Although the multiplication of bacteriophages (phages) has a substantial impact on the biosphere, comparatively little is known about how the external environment affects phage production. Here we report that sub-lethal concentrations of certain antibiotics can substantially stimulate the host bacterial cell’s production of some virulent phage. For example, a low dosage of cefotaxime, a cephalosporin, increased an uropathogenic *Escherichia coli* strain’s production of the phage \( \phi \) MFP by more than 7-fold. We name this phenomenon Phage-Antibiotic Synergy (PAS). A related effect was observed in diverse host-phage systems, including the T4-like phages, with \( \beta \)-lactam and quinolone antibiotics, as well as mitomycin C. A common characteristic of these antibiotics is that they inhibit bacterial cell division and trigger the SOS system. We therefore examined the PAS effect within the context of the bacterial SOS and filamentation responses. We found that the PAS effect appears SOS-independent and is primarily a consequence of cellular filamentation; it is mimicked by cells that constitutively filament. The fact that completely unrelated phages manifest this phenomenon suggests that it confers an important and general advantage to the phages.

**RESULTS AND DISCUSSION**

To determine the antibiotic sensitivity of the bacterium causing a urinary tract infection in a hospitalized child, a Petri plate was inoculated with a dilution of the patient’s urine and a series of antibiotic disks were placed on the surface of the agar. As suspected, the urine was contaminated with an uropathogenic *Escherichia coli* strain, but also with phages that infected it. Remarkably, with some of the antibiotics, the phage plaques were significantly larger in zones circumscribing the disks where there was a sub-lethal concentration of the drug. This effect was observed with disks containing the \( \beta \)-lactam antibiotics (aztreonam, monobactam, and cefixime, a cephalosporin), but not with antibiotics of other classes (tetracycline and gentamicin). Thus, a sub-lethal dosage of \( \beta \)-lactam antibiotics appeared to stimulate phage growth in this host-phage system. To further characterize this curious phenomenon, both a single a colony of the uropathogenic *E. coli* isolate (MFP) and a single phage plaque (\( \phi \) MFP) were isolated from the urine sample. The synergistic effect was highly reproducible with these purified isolates (Figure 1) and was not the result of induction of lysogens (no appearance of phage on control plate); we called this effect PAS (Phage Antibiotic Synergy). Other cephalosporins (cefotaxime, ceftriaxone, and cefazidime) produced a similar effect on \( \phi \) MFP growth in the *E. coli* MFP strain. Since this host strain is resistant to many types of penicillins (e.g. piperacillin, ticarcillin and amoxicillin), these drugs in the \( \beta \)-lactam family could not be tested.

Electron microscopy of the phage \( \phi \) MFP revealed morphology typical of a siphovirus [5]: a long, flexible, non-contractile tail structure and an isometric, icosahedral head of ~60 nm (data not shown). DNA sequencing of 25 random 500 bp segments of the \( \phi \) MFP genome indicated that it is related to the *Salmonella typhimurium* phage MB78 [6] (data not shown).

Since the PAS effect was only detectable in the zone adjacent to the disk where the drug was not completely inhibiting bacterial growth, we determined the drug concentration in Luria-Bertani (LB) agar that gave the optimal PAS effect. These plates were overlaid with soft LB agar containing the MFP indicator strain, a fixed quantity of the \( \phi \) MFP phage and various concentrations of the cephalosporin cefotaxime. Up to a concentration of 50 ng/mL, the PAS response (as assayed by plaque diameter) increased with the dose. As shown in Figure 2, this phage made quite small plaques with no cefotaxime and remarkably large ones with the...
Plaque size is largely determined by two properties of the phage infected cell: the phage burst size (the number of virions produced by each infected cell) and the time it takes for the phage to lyse an infected cell. To determine if the cefotaxime-mediated increase in plaque size was due directly to an increase in phage burst size, we compared phage ΦMFP production when the MFP host cells were grown in a liquid culture either with or without 20 ng/mL of cefotaxime. The data in Figure 3 show that total phage production in a single cycle of growth was greater in the presence of the cephalosporin. This difference was detectable throughout the latent period and was about 7-fold when the two cultures lysed. However, the lysis of the antibiotic-treated infected culture was also clearly affected, occurring more rapidly in the presence of the drug (data not shown). It should be recalled that some mutant phages lyses more rapidly and also make significantly larger plaques than their wild type ancestor [7].

A search of the phage literature revealed that a possibly related observation had been made in the classic T4 phage system. In 1969, Yamagami and Endo [8] observed that UV irradiation, mitomycin C and a variety of other chemical treatments that cause E. coli B to form filaments resulted in T4 plaques that were larger than normal. To determine if the PAS phenomenon also occurred in the T4-type phages, we identified two phages, RB32 and RB33 [9]. In the Toulouse collection that grow well on E. coli B, was chosen for further studies of the PAS effect because the strain AS19, an antibiotic permeability mutant [10] of E. coli, has long been known to cause an inhibition of bacterial cell division in response to DNA damage. Interestingly in the context of our results, it has recently been demonstrated that the inactivation of optimal concentration, 50 ng/mL. Concentrations of cefotaxime above 100 ng/mL inhibited the growth of the MFP bacterial strain to a level that made the analysis phage plaque size impossible.

