Boosting Bacterial Metabolism to Combat Antibiotic Resistance

Prerna Bhargava[1,2,3] **and James J. Collins**[1,2,3,*]
[1]Institute for Medical Engineering & Science, Department of Biological Engineering, and Synthetic Biology Center, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[2]Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA
[3]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA
*Correspondence: jimjc@mit.edu

The metabolic state of a bacterial cell influences its susceptibility to antibiotics. In this issue, Peng et al. (2015) show that resistant bacteria can be sensitized to antibiotic treatment through the addition of exogenous metabolites that stimulate central metabolic pathways and increase drug uptake.

The role of metabolism in the bacterial response to antibiotics has recently garnered interest because of a rapid rise in antibiotic resistance, a clear link between metabolic function and cell viability, and the lack of novel targets for hard-to-treat infections. A large body of evidence suggests that bacterial metabolism is closely tied to antibiotic susceptibility. Bacteria with reduced metabolism are resistant or tolerant to many classes of antibiotics, while increased drug sensitivity is linked to enhanced metabolism (Allison et al., 2011; Bryan and Van Den Elzen, 1977; Kohanski et al., 2007; Martínez and Rojo, 2011). Little is known, however, about the metabolic profiles of genetically resistant bacterial populations. Peng et al. address this topic in this issue of *Cell Metabolism*.

To investigate the metabolic state of resistant bacteria, Peng et al. (2015) evolved *Edwardsiella tarda* (*E. tarda*), an opportunistic pathogen in humans and fish, against the aminoglycoside antibiotic, kanamycin. Resistant *E. tarda* strains had altered metabolic profiles, with defects observed in central metabolic pathways (Figure 1A). Resistant strains exhibited the greatest deficiencies in glucose and alanine abundances. These findings are consistent with previous work in resistant strains of *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* (Alonso et al., 2004; Stickland et al., 2010), which were found to exhibit defects in central metabolism, specifically glucose and amino acid metabolism.

It is generally accepted that the acquisition of genetic resistance determinants results in a metabolic cost for bacteria, such that susceptible bacteria will effi-ciently outcompete resistant bacteria in the absence of selection pressures (Lázár et al., 2014). This notion is rooted in the fact that constantly replicating large plasmids containing resistance genes, or producing enzymes that inactivate antibiotics, would lead to a large metabolic burden. Several mechanisms of known resistance have been documented, ranging from drug-degrading enzymes to efflux pumps. These mechanisms afford different levels of protection for a population of bacteria, and the metabolic impact of each of these resistance mechanisms varies depending on their fitness cost (Martínez and Rojo, 2011). The bacterial strains studied by Peng et al. were evolved in vitro under the selection pressure of kanamycin. However, the authors did not sequence the strains to identify the mutations and underlying resistance mechanisms that arose. Therefore, it remains unclear if the metabolic deficiencies observed were specific to the evolved mechanism of resistance or whether they can be generalized across resistant strains that are mechanistically distinct.

Given the metabolic deficiencies observed in resistant strains of *E. tarda*, Peng and co-authors hypothesized that addition of the deficient metabolites could increase the susceptibility of the resistant bacteria to antibiotic treatment. Challenging kanamycin-resistant *E. tarda* with alanine and/or glucose plus kanamycin indeed sensitized the bacteria to the antibiotic. Additionally, cells treated with alanine and glucose had higher intracellular levels of kanamycin. This enhancement in drug uptake was shown to be a result of increased proton motive force, due to greater flux through the tricarboxylic acid (TCA) cycle (Figure 1B). This work indicates that metabolite-enabled increases in central metabolic flux can enhance drug uptake and bacterial killing in resistant bacteria.

Alanine and glucose supplementation was also able to increase aminoglycoside-induced killing in lab-evolved beta-lactam-resistant, quinolone-resistant, and tetracycline-resistant strains of *E. tarda*. The lethal effects of aminoglycoside treatment were also enhanced with the addition of these metabolites in several other human pathogens, including *Klebsiella pneumoniae*, methicillin-resistant *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Biofilms and persisters of clinical pathogenic isolates of *E. tarda* were also successfully targeted with the same approach. This is consistent with previous work by Allison et al., which showed that addition of exogenous metabolites (e.g., glucose, mannitol, fructose) could stimulate central metabolic pathways and enable aminoglycosides to eradicate *Escherichia coli* and *Staphylococcus aureus* persisters and biofilms (Allison et al., 2011). Together, these works show that metabolic stimuli can boost the bactericidal effects of aminoglycosides across many species in different physiological states.

