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Engineering Synthetic Bacteriophage to Combat Antibiotic-Resistant Bacteria

T.K. Lu^{*†} and J.J. Collins[†]

^{*}Harvard-MIT Division of Health Sciences and Technology, 77 Massachusetts Ave., E25-519, Cambridge, MA 02139

[†]Howard Hughes Medical Institute, Center for BioDynamics and Department of Biomedical Engineering, Boston University, 44 Cummington Street, Boston, MA 02215

Abstract—Antibiotic resistance is a rapidly evolving problem that is not being adequately met by new antimicrobial drugs. Thus, there is a pressing need for effective antibacterial therapies that can be adapted against antibiotic-resistant bacteria. Here, we engineered synthetic bacteriophage to combat antibiotic-resistant bacteria by overexpressing proteins and attacking gene networks which are not directly targeted by antibiotics. By suppressing the SOS network, our engineered phage enhance bacterial killing by quinolone antibiotics by several orders of magnitude *in vitro* and significantly increase the survival of infected mice *in vivo*. Our synthetic phage design can be extended to target non-SOS gene networks and overexpress multiple factors to produce additional effective antibiotic adjuvants. In addition, our synthetic phage act as strong adjuvants for other bactericidal antibiotics, improve the killing of antibiotic-resistant bacteria, and reduce the number of antibiotic-resistant bacteria that arise from antibiotic-treated populations. This work establishes a novel synthetic biology platform for translating identified targets into effective antibiotic adjuvants.

I. INTRODUCTION

Bacterial infections are responsible for significant morbidity and mortality in clinical settings [1]. Dramatic increases in antibiotic-resistant infections are responsible for sicker patients, longer hospitalizations, and increased costs [1]. New classes of antimicrobials are needed, but few are in pharmaceutical pipelines [1]. High-throughput systems biology studies have enabled the discovery of many potential drug targets [2, 3], but a significant amount of additional work and investment is needed to translate these targets into actual drugs. Moreover, antibiotic drugs typically do not take advantage of targets that need to be upregulated in order to achieve antimicrobial activity. Thus, there is a significant gap between target identification and drug development.

Natural phage have been used to kill bacteria since the early 20th century [4]. We engineered antibiotic-adjuvant phage to overexpress proteins to target non-essential gene networks that are not directly attacked by antibiotics in order to minimize evolutionary pressures. By using a combination of engineered phage and antibiotics, we reduce the evolution of antibiotic resistance and enhance bacterial killing [5-8].

II. TARGETING THE SOS DNA REPAIR SYSTEM

Bactericidal antibiotics (e.g., quinolones such as ofloxacin) induce the formation of hydroxyl radicals which cause DNA,

protein, and lipid damage, and ultimately, cell death [2]. DNA damage initiates the SOS response [9], which results in DNA repair and cell survival (Fig. 1A). To suppress the SOS network and enhance the effect of bactericidal antibiotics, we engineered M13mp18 phage to overexpress *lexA3*, a repressor of SOS [10]; we named this phage ϕ_{lexA3} (Fig. 1A) and the unmodified M13mp18 phage ϕ_{unmod} . M13mp18, a modified version of M13 phage, is a non-lytic filamentous phage and can accommodate DNA insertions into its genome [11].

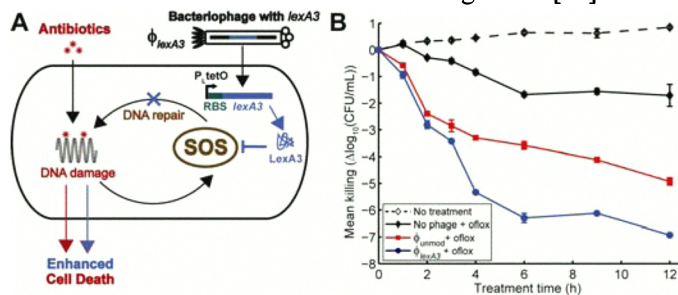


Fig. 1. Engineered ϕ_{lexA3} phage enhances killing of wild-type *E. coli* EMG2 bacteria by bactericidal antibiotics. (A) Engineered phage, with the *lexA3* gene (ϕ_{lexA3}) under the control of a promoter (P_{tetO}) and a ribosome-binding sequence (RBS) [12], act as antibiotic adjuvants by suppressing SOS and increasing cell death. (B) Killing curves for no phage (black diamonds), unmodified phage ϕ_{unmod} (red squares), and engineered phage ϕ_{lexA3} (blue circles) with 60 ng/mL ofloxacin [oflox] (solid lines, closed symbols). 10^8 plaque-forming-units/mL (PFU/mL) phage was used. A growth curve for EMG2 with no treatment is also shown (dotted line, open symbols). ϕ_{lexA3} greatly enhanced killing by ofloxacin by 4 hours of treatment.

