The Genome and Structural Proteome of YuA, a New *Pseudomonas aeruginosa* Phage Resembling M6[†]

Pieter-Jan Ceysens,¹ Vadim Mesyanzhinov,² Nina Sykilinda,² Yves Briers,¹ Bart Roucourt,¹
Rob Lavigne,¹ Johan Robben,³ Artem Domashin,² Konstantin Miroshnikov,²
Guido Volckaert,¹ and Kirsten Hertveldt^{1*}

Division of Gene Technology, Katholieke Universiteit Leuven, Kasteelpark Arenberg 21, Leuven B-3001, Belgium¹;
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Miklukho-Maklaya, Street 6/10, Moscow 117997,
Russia²; and Biomedical Research Institute, Limburgs Universitair Centrum, and School of Life Sciences,
University Hasselt, Diepenbeek B-3590, Belgium³

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Pseudomonas aeruginosa phage YuA (*Siphoviridae*) was isolated from a pond near Moscow, Russia. It has an elongated head, encapsulating a circularly permuted genome of 58,663 bp, and a flexible, noncontractile tail, which is terminally and subterminally decorated with short fibers. The YuA genome is neither Mu- nor λ -like and encodes 78 gene products that cluster in three major regions involved in (i) DNA metabolism and replication, (ii) host interaction, and (iii) phage particle formation and host lysis. At the protein level, YuA displays significant homology with phages M6, ϕ JL001, 73, B3, DMS3, and D3112. Eighteen YuA proteins were identified as part of the phage particle by mass spectrometry analysis. Five different bacterial promoters were experimentally identified using a promoter trap assay, three of which have a σ^{54} -specific binding site and regulate transcription in the genome region involved in phage particle formation and host lysis. The dependency of these promoters on the host σ^{54} factor was confirmed by analysis of an *rpoN* mutant strain of *P. aeruginosa* PAO1. At the DNA level, YuA is 91% identical to the recently (July 2007) annotated phage M6 of the Lindberg typing set. Despite this level of DNA homology throughout the genome, both phages combined have 15 unique genes that do not occur in the other phage. The genome organization of both phages differs substantially from those of the other known *Pseudomonas*-infecting *Siphoviridae*, delineating them as a distinct genus within this family.

Despite a decade of major sequencing efforts, many aspects of the genomic diversity among bacteriophages remain to be addressed. Recent metagenomic sequencing of uncultured viral communities from oceanic regions has shown that, although common patterns of genomic organization are present, up to 90% of marine-phage sequences are novel (6, 14). Other phage-sequencing projects revealed distinct levels of genomic diversity among phages infecting different bacteria. For example, the diversity of phage types infecting mycobacteria (37) contrasts sharply with dairy phages, which constitute a close-knit group (15).

In recent years, genome sequencing efforts for phages infecting *Pseudomonas aeruginosa* have revealed this group as strongly diverse at the genome organizational level, which is consistent with their reported diversity in propagation, host interaction, and particle structure. The phages of *P. aeruginosa* are under investigation to determine the scope of their therapeutic potential and to unravel their dynamic interaction with their pathogenic host. Moreover, insight into the genome content of *P. aeruginosa* phages allows insight into the evolutionary aspects of these phages. At present, 27 complete genome sequences of phages infecting *P. aeruginosa* have been deposited

in public databases (2). Among the siphoviruses infecting *P. aeruginosa*, phage D3112 is probably the best studied. With the exception of a DNA modification module and a structural region coding for tail morphogenesis proteins, phage D3112 shares its overall genome organization and transposable nature with phage Mu. Its tail, however, resembles the flexible tails of lambda-like particles, which is in contrast to the rigid, contractile tails of Mu-like particles (49). This mosaicism is relatively common among temperate phages and suggests horizontal evolution. Phage B3 is another transposable *P. aeruginosa*-infecting phage but is more distantly related to phage Mu than to phage D3112 (12). Phage DMS3 shares DNA similarity with phage D3112 and is able to transduce DNA between *P. aeruginosa* strains PA14 and PAO1 (17). Phage D3 resembles phage λ both from an organizational and a morphological point of view (25).

