Phase Variation in the Phage Growth Limitation System of
*Streptomyces coelicolor* A3(2)

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The phase-variable phage growth limitation (Pgl) system of *Streptomyces coelicolor* A3(2) is an unusual bacteriophage resistance mechanism that confers protection against the temperate phage φC31 and homologous relatives. Pgl is subject to phase variation, and data presented here show that this is at least partially due to expansion and contraction of a polyguanine tract present within the putative adenine-specific DNA methyltransferase gene, *pglX*. Furthermore, the *pglX* paralogue SC6G9.02, here renamed *pglS*, was shown to be able to interfere with the Pgl phenotype, suggesting that PglS could provide an alternative activity to that conferred by PglX.

Bacteria utilize a number of methods to protect themselves from infection by bacteriophages, and perhaps the most common of these are restriction-modification (R-M) systems (1, 7, 22). In type I R-M systems the HsdS subunit is required for target sequence recognition, and in some cases the sequence specificity of such subunits can be altered by natural processes (1, 12, 28, 30). Thus, the products of different *hsdS* alleles can confer novel R-M specificity when they interact with the other subunits of the system, HsdR (required for restriction) and HsdM (required for modification) (12, 28, 30). R-M systems are also occasionally subject to high-frequency phase variation (11, 12, 30). For example, the *mod* gene of the *Haemophilus influenzae* type III R-M system contains 40 tetranucleotide (AGTC) repeats which expand and contract, resulting in frameshifts (11). While the alteration of the sequence specificity of an R-M system may be viewed as a response to an evolving phage population, the advantage provided to bacteria by phase-variable R-M systems is not clearly understood (12).

*Streptomyces coelicolor* has an unusual phage resistance mechanism termed the phage growth limitation (Pgl) system (9). The phenotype is characterized by the ability of the infecting phage (e.g., φC31) to undergo a single phase burst, which produces progeny that are severely attenuated in a second infectious cycle. The model proposed by Chinenova et al. postulates that Pgl+ strains modify phage, which are then restricted during the second cycle (9). This modification is not lethal to the phage as progeny from a Pgl+ strain can infect and form plaques on Pgl– strains (9). Two loci, *pglYZ* and *pglWX*, have been identified as being necessary and sufficient for production of a Pgl+ phenotype in *S. coelicolor* (3, 19, 31). Two classes of Pgl+ mutants are evident; class A mutants can be complemented by *pglYZ* (19), and class B mutants can be complemented by *pglWX* (31). PglW shows homology to eukaryotic-like serine/threonine protein kinases, while PglX resembles adenine-specific DNA methyltransferases. PglX is hypothesized to fulfill the modification component of Pgl through DNA methylation (31). PglY is distantly related to the AAA+ group of proteins thought to be required for the assembly, maintenance, and disassembly of protein complexes (23), a role that is consistent with the preliminary evidence that the Pgl proteins act as a complex (31). PglZ contains no recognizable functional motifs (3). A recent study suggested that PglZ could be phosphorylated in uninfected cells (15), possibly through the activity of PglW. Pgl may therefore act via phosphoproteins that in the first infectious cycle modify the progeny phage DNA through methylation, and in a subsequent infectious cycle the infection is somehow aborted (31). Unusually for a phage resistance system, Pgl has a very limited range of sensitive phages (i.e., φC31 and its homologous relatives) (19).

The Pgl system has been shown to be phase variable, with Pgl+ mutants arising at frequencies of $10^{-4}$ to $10^{-5}$ per spore (19) and reversion occurring at similar frequencies. Previously, the phase variation mechanism of Pgl was examined by genomic Southern blotting of the *pgl* loci from different Pgl+ and Pgl– strains (19, 31). With the exception of the IS1648 insertion into *pglW* in strain B135 Pgl– (and its derivative J1974), no large-scale structural rearrangements were identified in any Pgl– strain that could account for the phase variability. While insertion sequences have been reported to be potentiaters of phase variation (34), this is not thought to be the case with the IS1648 insertion in B135 Pgl– as this strain is believed to be a UV-induced mutant rather than a natural phase variant (Helen Kieser, personal communication).