The PAS response appears to be explained by a simple mechanism. There is a striking effect on cell morphology when sensitive strains of E. coli grow in the presence of even very low levels of β-lactam antibiotics. The mode of action of these antibiotics is to block cell division [3] and, even with the sub-lethal levels of β-lactams also acts by inhibiting bacterial cell division, but by a different mechanism [12]; as do mitomycin C [13] and UV irradiation [14]. The SOS response in E. coli has long been known to cause an inhibition of bacterial cell division in response to DNA damage. Interestingly in the context of our results, it has recently been demonstrated that the inactivation of

Figure 2. Plaque sizes of phage ΦMFP on E. coli MFP with and without 50 ng/mL of cefotaxime (CTX) in Luria agar plates.

doi:10.1371/journal.pone.0000799.g002
the βl gene product, the penicillin binding protein III, by β-lactam antibiotics also induces the expression of the SOS system via a two-component signal transduction system [15]. Among the numerous genes in the SOS regulon is the sulA (βlA) gene that encodes an inhibitor of cell division. Thus, the bacterial SOS response to β-lactam antibiotics results in filamentation that allows the bacteria to reduce the lethal effects of the drugs [15].

Obvious questions arise concerning a role of the bacterial SOS response, and filamentation in general, in the PAS phenomenon. To address these questions we employed a series of E. coli mutant hosts, that were either defective in the SOS pathway or had an altered filamentation response (constitutive filamentation), and determined if they gave the PAS effect or not (Fig. 5). These results indicate that the PAS effect is SOS pathway-independent as larger plaques around the antibiotic halos were clearly present in the sulA mutant, as well as in a lexA mutant which cannot de-repress the SOS pathway genes (and hence should have no SOS response). A recA mutant, defective for a major component of the SOS system, showed a positive, but slightly dimished, PAS response (data not shown). Thus, the SOS system does not seem to play a primary role in the PAS response, it could nevertheless still play some minor supporting role. However, the primarily SOS-independent nature of the PAS effect may not be surprising given that filamentation can still be induced by antibiotics in an SOS-independent manner [16]. Turning to filamentation itself—if the PAS effect is primarily due to the physiological changes in filamenting cells, a mutant E. coli strain that is able to filament without the activity of antibiotics should have a similar response. That is exactly what is observed (Fig. 5) in an ftsZ mutant which filaments due to its defective cell division apparatus. Phage plaques on this mutant are uniformly bigger than the isogenic control strain throughout the host lawn with only a very modest increase in size near the zone of antibiotic activity.

The phenotype of filamentation therefore seems to play a significant role in the PAS effect, probably through multiple pathways. The altered physiological state of filamenting cells obviously permits much faster phage assembly, possibly by making larger/altered pools of precursors available and/or remedying certain rate-limiting steps in assembly. We suggest that the antibiotics also accelerate the timing of cell lysis given that

Figure 3. Increase in phage titer in the presence of the cephalosporin cefotaxime (CTX). E. coli strain MFP was infected with phage ΦMFP in Luria liquid medium supplemented at the time of infection with 20 ng/mL of cefotaxime, or left untreated. The multiplicity of infection was 5. Chloroform was added at various times after infection to lyse the infected cells.
doi:10.1371/journal.pone.0000799.g003

Figure 4. Plaque sizes of various coliphages with and without cefotaxime (CTX) in Luria agar plates. Phages RB32 and RB33 were grown on E. coli strain MFP (50 ng/mL CTX) at 37 °C; and T4, T3 and T7 were grown on E. coli strain AS19 (30 ng/mL CTX) at 25 °C. All plaques were photographed at identical magnifications.
doi:10.1371/journal.pone.0000799.g004