Here, we report the characterization of the new *P. aeruginosa* phage YuA on the morphological, genomic, and proteomic levels. Phage YuA was isolated in a pond near Moscow, Russia, and belongs to the *Siphoviridae* family. Morphological data suggest that it is related to phage M6 from the Lindberg typing set (1, 28). Both YuA and M6 are significantly different from other *Pseudomonas*-infecting siphoviruses deposited in the public databases. Therefore, an in-depth analysis of the phage YuA genome sequence and its particle protein content was performed.

MATERIALS AND METHODS

Bacteriophage isolation, bacterial strains, media, and plasmids. Bacteriophage YuA was isolated from an environmental water sample, using standard enrichment methods (3). The host bacterium, *P. aeruginosa* PAO1, and 73 other

* Corresponding author. Mailing address: Division of Gene Technology, Katholieke Universiteit Leuven, Kasteelpark Arenberg 21, Leuven B-3001, Belgium. Phone: 32 16 32 96 71. Fax: 32 (0)16 32 19 65. E-mail: kirsten.hertveldt@biw.kuleuven.be.

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amplified fragment length polymorphism-typed clinical *P. aeruginosa* strains were kindly provided by J. P. Pirnay (38). The *P. aeruginosa* PAO1 *rpoN* mutant strain (*rpoN::tet*) (7) was kindly provided by H. Arai. Bacterial strains were grown in standard Luria-Bertani medium. Electrocompetent *P. aeruginosa* PAO1 cells were prepared by washing a 2-ml overnight culture with 1 ml of ice-cold ultrapure water in six consecutive steps and finally resuspending the culture in 50 μ l H₂O.

Phage purification and electron microscopic imaging. High-titer stocks of YuA were obtained by an overnight incubation of 10⁶ PFU in the presence of *P. aeruginosa* PAO1 cells using the standard soft agar overlay technique (3). Lawns of soft agar were collected, resuspended in 20 ml of phage buffer (150 mM NaCl, 10 mM MgSO₄, 10 mM Tris · HCl [pH 8]), and briefly vortexed. Phage particles were collected by centrifugation (20 min; 4,000 $\times$ g), concentrated in the presence of polyethylene glycol 8000 (8%, wt/vol), and purified by two successive rounds of CsCl density gradient centrifugation. Purified phage particles were negatively stained with uranyl acetate (2%, wt/vol) and visualized by transmission electron microscopy.

DNA isolation, characterization, and sequencing. Phage DNA was isolated as described elsewhere (35). Restriction digests were performed according to the manufacturer's protocol. Initial sequence data were obtained from a shotgun library of phage DNA in pUC18. Several consecutive rounds of primer walking were performed directly on phage DNA, until the sequence assembled into a single contig with an average fourfold redundancy. Open reading frames (ORFs) were predicted using Genemark HMM (31) and visually inspected for the presence of convincing ribosome binding sites. Translated ORF sequences were compared with known proteins using the BLASTP (5) and PSI-BLAST (4) algorithms against the nonredundant GenBank protein database. In addition, smaller, nonpredicted ORFs which are conserved between YuA and M6 were considered functional ORFs, based on tBLASTx comparisons between both phage genomes. Prokaryotic promoters were predicted by using the BDGP (39) and SAK (22) prediction programs and by scanning the genome for conserved intergenic motifs using the MEME/MAST algorithm (8). Putative terminators were searched using Transterm (19), and transmembrane helices were detected using the TMHMM algorithm (33). Finally, tRNA genes were searched by using the tRNAscan-SE program (30).

Experimental promoter identification. Purified YuA DNA was randomly sheared by sonication. Fragments ranging from 200 to 400 bp were recovered from agarose gel, end repaired, phosphorylated, and ligated into the SmaI-digested and dephosphorylated vector pTZ110, a promoterless broad-host-range vector with a *lacZ* operon fusion (44). A threefold-redundant promoter library was obtained after electroporation of the ligation mixture into freshly prepared electrocompetent *P. aeruginosa* PAO1 cells (1.8 kV, 25 μ F, 250 Ω) and plating onto LB plates supplemented with 125 μ g/ml carbenicillin and 40 μ g/ml X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside). Plasmid DNA from blue colonies was isolated by the alkaline lysis method (41), and inserts were sequenced using a vector-specific primer (5'-GCCACCTGACGTCTAAGAAAC-3'). The specific β -galactosidase activities of these selected clones were confirmed and quantified in liquid cultures (32).