Data presented here show that the phase variation is at least partially due to expansion and contraction of a polyguanine tract present in the putative adenine-specific DNA methyltransferase gene, *pglX*. Furthermore, the *pglX* paralogue SC6G9.02, here renamed *pglS*, was shown to be able to interfere with the Pgl phenotype, suggesting that PglS may provide an alternative activity to that conferred by PglX.
TABLE 1. Pgl status and length of the G tract in pglX of strains used in this study

<table>
<thead>
<tr>
<th>S. coelicolor strain(s)</th>
<th>Pgl status</th>
<th>Length of G tract (bases)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A200</td>
<td>+</td>
<td>8</td>
<td>33</td>
</tr>
<tr>
<td>B135 Pgl−</td>
<td>pglW::IS1648 (class B)</td>
<td>8</td>
<td>18, 31</td>
</tr>
<tr>
<td>B140</td>
<td>+</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>B140 Pgl−</td>
<td>pglWX (class B)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>J1501</td>
<td>pglYZ defective (class A)</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>J1902</td>
<td>pglY and pdgY and/or pglZ (class AB)</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>J1903</td>
<td>pglX and pdgY or pglZ (class AB)</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>J1914</td>
<td>− (class B)</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>J1929</td>
<td>ΔpglY (class A)</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>J1973</td>
<td>pglX (class B)</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>JF3</td>
<td>+</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>JF3 Pgl−</td>
<td>−</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>JF4</td>
<td>+</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>JF4 Pgl−</td>
<td>pglYZ (class A)</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>M130</td>
<td>+</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>M145</td>
<td>+</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>M145 Pgl−</td>
<td>pglX (class B)</td>
<td>J. White</td>
<td>9</td>
</tr>
<tr>
<td>PglI</td>
<td>N600−</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td>N600− PglI</td>
<td>+</td>
<td>8</td>
<td>This study</td>
</tr>
<tr>
<td>N703</td>
<td>pglX (class B)</td>
<td>NAa</td>
<td>31</td>
</tr>
<tr>
<td>TK18</td>
<td>pglYZ (class A)</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>PglI</td>
<td>1147</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td>1190</td>
<td>+</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>1915</td>
<td>pglX (class B)</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>2147</td>
<td>pglY (class A)</td>
<td>8</td>
<td>20</td>
</tr>
</tbody>
</table>


b NA, not applicable.

MATERIALS AND METHODS

Strains, bacteriophages, and plasmids. The S. coelicolor strains used are listed in Table 1. Conjugal transfer from the nonmethylating Escherichia coli strain ET15256 (pUZ2002) dam ccm hsdM (21) was performed as described previously (17). E. coli DH5α was routinely used as the cloning host (26). High-titer stocks of the clear-plaque phage φC31cΔ25 (29) or the extended-host-range mutant φC31bhc (10) were prepared as previously described (17). Plaque tests were performed by overlaying 1 ml of soft YT that was seeded with approximately 5 × 10^10 spores onto a Difco nutrient agar plate containing 10 mM MgSO_4 and 8 mM Ca(NO_3)_2 spread with dilutions of phage (17). The antibiotic concentrations used in this study were isopropyl-β-D-thiogalactopyranoside (IPTG), kanamycin, ampicillin, streptomycin, and chloramphenicol. Carbenicillin (100 μg/ml) was routinely used as the cloning host (26). High-titer stocks of the clear-plaque phage φC31cΔ25 were obtained by phage transduction from E. coli DH5α (21). The addition of 4% DMSO to the soft YT agar plate was found to increase the number of plaques formed per ml of phage suspension. The DNA sequencing, based on the method of Sanger et al. (27), was performed by the Biopolymer Synthesis and Analysis Unit, University of Nottingham, with an ABI 373 DNA sequencer. Instructions to clean DNA fragments after enzymatic reactions or PCRs. A clean-up or nucleotide removal kits were used according to the manufacturer’s instructions to clean DNA fragments after enzymatic reactions or PCRs. A clean-up or nucleotide removal kits were used according to the manufacturer’s instructions to clean DNA fragments after enzymatic reactions or PCRs.