Figure 5. The PAS effect of phage T4 on various E. coli SOS and filamentation mutant strains. T4 was grown on E. coli sulA-inactivated and lexA non-inducible mutant strains (defective SOS systems), as well as an ftsZ-inactivated mutant strain (non-antibiotic induced filamentation), in the presence of disks of cefotaxime (CTX). Isogenic wild-type strains (wt) are also included and representative plaques demonstrating the PAS effect are indicated by red arrows. All plates were photographed at identical magnifications.
doi:10.1371/journal.pone.0000799.g005
filamentation induces perturbations in the peptidoglycan layer and this probably causes a greater sensitivity to the action of phage lysis genes (e.g. lysozymes, holins). The interplay of these two effects, faster lysis and increased rate of phage production, cause the PAS effect and it is not surprising that different phage-host systems, with their different developmental programs and molecular mechanisms of lysis, respond slightly differently. For example, with their different developmental programs and molecular faster lysis and increased rate of phage production, cause the PAS genes (e.g. lysozymes, holins). The interplay of these two effects, filamentation induces perturbations in the peptidoglycan layer and cause the PAS has on the microbial ecosystem. With respect to the clinical population balances in the microfauna of biofilms, for example, antibiotic-producing cells could play a role in determining bacterial cells producing the antibiotics and PAS-enabled phages to situations can be viewed as a mutualism between the fungal or burst of phage out of them before they do so is to the advantage of since the host cells are likely to die anyway and getting a last, quick situation can be viewed as a mutualism between the fungal or phage life-cycles, the ability to successfully adapt to a “sub-optimal” environment for bacterial growth. The presence in the environment of low levels of antibiotics that interfere with cell wall synthesis represents a major threat to bacteria and it was to be expected that they would develop strategies to minimize the consequences of such agents. The SOS system induction of filamentation can reduce the danger posed by such compounds [15] and is thus evolutionarily advantageous. Phage of both the temperate (induction of lysogens [17]) and virulent (PAS-enabled) types have adapted to take advantage of the altered physiology of such stressed cells and can propagate adventitiously in them, producing more progeny than they would in “healthier” situations. This makes sense from an evolutionary perspective since the host cells are likely to die anyway and getting a last, quick burst of phage out of them before they do so is to the advantage of the phage. From an ecological perspective, this seemingly bizarre situation can be viewed as a mutualism between the fungal or bacterial cells producing the antibiotics and PAS-enabled phages to more efficiently compete with antibiotic- and phage-sensitive bacterial cells. This synergistic interaction between phages and antibiotic-producing cells could play a role in determining population balances in the microfauna of biofilms, for example. Clearly, more investigation will be required to assess the impact that PAS has on the microbial ecosystem. With respect to the clinical setting, there is perhaps a lesson to be learned here from nature–if your objective is to kill bacteria, then giving them a tad of β-lactam antibiotics combined with a dash of phage may be better at accomplishing this task than either treatment individually.

**REFERENCES**

1. Karam J, ed (1994) Molecular biology of bacteriophage T4. Washington DC: American Society for Microbiology Press. pp 633.

2. Oppenheim AB, Kobiler O, Stavans J, Court DL, Adhya S (2005) Switches in bacteriophage lambda development. Annu Rev Genet 39: 409–429.

3. Gottfredson M, Erlenkötter H, Sigfússon A, Gudmundsson S (1998) Characteristics and dynamics of bacterial populations during postantibiotic effect determined by flow cytometry. Antimicrob Agents Chemother 42: 1005–1011.

4. Piddock LJ, Walters RN, Diver JM (1990) Correlation of quinolone MIC and inhibition of DNA, RNA, and protein synthesis and induction of the SOS response in Escherichia coli. Antimicrob Agents Chemother 34: 2331–2336.

5. Ackermann H-W (2003) Bacteriophage observations and evolution. Res Microbiol 154: 245–251.

6. Joshi A, Siddiqui JZ, Rao GRK, Chakravorty M (1982) MB78, a virulent bacteriophage of Salmonella typhimurium. J Virol 41: 1035–1043.

7. Follmann P, Aholou ST, Dressman HK, Gallrath K, Tracey J, et al. (1998) The role of the bacteriophage T4 t r genes in lysis inhibition and fine-structure genetics: A new perspective. Genetics 148: 1539–1550.

8. Yamagami H, Endo H (1969) Loss of lysis inhibition in filamentous Escherichia coli infected with wild-type bacteriophage T4. J Virol 3: 343–349.

9. Mono G, Roppola F, Kuttadathere M, Ttanta F, Krüger HM (1997) The genome of the T-even bacteriophages, a diverse group that resembles T4. J Mol Biol 267: 237–249.

10. Seliguchi M, Iida S (1967) Mutants of Escherichia coli permeable to acacinonin. Proc Natl Acad Sci USA 58: 2313–2320.

11. Dressman HK, Drake JW (1999) Lysis and lysis inhibition in bacteriophage T4: F mutations reside in the holin t gene. J Bacteriol 181: 4391–4396.

12. Andersson M, MacGowan AP (2003) Development of the quinolones. J Antimicrob Chemother 51, Suppl S1: 1–11.

13. Suzuki H, Kilgore WW (1967) Effects of mitomycin C on macromolecular synthesis in Escherichia coli. Proc Natl Acad Sci USA 58: 2313–2320.

14. Burton P, Holland IB (1983) Two pathways of division inhibition in UV-irradiated E. coli. Mol Gen Genet 190: 309–314.

15. Miller C, Thomsen LE, Gaggero G, Mosseri R, Ingmer H, et al. (2004) SOS response induction by β-lactams and bacterial defense against antibiotic lethality. Science 305: 1629–1631.

16. Liu G, Begg K, Geddes A, Donachie WD (2001) Transcription of essential cell synthesis in Escherichia coli. Mol Microbiol 40: 909–916.

17. Geerse C, Koller J, Wetz C (2006) Ciprofloxacin and trimethoprim cause phage induction and virulence modulation in Staphylococcus aureus. Antimicrob Agents Chemother 50: 171–177.