Mass spectrometry. For the identification of structural YuA proteins, 10 μ l of concentrated phage solution (10¹¹ PFU) was reduced in 2 mM β -mercaptoethanol, heat denatured (95°C, 5 min), and loaded onto a standard 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel. The entire lane was cut into slices, which were subjected to trypsin digestion (46). For the alternative whole-phage shotgun approach, equal amounts of phages were destabilized by four successive rounds of freezing and thawing and sonication, heated for 10 min at 95°C, and reduced in the presence of 10 mM dithiothreitol for 1 h at 56°C. Disulfide bonds were blocked by alkylation with 10 mM iodoacetamide, followed by an overnight trypsinization of the whole reaction mixture at 37°C. Peptides generated by the two methods were separated by liquid chromatography with a linear 5 to 60% (vol/vol) acetonitrile gradient and subsequently identified using electrospray ionization-tandem mass spectrometry (MS/MS) as described previously (27).

Nucleotide sequence accession number. The genome of bacteriophage YuA was deposited at GenBank under accession number AM749441.

RESULTS AND DISCUSSION

General features of phage YuA and its genome. Bacteriophage YuA propagates on *P. aeruginosa* PAO1 and infects 13 out of 73 diverse *P. aeruginosa* strains from an amplified fragment length polymorphism-typed library collected worldwide. YuA displays a small (1-mm diameter) and turbid plaque mor-

FIG. 1. Electron microscopic image of phage YuA particles. Scale bar = 100 nm. Phage YuA has an elongated head and a flexible tail.

phology, suggesting a temperate nature. The phage does not infect *Pseudomonas putida*, *Pseudomonas fluorescens*, or other gram-negative bacteria like *Escherichia coli* and *Shigella* and *Salmonella* species but is able to lyse *Burkholderia solanacearum* at a low efficiency (efficiency of plating, 10⁻⁷).

Electron microscopic imaging revealed YuA as a typical member of the *Siphoviridae* family of double-stranded DNA bacteriophages (*Caudovirales*) having a flexible, noncontractile tail (Fig. 1). In contrast to the well-known *Pseudomonas*-infecting *Siphoviridae* phages D3, B3, and D3112, which resemble phage λ , phage YuA has an elongated head (B2 morphotype) resembling *P. aeruginosa* phage M6 (1). The phage YuA head size is $\sim$ 72 by $\sim$ 51 nm, and the tail length is $\sim$ 145 nm. Besides an elongated head, both YuA and M6 have striated tails which are terminally and subterminally decorated with short fibers. Phage M6 is reported to be morphologically identical to *Xanthomonas oryzae* phage XP12 (1) and has been shown to adsorb to nonretractile host pili (11).

YuA genomic DNA is insensitive to the activities of 10 out of 13 tested restriction enzymes, including many common enzymes like HindIII, BglII, and EcoRI and methylation-dependent DpnI (GA^m/TC). Given YuA's sensitivity to digestion with Sau3A (/GATC) and the methylation-sensitive SmaI (CsCCs/GGG), it can be concluded that YuA contains unmethylated adenine and cytosine residues. This is in contrast with the morphologically related phage XP12, which is known to contain a 5-methylcytosine instead of cytosine in its genome (20). In silico analysis revealed the absence of 9 out of 10 recognition sites, although 4 EcoRI sites are present despite YuA's insensitivity to that restriction enzyme. Furthermore, it became clear during genome sequencing that isolated YuA DNA is rather inaccessible to standard PCR amplification using various primers, annealing temperatures, and commercially available DNA polymerases. These observations suggest the presence of another base substitution or modification, as discussed below. Resistance to restriction during phage infection could also be provided by gene product 45 (gp45) of YuA, which displays high similarity to the ArdB antirestriction protein (Pfam E value of 10⁻³⁰). This plasmid-encoded protein inhibits efficient restriction by members of the three known families of type I restriction endonucleases (10).