RESULTS

Identification of a phase variation mechanism for Pgl. By using the genome sequence of S. coelicolor (4) (available at www.sanger.ac.uk/Projects/S_coelicolor/), the coding and promoter regions of pglWX and pglYZ were analyzed for candidate sequences that might be the basis for the phase variation mechanism (e.g., inverted repeats or simple sequence repeats). Two possibilities were identified, an 8-base G tract within pglX which may be a target for slipped-strand mispairing during DNA replication (located 777 to 784 nucleotides from the start of the pglX open reading frame) and 14-bp inverted repeats within pglY which may cause phase variation by DNA inversion (located 1,019 and 1,394 nucleotides from the start of the pglY open reading frame). The region of pglY containing the inverted repeats was amplified by PCR, and the sequences of this region in four strains known to be complemented by the pglYZ locus (class A mutant strains J1501, J44 Pgl−, TK18 Pgl−, and 2147) and in two strains believed to have mutations in both the pglYZ and pglWX loci (class AB strains J1902 and J1903) were determined. In no case was the Pgl− phenotype attributable to DNA inversion between these inverted repeats (data not shown).

A similar strategy was used to determine whether Pgl− strains defective at the pglWX locus contained an expanded or contracted G tract within pglX. Eight different Pgl− strains were sequenced and were shown to contain the expected 8-base G tract (Table 1 and Fig. 1). However, the class B Pgl− strains, M145 Pgl− (kindly provided by J. White, John Innes Centre) and 1915 (and also its derivative J1973), contained 9-base G tracts (Fig. 1 and Table 1). In addition, the spontaneously arising double Pgl− mutants J1902 and J1903 had 7- and 9-base G tracts, respectively (Fig. 1 and Table 1). These data indicate that in the JIC strain collection there have been four apparently independent events of expansion or contraction of the G tract to generate a Pgl− phenotype (1915, J1902, J1903, and M145Pgl−).

Selection for Pgl phase variants from Pgl− to Pgl+. To observe phase variation in the Pgl− to Pgl− direction, spores of J1973, a Pgl− strain complemented by plasmids encoding pglX and containing a 9-base G tract, were plated onto nutrient agar containing divalent cations and a high titer (~10^10) of the clear-plaque phage φC31cΔ25 (29) to select for Pgl+ phase variants. Ten phage-resistant J1973 colonies (N600 to N609) were isolated, and the spores were amplified for use in plaque tests. To eliminate the possibility that the isolated colonies...
A contraction of the G tract in pglX confirmed that each isolate had indeed regained an 8-base G tract in the equivalent position to that in pglX. pglS does, however, have a run of seven G residues 487 bp downstream. While previously we showed that pglS is not required for the Pgl phenotype (31), we did not test whether PglS could interact with other Pgl components in a manner reminiscent of the interaction of variable HsdS proteins that interact with HsdR and HsdM proteins in the type I R-M systems (12, 28, 30). If PglS can indeed interact with the other Pgl proteins and thereby change the phase specificity of the resistance phenotype, when we overexpress pglS, we would expect a Pgl− phenotype with φC31 and enhanced resistance to a subset of other phages. Plasmid pPS8001 expresses pglS fused to an N-terminal six-His tag from the thiostrepton-inducible promoter (ptipA). After introduction of pPS8001 or pPS8003 (expressing His-tagged PglX from ptipA) into Pgl− strain M145 by conjugation, eight separate exconjugants were isolated from each conjugation, and spores were amplified for use in plaque tests. In the presence of 20 μg of thiostrepton per ml, M145 derivatives containing pPS8001 produced faint plaques, compared to no plaques obtained with M145 containing pPS8003 (Fig. 3). In a separate experiment pPS8001 did not confer a Pgl+ phenotype to pglX strain N703, whereas pPS8003 did (31; data not shown), indicating that PglX complements the pglX lesion but PglS does not. To explain the effect of PglS overexpression in M145, we propose that PglS is able to interact with other Pgl proteins, titrating them away from PglX and resulting in a weak Pgl− phenotype.

