the *int* and *soj* genes, at least some of the homologs are responsible for the mobilization, transfer, and stabilization of the island (Fig. 3). In other words, genes of the cassette of conserved homologs should mediate lateral gene transfer, whereas the other half of the island would represent the individual cargo that endows the recipient with strain-specific metabolic properties. The forthcoming genome projects will resolve whether or not this peculiar type of gene island with its mosaic structure of individual cargo and of conserved homologs is obligatorily associated with $tRNA^{Gly}$ genes. These potentially transmissible islands seem to be rather common among metabolically versatile proteobacteria that initially had been classified as pseudomonads by physiology-oriented taxonomists. We have preliminary evidence from ongoing Southern and in silico analyses that homologs of PAGI-2 or PAGI-3 or conserved ORFs thereof exist not only in *R. metallidurans* CH34 and *X. fastidiosa* but also in other *P. aeruginosa* strains, type I pseudomonads, and *Burkholderia* spp.

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