FIG. 2. Circular representation of the YuA genome. The outer circle represents the YuA ORFs, and their predicted functions in DNA metabolism and replication, host interaction, particle formation, and host lysis are indicated. Experimentally confirmed structural proteins are marked with an asterisk, and confirmed phage promoters are indicated with black arrows. Predicted (nonconfirmed) promoters and terminators are indicated with open arrows and stem-loop structures, respectively. The inner circles represent similar ORF regions of phages 73 (purple), ϕ JL001 (blue), and B3 and D3112 (red). The corresponding E values are indicated for ϕ JL001, B3, and D3112.

YuA genome sequence and similarity to other phages. The genome of YuA comprises 58,663 bp and has a G+C content of 64.3%, strongly resembling the G+C average (65%) of its host. In total, 78 ORFs (ORFs 1 to 77 and ORF 60.1) were predicted from the circular genome map (Fig. 2; Table S1 in the supplemental material), all oriented in the same direction and leaving only 4% of the YuA genome as noncoding. No tRNA genes were predicted. The genome of YuA is neither Mu- nor λ -like and can be roughly divided into three functional

regions, containing gene products involved in (i) nucleotide metabolism and DNA replication, (ii) host interaction, and (iii) particle structure, packaging, and host lysis (Fig. 2).

The entire YuA genome displays 91% DNA similarity to phage M6 (GenBank accession no. NC_007809) (26), which results in >80% amino acid identity with 92% of the predicted ORFs of M6. The data obtained for YuA confirm the recent gene predictions made for M6 in GenBank. Only six genome regions contain unique YuA or M6 sequences, accounting for

FIG. 3. Pairwise comparison of bacteriophages YuA and M6. The predicted ORFs and their mutual amino acid identities are indicated in red (>90% identity), orange (>80% identity), and yellow (>50% identity). ORFs unique to YuA (4) and M6 (11) are hatched and purple, respectively, and predicted functions are indicated. Nucleotide identity throughout both genomes is illustrated by the middle graph, comparing both phages ORF by ORF using a sliding window of 60 bp.

15 differential gene products in total, 4 of which occur in YuA and 11 in M6. Further comparative analysis between YuA and M6 also revealed YuA gp70 and gp71 as the most deviating proteins in the structural region, hinting at a role for these proteins in host recognition and surface adhesion (Fig. 3).

Comparison of YuA proteins to those of other phages reveals significant similarity between 29 predicted YuA proteins and proteins encoded by ϕ JL001 (63,469 bp), a phage that infects an uncharacterized marine alphaproteobacterium, JL001. Phage ϕ JL001 is reported to be a temperate phage, shares roughly the YuA particle morphology, and appears to lack the ability to form stable lysogens (29). In addition, 18 particle-structure-related YuA proteins share amino similarity to gene products of *P. aeruginosa* phage 73 from the Lindberg typing set (28). Despite major similarities in head morphogenesis genes, phage 73 is morphologically identical to phage D3112 (1) and does not show the elongated head morphology typical of YuA, M6, and ϕ JL001 particles. This might be explained by the smaller genome content of phage 73 (42,999 bp) than those of the last-named phages (60 kb). Finally, eight YuA proteins (gp70 to gp77), which are most probably involved in host attachment and interaction, share sequence similarity with the transposable and pilus-specific *P. aeruginosa* phages B3, DMS3, and D3112 (Fig. 2; Table S1 in the supplemental material). The gene products of these phages also appear in several bacterial genome sequences as prophage or cryptic phage elements, e.g., in *Hahella chejuensis*, *Xylella fastidiosa*, *Burkholderia cepacia*, and *Haemophilus ducreyi* and in *Burkholderia cepacia* phage BcepNazgul (GenBank accession no. NC_005091; 57,455 bp).