As an initial attempt to discover whether the overexpression of PglS in M145 resulted in increased resistance to a subset of phages other than φC31 and its homologs, the sensitivity of M145(pPS8001) to phages R4 and φHAU3 and a number of novel phages isolated from soil was tested. No change in the efficiency of plating or plaque morphology was observed (data not shown).

**DISCUSSION**

The phage growth limitation phenotype of *S. coelicolor* A3(2) has a paralogue of pglX, SC6G9.02 (here renamed pglS) which encodes a protein with 76% identical amino acids (Fig. 2). The level of DNA identity is also 76%, but it is noteworthy that pglS does not contain an 8-base G tract in the equivalent position to that in pglX. pglS does, however, have a run of seven G residues 487 bp downstream. While previously we showed that pglS is not required for the Pgl phenotype (31), we did not test whether PglS could interact with other Pgl components in a manner reminiscent of the interaction of variable HsdS proteins that interact with HsdR and HsdM proteins in the type I R-M systems (12, 28, 30). If PglS can indeed interact with the other Pgl proteins and thereby change the phase specificity of the resistance phenotype, when we overexpress pglS, we would expect a Pgl− phenotype with φC31 and enhanced resistance to a subset of other phages. Plasmid pPS8001 expresses pglS fused to an N-terminal six-His tag from the thiostrepton-inducible promoter (ptipA). After introduction of pPS8001 or pPS8003 (expressing His-tagged PglX from ptipA) into Pgl− strain M145 by conjugation, eight separate exconjugants were isolated from each conjugation, and spores were amplified for use in plaque tests. In the presence of 20 μg of thiostrepton per ml, M145 derivatives containing pPS8001 produced faint plaques, compared to no plaques obtained with M145 containing pPS8003 (Fig. 3). In a separate experiment pPS8001 did not confer a Pgl+ phenotype to pglX strain N703, whereas pPS8003 did (31; data not shown), indicating that PglX complements the pglX lesion but PglS does not. To explain the effect of PglS overexpression in M145, we propose that PglS is able to interact with other Pgl proteins, titrating them away from PglX and resulting in a weak Pgl− phenotype.

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**DISCUSSION**

The phage growth limitation phenotype of *S. coelicolor* A3(2) exhibits a high-frequency of phase variation from Pgl to Pgl and vice versa. We show here that Pgl phase variation can occur by expansion and contraction of a G tract located within pglX. Pgl+ strains have an 8-base G tract, whereas several Pgl− strains have either seven or nine G residues that produce truncated proteins consisting of 282 or 336 amino acids, and these should be compared to a nonframeshifted pglX protein consisting of 1,211 amino acids. The variation in the length of the G tract is likely to be due to slipped-strand mispairing during DNA replication and is a common mechanism for phase variation of cell surface components in pathogenic bacteria and of R-M systems (11, 12, 14). The frequency of the Pgl−-to-Pgl+ phase variation in 1915 was measured previously by Laity et al. and was found to be 3.9 × 10−4 Pgl+ CFU/spore (19). The fact that Pgl− strains B140 Pgl−, J1914, J1501, 2147, JF4 Pgl−, and TK18 Pgl− do not contain an expanded or contracted G tract suggests that there may be other mechanisms for phase variation. Indeed, Laity et al. (19) demonstrated that J1501, a Pgl− strain now known to contain
FIG. 2. Alignment of PglX and PglS. PglX and PglS were aligned to identify regions of similarity. A black background indicates identical amino acids, a grey background indicates functionally similar amino acids, and a white background indicates functionally dissimilar amino acids. The alignment was created by using the PileUp program from GCG. The region enclosed by a dashed box corresponds to the variable region in PglX and PglS and to the low-G+C-content region in the corresponding gene sequences. The S-adenosyl methionine binding site and the N6-adenine DNA methylase signature are indicated by one and two grey lines, respectively, above the amino acid sequence.
an 8-base G tract, could switch phase from Pgl− to Pgl+ with a high frequency (1.5 × 10−4 Pgl+ CFU/spore).

