Clarifying the Role of Two-Component Regulation in Antibiotic Killing

Colin Manoil

Department of Genome Sciences, University of Washington, Seattle, Washington, USA

Understanding how antibiotics kill bacteria is of vital importance because it may identify new therapies that enhance the efficacy of established drugs. The most provocative work of the last decade in this area was a systems-level analysis of *Escherichia coli* that suggested that bactericidal antibiotics with different targets act through a common pathway involving reactive-oxygen-based killing (1, 2).

The three classes of antibiotics examined had previously been thought to act by fully independent mechanisms—aminoglycosides through targeting protein synthesis, beta-lactams on cell wall assembly, and fluoroquinolones on DNA replication. The common pathway model postulated that after acting on their primary targets, the three antibiotics activate envelope stress and redox sensing two-component responses mediated by CpxRA and ArcAB to set off a cascade of events culminating in hydroxyl radical formation and cell death. More-recent studies suggest that hydroxyurea, a ribonucleotide reductase inhibitor, may kill cells by related mechanisms (3).

The common pathway model incorporated data from genome-scale expression profiling and mutant screening and linked diverse physiological processes associated with antibiotic action. The strengths of this systems-level approach are that it can bypass the traditional step-by-step process of hypothesis generation and testing and identify previously unsuspected mechanisms or associations. However, if unaccompanied by focused validation studies, the approach has more limited ability to rigorously establish cause-and-effect relationships or to eliminate alternative explanations for systems-level observations (4).

In the current issue of the *Journal of Bacteriology*, Mahoney and Silhavy (5) report a thorough genetic analysis of a fundamental element of the common pathway model, the action of the Cpx two-component response. Their work shows that rather than promote killing as postulated in the model, activation of CpxRA actually helps bacteria resist aminoglycoside and hydroxyurea lethality. The work thus contradicts a counterintuitive part of the common pathway model in which a normally protective stress response was proposed to play an opposite role.

The Mahoney and Silhavy study (5) first reproduced the key previous observation that deletions of the Cpx sensor gene (*cpxA*) made bacteria more resistant to aminoglycosides and hydroxyurea, a result that had been interpreted as indicating that activation of the Cpx response contributes to killing (2, 3). However, two-component sensors like CpxA have inhibitory (phosphatase) activities as well as activator (kinase) activities acting on cognate response regulators (CpxR in this case) (6). Accordingly, deletions of sensor genes can either eliminate or activate the corresponding responses.

To distinguish between these alternatives, Mahoney and Silhavy examined the effects of activating the Cpx response using *cpxA* mutations that selectively reduce phosphatase activity. The

mutations enhanced aminoglycoside and hydroxyurea resistance, indicating that activating the Cpx response was protective. Activating the Cpx response by overproduction of an outer membrane protein also increased resistance. The increased resistance phenotypes of bacteria with activated CpxA required CpxR function, ruling out significant off-pathway effects.

Mahoney and Silhavy (5) also examined the effects of blocking the Cpx response by altering either the phosphorylation or DNA binding sites of the CpxR response regulator. Both changes failed to enhance resistance. Previous studies of *cpxRA* deletion mutants also implied that the Cpx response protects, rather than sensitizes, cells to aminoglycosides (7), as did studies of mutations inactivating a functional homologue of CpxRA in *Pseudomonas aeruginosa* (8). In conclusion, although it remains possible that there are special conditions in which CpxRA action sensitizes cells to aminoglycosides and hydroxyurea, the combined evidence strongly implies that the response is primarily protective.

The common pathway model associates CpxRA action with killing by fluoroquinolone and beta-lactam as well as aminoglycoside antibiotics. However, Mahoney and Silhavy (5) found no effect of Cpx activating mutations on resistance to fluoroquinolone and beta-lactam antibiotics. Thus, if there are common steps in killing by the three antibiotics, they evidently do not include Cpx action. A recent study by Liu and Imlay (9) found that oxidative damage is not required for killing by any of the three bactericidal antibiotics, indicating that oxidative stress is also not a necessary part of a common pathway.

The protective effect of the CpxRA response toward aminoglycosides can be readily understood in terms of the classic model of Davis et al. (10) for aminoglycoside lethality. They proposed that streptomycin-induced mistranslation products compromise the cytoplasmic membrane, leading to enhanced antibiotic uptake, translation shutdown, and cell death. Enhanced membrane proteolysis and other protective functions induced by CpxRA activation should help eliminate the harmful mistranslation products and contribute to aminoglycoside resistance. The mechanism by which CpxRA activation protects bacteria from hydroxyurea is more mysterious.

The common pathway model for antibiotic lethality links broad areas of bacterial physiology and accounts for large sets of genome-scale data. The model was thus a plausible working hy-

Published ahead of print 22 February 2013

Address correspondence to manoil@u.washington.edu.

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doi:10.1128/JB.00190-13

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pothesis with experimentally testable predictions. The studies of Mahoney and Silhavy elegantly illustrate how detailed analysis of key components of such a large-scale model can distinguish verifiable elements from those that need to be modified or discarded. This example illustrates how broad-based systems approaches can suggest novel mechanisms that would be difficult to formulate in other ways. Traditional reductionist analysis is then needed to rigorously test and, if necessary, revise the ideas generated. When used together, these approaches provide a powerful complementarity.

ACKNOWLEDGMENT

Pradeep Singh provided thoughtful suggestions.

REFERENCES

1. Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ. 2007. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* 130:797–810.
2. Kohanski MA, Dwyer DJ, Wierzbowski J, Cottarel G, Collins JJ. 2008. Mistranslation of membrane proteins and two-component system activation trigger antibiotic-mediated cell death. *Cell* 135:679–690.
3. Davies BW, Kohanski MA, Simmons LA, Winkler JA, Collins JJ, Walker GC. 2009. Hydroxyurea induces hydroxyl radical-mediated cell death in *Escherichia coli*. *Mol. Cell* 36:845–860.
4. Hassett DJ, Imlay JA. 2007. Bactericidal antibiotics and oxidative stress: a radical proposal. *ACS Chem. Biol.* 2:708–710.
5. Mahoney TF, Silhavy TJ. 2013. The Cpx stress response confers resistance to some, but not all, bactericidal antibiotics. *J. Bacteriol.* 195:1869–1874.
6. Raivio TL, Silhavy TJ. 1997. Transduction of envelope stress in *Escherichia coli* by the Cpx two-component system. *J. Bacteriol.* 179:7724–7733.
7. Zhou L, Lei XH, Bochner BR, Wanner BL. 2003. Phenotype microarray analysis of *Escherichia coli* K-12 mutants with deletions of all two-component systems. *J. Bacteriol.* 185:4956–4972.
8. Lee S, Hinz A, Bauerle E, Angermeyer A, Juhaszova K, Kaneko Y, Singh PK, Manoil C. 2009. Targeting a bacterial stress response to enhance antibiotic action. *Proc. Natl. Acad. Sci. U. S. A.* 106:14570–14575.
9. Liu Y, Imlay JA. Cell death from antibiotics without the involvement of reactive oxygen species. *Science*, in press.
10. Davis BD, Chen LL, Tai PC. 1986. Misread protein creates membrane channels: an essential step in the bactericidal action of aminoglycosides. *Proc. Natl. Acad. Sci. U. S. A.* 83:6164–6168.