The choice of the YuA genome sequence zero point was based on genome comparisons with phages ϕ JL001, D3112, DMS3, and B3; predicted gene functions; and promoter prediction/identification in phage YuA. The YuA zero point differs from the phage M6 zero point, which might be reconsidered for consistency among these related phages.

Regulatory elements. Motif searches led to the identification of two different conserved intergenic motifs that could be involved in the transcription regulation of phage YuA (Fig. 2). To experimentally identify host promoter sequences, promoter activity was determined quantitatively in *P. aeruginosa* cells by measuring the β -galactosidase activities of individual clones of the constructed promoter trap library (32, 44). We identified five YuA regions from which transcription of the vector-borne *lacZ* gene was initiated by the *P. aeruginosa* transcriptional machinery (Fig. 4). Two different promoter types were distinguished based on sequence information and promoter strength. The first type, found in front of genes 2 and 50, has a clear σ^{70} -like consensus sequence (TTAGGT-N₁₇-TtaAAT) and yields 1,021 Miller units of β -galactosidase activity. The second promoter type is located in the genome region involved in particle formation and host lysis. It precedes genes 55, 58, and 68 and displays approximately twofold more activity (2,152 Miller units) than that of the first promoter. Conserved GG and GC elements in this second promoter type are separated by a DNA stretch corresponding to one helical turn, resembling σ^{54} binding sites (9). This finding was further investigated using an *rpoN* deletion mutant of *P. aeruginosa* PAO1, which is unable to produce the σ^{54} transcription factor (7). Infection studies (multiplicity of infection, 0.1 to 10⁸) revealed the in-

FIG. 4. Promoter assay of the YuA fragment library. Five promoter regions were identified and are named after the genes that they precede. Measurements of β -galactosidase activity in wild-type *P. aeruginosa* PAO1 and the *rpoN* mutant are indicated in black and gray, respectively. Data represent averages and standard deviations for six samples per promoter construct for accurate quantification of promoter activity by the Miller assay.

ability of YuA to replicate inside this mutant. In a second step, five pTZ110 constructs containing the identified promoter regions (Fig. 4) were electroporated into this mutant, and the transformants were assayed for β -galactosidase activity. In contrast to the two identified σ^{70} promoters (P₂ and P₅₀), which largely maintained their activities, transcription of the reporter β -galactosidase gene downstream from the putative σ^{54} promoters was absent (Fig. 4). This proves that the three promoters preceding genes 55, 58, and 68 specifically require the σ^{54} binding factor to initiate transcription of the downstream genes. This is, to our knowledge, the first time that this has been demonstrated for a bacteriophage and implies the dependency of YuA on additional transcriptional factors (enhancer binding proteins) to initiate RNA synthesis of the late phage proteins (34).

No promoter activity could be associated with a third, perfectly conserved intergenic motif (CTTTACTTACTTCGG-N₉-TATACTT) preceding genes 8, 12, 28, 42, and 45 (Fig. 2), which might indicate the need for one or more phage-encoded proteins to control the expression of these YuA genes. Alternatively, another bacterially encoded transcription factor that is present only under certain circumstances might be required to initiate transcription.

In addition, four p-independent terminators were predicted, downregulating transcription beyond genes 5 (with an unknown function), 12 (encoding a predicted DNA repair enzyme), 55 (encoding a structural protein), and 56 (encoding a major capsid protein) (Fig. 2).

Gene products involved in DNA metabolism and genome replication. The YuA genome region from ORFs 2 to 23 encodes several proteins which are predicted to be involved in nucleotide metabolism (gp17, gp22, and gp23) and DNA replication (gp7, gp13, gp21, and gp41). Gene 22 encodes a putative ribonucleotide reductase which catalyzes the committed step to DNA synthesis by the reduction of ribonucleoside diphosphates to the corresponding deoxyribonucleoside diphosphates. dCMP deaminase (gp23) catalyzes the deamination of dCMP to deoxyuridylate (dUMP). Interestingly, a dUMP hydroxymethylase (dUMP-HMase) function is predicted for gp17. In this enzymatic reaction, dUMP serves as a substrate for the addition of a hydroxymethyl group, using CH₂H₄ folate as a cofactor and generating the modified base hydroxymethyl-dUMP (43). The presence of this modified base was shown in