As the variable G tract is located in pglX, we studied the role of the pglX parologue pglS in the Pgl phenotype. Overexpression of PglS can confer a weak Pgl− phenotype (faint, cloudy plaques) in M145, a strain that is otherwise Pgl+. We propose that this effect is due to PglS outcompeting PglX for interaction with the other Pgl proteins. The PglX and PglS proteins are predicted to be DNA adenine methyltransferases. The current model for the mechanism of Pgl proposes that PglX modifies φC31 DNA so that during infection of the host with modified phage, the infection is aborted (31). If this were the case, the presence of a close homologue, pglS, in combination with the phase variation mechanism in pglX could potentially enable S. coelicolor to switch the specificity of the Pgl system to a different target. Our inability to detect enhanced resistance to several phages tested on M145 overexpressing pglS may simply have been because we did not test enough phages. Currently, there is no information on the transcription of pglS or regulation of its gene product, both of which also need to be considered in any switch mechanism.

A curious observation is that while PglS interferes with the activity of Pgl proteins in M145, higher levels of PglS apparently have the opposite effect on the Pgl phenotype of J1501. Introduction of pglS into J1501 on a low-copy-number, replicating vector has previously been shown to reduce the efficiency of plating of φC31cJ phage (2). It is not known how significant this is considering that of the many strains tested, J1501 was the only strain that showed this effect with the pglS-encoding construct. Another observation with strain J1501 is that its pglYZ genes appear to be only partially active, so that increasing the copy number confers a weak Pgl− phenotype to J1501 and the ΔpglY mutant J1929 (2). One possible explanation for the behavior of J1501 is that this strain is less able to assemble stable, active Pgl complexes and extra copies of the PglY and PglZ or PglS proteins could rectify this defect, either directly through the proposed activity of PglY or via provision of additional scaffolds, as could be the case with PglS.

Analysis of the codon usage of pglWYZ showed that for the entire length, the G+C content is close to that observed for the average Streptomyces gene (72.1%) (4). In contrast, pglX and pglS both have much lower G+C contents (63.5 and 62.9%, respectively), particularly in the central regions. Moreover, alignment of the amino acid sequences of PglX and PglS showed that the proteins are virtually identical at their termini but diverge greatly in the middle (Fig. 2). The divergence in the central region was confirmed by alignment of the DNA sequences, indicating that sequencing errors do not provide a trivial explanation (data not shown). A significant change in base content compared to the overall average content is often an indication that the DNA has been horizontally acquired, and we speculate that pglX and pglS are mosaics. The differences in the activities of PglX and PglS could conceivably be localized to these variable regions.

Recently, homologues of the pgl genes have emerged in the microbial genome databases. A cluster encoding pglWYZ-like genes is present in the preliminary genome sequence of the thermophilic actinomycete Thermohibidula fusca (http://www.jgi.doe.gov/JGI_microbial/html/index.html). There are, in addition, two putative DNA methyltransferase genes homologous to pglX and pglS, and one of these has a low-G+C-content central region like pglX and pglS. However, alignment of the T. fusca PglX and PglS homologues did not reveal a highly variable region like that seen with PglX and PglS. Bacteriophages have been reported for T. fusca, but there is insufficient information to determine if there is a Pgl phenotype (25). More distant relatives are found in the Nostoc punctiforma genome, which has a cluster encoding pglZ, pglXS, and pglY-like genes and another homologue of pglX/S at a distal location (http://www.jgi.doe.gov/JGI_microbial/html/nostoc/nostoc_homepage.html).

In summary, we describe a mechanism that at least partially explains the high-frequency phase variation in the Pgl system in S. coelicolor A3(2). A search of the S. coelicolor genome sequence for additional G tracts indicated that there are 28 8-base G tracts, eight loci with 9-base G tracts, one locus with 10 bases, and one locus with 12 bases (4). Only four of these loci are intragenic, lying within pglX, SCO1006, SCO5902, and SCO6313. SCO5902 is annotated as a transposase pseudogene, while the functions of SCO1006 and SCO6313 are not known. In general, it appears that G tracts are therefore avoided within gene sequences in Streptomyces and expansion and contraction of the G tract in pglX are likely to have been selected. The ability of PglS to interact with the Pgl system implies that it may be able to provide an activity that is an alternative to PglX activity, adding to the versatility of this unusual phase resistance phenotype.

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REFERENCES