The addition of exogenous metabolites to restore metabolic deficiencies offers an attractive approach to treat drug-resistant pathogens in combination with antibiotics that would otherwise be ineffective (Murima et al., 2014). It is important to note, however, that the metabolic burden on a resistant pathogen is highly dependent on the bacterial microenvironment and the metabolic adaptations required for
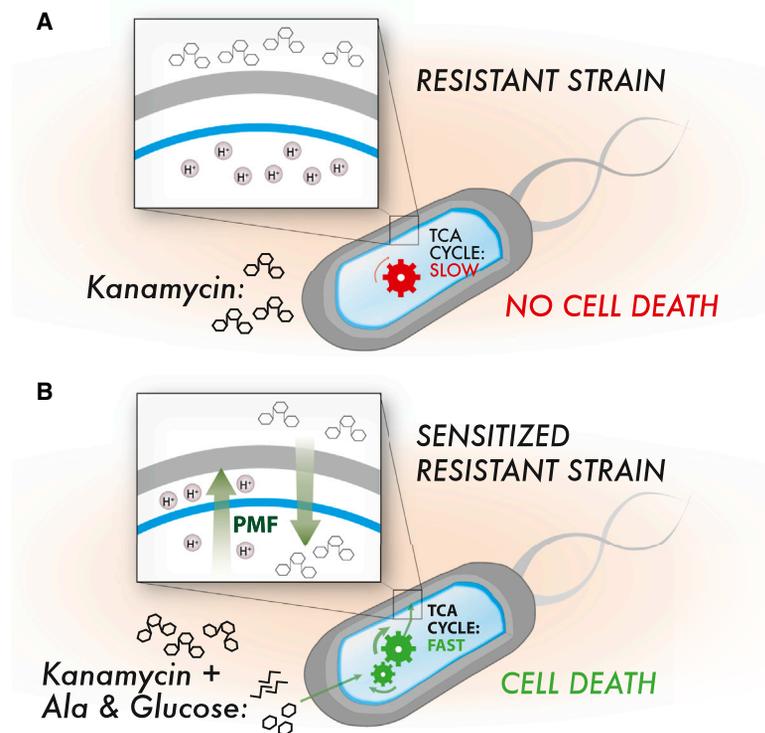
Figure 1. Exogenous Addition of Metabolites Enhances Antibiotic Uptake, Leading to Cell Death

(A) Antibiotic-resistant cells have lower metabolite abundances, leading to reduced PMF, inhibited drug uptake, and survival.

(B) Exogenous addition of alanine and/or glucose can enhance drug uptake and restore cell sensitivity.

colonizing such a habitat. The work by Peng et al. raises many questions about the role of environmental metabolic signals in the efficacy of antibiotics. Bacteria generate a large number of metabolites, many of which have unknown or incompletely understood biological functions, and metabolites produced by one class of bacteria can influence the antibiotic susceptibility of neighboring bacteria within a niche (Vega et al., 2013). Further studies are needed to understand more completely the role of metabolites in in vivo infection microenvironments. Along these lines, little is known about the role of host metabolites in the regulation of antibiotic efficacy. Interestingly,

severe and recurrent infections tend to manifest more frequently within hosts that are immunocompromised, suggesting that the host environment, including the metabolome, could significantly influence the rate of infection, the efficacy of antibiotics, and the generation of resistance. Expanding on these studies will provide insights into how bacterial and host metabolism can influence antibiotic efficacy, potentially leading to personalized infection control strategies based on a patient's metabolic state.

Investigating the relationships between bacterial metabolism and antibiotic sensitivity can help to uncover novel strategies for treating infections. The report by Peng

et al. highlights the significance of the metabolic environment in antibiotic resistance and treatment strategies (Peng et al., 2015). It will be important to build upon this work and examine how the metabolic state varies with different resistance mechanisms and across different environmental conditions. Further studies may allow us to develop generalized metabolic therapeutics as co-treatments for already-prescribed antibiotics, thereby expanding a rapidly shrinking arsenal of effective therapies against resistant and persistent infections.

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