Promoter	YuA Region	Core sequence
P ₂	489-568	CTTAGGTTACTTCACTTTCTCCAGTTCAATCCTA
P ₅₀	33014-33160	CTTAGGTTACTTGGGTTTCTTACCTGAAATAAAT -35 -10
P ₅₅	38392-38570	GGCACAACACTGGCGGGTGATCTGCCGACAATGA
P ₅₈	40888-41035	GACGATGCCCTGGCGGACGAGAAGCTGGAAGTGT
P ₆₈	47152-47312	GATCAAGCCCTGGTGTCTGGGACGCCCTGGGACA -24 -12

Bacillus phage SPO1 (50) and is also predicted for ϕ JL001 and M6. dUMP-HMases share similarity with the more widespread and highly conserved group of thymidylate synthases, which use the same cofactor in the reduction of dUMP to dTMP. Sequence alignment of YuA gp17 with the dCMP-HMase of T4 and the corresponding gp17-homologous proteins in ϕ JL001 and SPO1 (Figure S1 in the supplemental material) clearly shows the conservation of catalytically important residues (Glu60, Cys148, and Asp179), deoxyribose-binding residues (His216 and Tyr218), and phosphate-binding residues (Lys28, Arg123, Arg124, Arg168, and Ser169) (47).

Host interaction. The YuA genome region bearing genes with predicted functions in host interaction ranges from ORFs 25 to 45. Unique to YuA is the presence of a diguanylate cyclase or GGDEF domain (ORF 44), which is widespread in bacterial proteins, functioning as a global second messenger controlling motility and adhesion in bacterial cells (21). The putative repressor (gp25) contains helix-turn-helix motifs similar to those of the phage λ repressor, while the predicted integrase (gp26) differs significantly from the two major families of tyrosine and serine site-specific recombinases (23). Thus far, we have not been able to isolate a stable, lysogenic *P. aeruginosa* PAO1 strain. Our assays involved the isolation of phage-resistant *P. aeruginosa* PAO1 clones from turbid plaques and the detection of phage sequences by PCR and DNA restriction analysis. Neither stably integrated nor stably nonintegrated phage genome sequences could be detected. Similar results were reported for phage ϕ JL001 (29) and *Vibrio parahaemolyticus* phages VP16T and VP16C (45). Strikingly, the YuA integrase exhibits 32% amino acid identity with the integrases of vibriophages VP5 and VP2, which share similarity with the VP16T and VP16C integrases. Apparently, these vibriophage-like integrase proteins are also unrelated to the well-studied tyrosine or serine recombinase families and exhibit distinct integrase behavior. The YuA integrase may require specific—but not yet determined—physiological conditions or a different host strain for stable lysogenic establishment.

Structural proteome. Electrospray ionization-MS/MS analysis of gel-separated phage particle proteins led to the experimental identification of 16 predicted proteins, reaching sequence coverages up to 66.1% (Table 1). The two most abundant proteins were identified as gp56 and gp66, suggesting

TABLE 1. Identification of the structural proteins of YuA

Gene	Molecular mass (kDa)	1-DE approach ^a		Whole-phage shotgun approach	
		No. of identified peptides	Sequence coverage (%)	No. of identified peptides	Sequence coverage (%)
52	56.1	13	28		
54	45.0	14	32.3	2	10.4
55	27.9	4	14.6		
56	32.2	20	66.1	22	88.2
57	19.1	2	22.9	2	23.4
59	5.5	2	35.7	3	46.4
63	18.6	8	36	3	29.7
64	13.4			3	63.1
65	16.4	3	22.7	1	10.4
66	54.0	33	63.3	21	66.1
69	100.4	24	26.3		
70	56.2	6	13.2		
71	35.7	9	40.6	4	24.5
72	62.2	9	17.6	3	15.1
73	30.1	6	24.5		
74	8.2			1	37.5
76	76.9	16	27.0	2	3.4
77	17.1	3	36.6		