To test ϕ_{lexA3} 's antibiotic-enhancing effect, we obtained time courses for killing of *E. coli* EMG2 bacteria with phage and/or ofloxacin treatment. We calculated viable cell counts by counting colony-forming units (CFUs) during treatment (Fig. 1B). After 6 hours, bacteria exposed to ofloxacin only were reduced by about 1.7 \log_{10} (CFU/mL), reflecting the presence of persister cells not killed by the drug (Fig. 1B). By 6 hours, ϕ_{lexA3} improved ofloxacin's bactericidal effect by 2.7 orders of magnitude compared to unmodified phage ϕ_{unmod} (~99.8% additional killing) and by over 4.5 orders of magnitude compared to no phage (~99.998% additional killing) (Fig. 1B). With combination phage and antibiotic treatment, no significant bacterial regrowth was apparent (Fig. 1B).

We also tested our synthetic phage along with other classes of bactericidal antibiotics, including gentamicin, an aminoglycoside, and ampicillin, a β -lactam. ϕ_{lexA3} increased

gentamicin's bactericidal action by over 2.5 and 3 orders of magnitude compared with ϕ_{unmod} and no phage, respectively. ϕ_{lexA3} improved ampicillin's bactericidal effect by over 2 and 5.5 orders of magnitude compared with ϕ_{unmod} and no phage, respectively. For both gentamicin and ampicillin, ϕ_{lexA3} 's strong adjuvant effect was noticeable after 1 hour of treatment. These results show that engineered phage can act as adjuvants for the three major classes of bactericidal drugs. Using this design, we have also created synthetic phage that target non-SOS genetic networks and/or overexpress multiple factors and have shown that they act as effective antibiotic adjuvants [13].

III. KILLING ANTIBIOTIC-RESISTANT BACTERIA

In addition to enhancing the killing of wild-type bacteria, engineered phage can improve the killing of bacteria that have already acquired antibiotic resistance and may therefore have the potential to revive defunct antibiotics. We applied ϕ_{lexA3} with ofloxacin against *E. coli* RFS289, which carries a *gyrA111* mutation that renders it resistant to quinolones. ϕ_{lexA3} increased bacterial killing by ofloxacin by over 2 and 3.5 orders of magnitude compared with ϕ_{unmod} and no phage, respectively. Furthermore, we have shown that synthetic phage significantly reduce the number of antibiotic-resistant bacteria that evolve from antibiotic-treated populations [13].

IV. RESCUING MICE INFECTED WITH BACTERIA

To demonstrate the clinical efficacy of antibiotic-enhancing phage *in vivo*, we applied our engineered phage with ofloxacin to prevent death in mice infected with bacteria (Fig. 2). Mice were injected with *E. coli* intraperitoneally 1 hour prior to receiving intravenous treatments (Fig. 2A). Eighty percent of mice treated with ϕ_{lexA3} plus ofloxacin survived, compared with 50% and 20% for mice that received ϕ_{unmod} plus ofloxacin or ofloxacin alone, respectively (Fig. 2B).

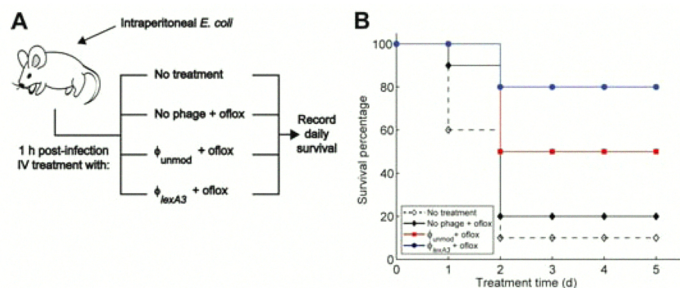


Fig. 2 Engineered ϕ_{lexA3} phage increases survival of mice infected with bacteria. (A) Female Charles River CD-1 mice were inoculated with 8.8×10^7 CFU/mouse *E. coli* EMG2. Treatments included 0.2 mg/kg ofloxacin and/or 10^9 PFU/mouse phage as indicated. Deaths were recorded at the end of each day. The mouse drawing was used under a Creative Commons Attribution 2.5 License [14]. (B) Survival curves for infected mice show that engineered phage ϕ_{lexA3} plus ofloxacin (solid line, closed blue circles) significantly increases survival compared with unmodified phage ϕ_{unmod} plus ofloxacin (solid line, closed red squares), no phage plus ofloxacin (solid line, closed black diamonds), and no treatment (dashed line, open black diamonds).

V. CONCLUSIONS

Our phage platform enables the development of effective antibiotic adjuvants and is a practical example of how synthetic biology and systems biology can be applied to

solve important real-world biomedical problems. Synthetic biology is focused on the rational and modular engineering of organisms to create novel behaviors. Synthetic phage are well-suited for incorporating targets, identified via systems biology, in a modular fashion to create effective antibiotic adjuvants. With current DNA sequencing and synthesis technologies, an entire engineered phage genome carrying multiple constructs that target different gene networks could be synthesized for less than \$10,000 [15]. These ever-improving technologies will enable large-scale modifications of phage libraries to produce antibiotic-adjuvant phage that target different gene networks and that can be applied with different antibiotic drugs against a wide range of bacteria.

Our *in vitro* and *in vivo* work demonstrates that synthetic phage can be engineered to act as effective antibiotic adjuvants and may help close the gap between antimicrobial target identification and implementation. Thus, combination therapy which couples antibiotics with engineered antibiotic-adjuvant phage is a promising antimicrobial strategy for the future.

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