^a 1-DE, one-dimensional electrophoresis.

functions as major capsid and tail proteins. The abundance of these two proteins was confirmed by denaturation and subsequent MS analysis of whole phage particles, as described in Materials and Methods. The latter method allowed the detection of additional peptides which were not found in the first analysis, thus increasing the overall coverage. Moreover, two additional YuA proteins (gp64 and gp75) were experimentally confirmed and identified as part of the virion particle (Table 1). These results roughly delimit the YuA genome region involved in particle formation and host release to genes 52 to 77 and corroborate the complementarity of the two MS approaches. It also supports our previous finding that the whole-phage shotgun approach is well suited to trace small or less abundant phage proteins (27), and it provides an experimental annotation of the particle proteins, accounting for 23% of the predicted ORFs.

YuA lysis cassette. Bacteriophage YuA encodes a delineated cluster of four overlapping lysis genes as typically observed in lambdoid phages, P22-like phages, and the more closely related phage B3 (51). The YuA lysis cassette is located within the genome region encoding phage particle proteins, presumably between the head and tail morphogenesis genes. Compared to the genes of the lysis cassette in lambdoid phages, the genes within this cassette in YuA are rearranged, since the putative endopeptidase *Rz* (gp60) gene and the embedded *Rz1* (gp60.1) (reading frame +1) gene precede the holin (gp61) gene and the endolysin (gp62) gene (16, 52). YuA *Rz* and *Rz1* proteins share similarity with the M6 gene products gp21 and gp21.1 (the last is newly predicted based on its homology to the YuA *Rz1* protein), respectively. *Rz1* has a typically high proline content (10/66 amino acids) and is predicted as a lipoprotein (LipoP 1.0; fatty acid lipidation at Cys15). YuA gp61 does not show homology to known proteins, with the exception of phage M6 gp22. However, its position in the putative lysis cassette, small size, and three transmembrane domains with a

topology inside the N terminus strongly suggest its function as a class I holin (48). Gp62 (the predicted endolysin) has a putative signal peptide sequence (SignalP 3.0; $P = 0.951$) with a cleavage site at position 23 (ALA-QD). The presence of a signal peptide and the lytic activity of gp62 are supported by the observation of the cellular toxicity of recombinantly expressed gp62 in the absence of sodium azide, an inhibitor of the Sec secretion complex. Expression in the presence of sodium azide largely elevates the toxicity of gp62 expression (unpublished results). The presence of a signal peptide sequence suggests the need for an inhibitory mechanism for the exported lysins to prevent early lysis of the host cells. Recent research showed that the full activity of the previously exported fOg44 lysin is achieved only after sudden, non-ion-specific dissipation of the proton motive force, an event undertaken by the holin (24, 36, 42). In this way, holins still function as time regulators of lysis.

General conclusions. With the estimated 10 million tailed-phage species in the environment (40), it is doubtful that we will ever reach the point at which sequencing more phage genomes will fail to add significantly to our understanding of phage diversity and evolution. It is compelling, however, that, despite evident genetic mosaicism, a limited (but steadily increasing) number of phage genera can be delineated, like the M6-like *Siphoviridae* investigated in this study. At this point, it remains unclear whether this clustering is an artifact of current sampling or whether these groups actually exist (18). Nevertheless, the sequencing of phage YuA supports the previously stated hypothesis that local viral diversity seems to be high but that global diversity is relatively low because of the movement of viruses between environments (13).

One must also consider the fact that research which is limited to genome sequencing generates vast numbers of genes with unknown functions in public databases, and functional studies involving nonmodel phages lag far behind. With well-chosen experimental studies, these gaps can be (partially) closed quite efficiently. For example, the experimental identification of 18 YuA particle proteins (Table 1) allows tentative functional annotations of their corresponding proteins in respective phage particles of phages M6, ϕ JL001, D3112, B3, DMS3, 73, BcepGomr, BcepNazgul, XP15, SETP3, and KS7. Novel studies unraveling unknown gene functions can give valuable insights into the diversity of lifestyle strategies and should be encouraged.

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