Persister Cells and the Paradox of Chronic Infections

Dormant persister cells are tolerant to antibiotics and are largely responsible for recalcitrance of chronic infections

Kim Lewis

Chronic infections are often caused by pathogens that are susceptible to antibiotics, but the disease may be difficult or even impossible to eradicate with antimicrobial therapy. This paradox has been known for a very long time, and a solution appears to be in sight.

Many recalcitrant chronic infections are associated with biofilms—communities of microorganisms that tend to adhere to surfaces and are enclosed in a layer of exopolymers. Biofilms form on indwelling medical devices or within tissues and are responsible for infections of catheters, orthopedic prostheses, heart valves, middle ear (otitis), unhealing wounds, and lungs of patients with cystic fibrosis (CF). These infections may require surgery to remove a heart valve or prosthesis, and ultimately cause the death of CF patients. How are biofilms able to resist killing by antibiotics?

Early attempts to explain biofilm resistance to killing failed to pinpoint a specific mechanism, resulting in the popular idea that recalcitrance is multicomponent. The usual suspects include retarded penetration of drugs through the exopolymer matrix, slow growth of cells, and antibiotic resistance mechanisms that are specifically expressed in the biofilm. Yet it appears that the main culprit may have been overlooked.

A decade ago, our group was studying killing of biofilms of *Pseudomonas aeruginosa*, the principal pathogen in CF infections, by antibiotics and noticed that death of cells was distinctly biphasic (Fig. 1A). Most cells in the biofilm were readily killed at low concentrations of antibiotics, but a small subpopulation appeared invincible. It seemed clear that these persister cells, rather than the bulk, were responsible for the infection's recalcitrance.

Originally described by Joseph Bigger in 1944 in the study of a planktonic population of *Staphylococcus*, persister cells remained a mere curiosity to the small number of experts who knew of their existence. Rediscovering persisters in biofilms, and the intriguing possibility that these cells are the main culprit of recalcitrant chronic infections, renewed our interest in understanding the nature of these unusual cells.

Summary

- Both bacteria and fungi produce small numbers of dormant persister cells whose function is survival.
- Persisters are not mutants, but phenotypic variants of the wild type, and are tolerant to killing by antibiotics.
- Toxin/antitoxin modules function as persister genes.
- Antimicrobial therapy selects for high persistence mutants.

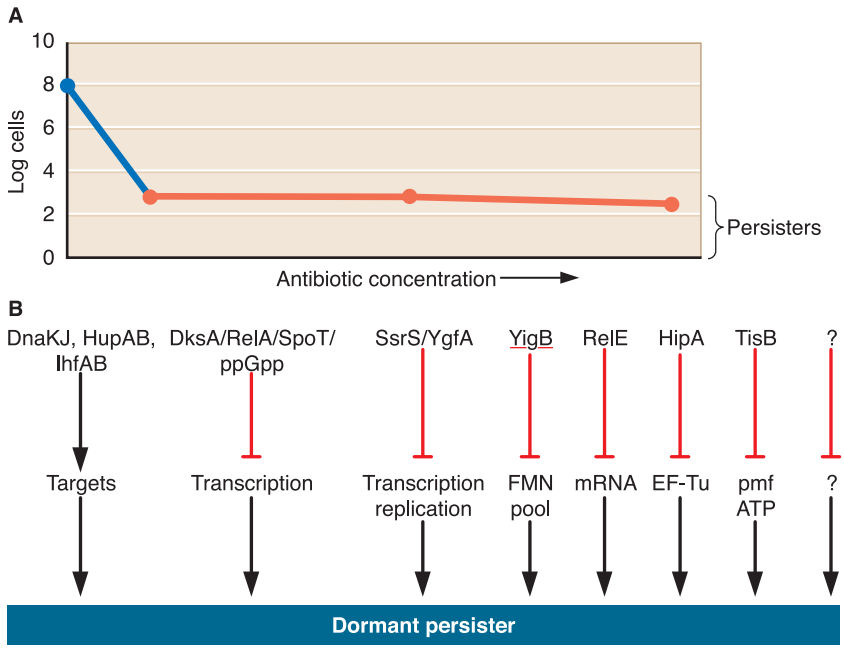
Why Are Persisters Tolerant to Antibiotics?

If some cells in a clonal population of a pathogen were resistant to killing by antibiotics, our first guess would be that this population includes resistant mutants. Where persisters are involved, this guess would be incorrect—regrowing surviving cells produces a culture with the same small subpopulation of survivors, from 10^{-5} to 10^{-2} of the original population. This simple experi-

Kim Lewis is a Professor in the Department of Biology, and Director of the Antimicrobial Discovery Center, Northeastern University, Boston.

Downloaded from www.asmscience.org by
IP: 137.142.46.113
On: Wed, 18 Jan 2017 18:29:59

FIGURE 1



(A) Dose-dependent killing with a bactericidal antibiotic reveals a small subpopulation of tolerant cells, persisters. (B) Candidate persister genes of *E. coli*. Persisters are formed through parallel redundant pathways.

ment shows that phenotypic variants are produced within a population of genetically identical cells by an apparently stochastic process. Random fluctuations in the expression of persister genes (Fig. 1B) will send some cells down a particular development pathway. There is an increasing appreciation of this type of phenotypic variation, known as bistability, which has been described for chemotaxis, competence, and sporulation.

Persisters neither die nor grow in the presence of antibiotic, which suggests that they are dormant. Natalie Balaban and her coauthors at Rockefeller University tracked individual cells of an *E. coli hipA7* ("high persister") mutant and found that persisters did not grow and, unlike regular cells from the same population, were not lysed by ampicillin. Guessing that persisters are dormant and probably have low levels of protein synthesis allowed us to separate them from the bulk of the population by using a strain of *E. coli* that has a degradable GFP gene cloned under the control of a ribosomal promoter. In a growing population, regular cells of this strain are green, but any cell that entered into dormancy will become dim, since GFP degrades and is not replenished (Fig. 2). Indeed, such a culture splits into two subpopulations—a bright one, sensitive to antibiotics, and a small, dim one that is not killed by the same agents.

How would dormancy help a microorganism survive exposure to antibiotics? One key is that bactericidal antibiotics kill not by merely inhibiting targets, but by corrupting them, leading to production of toxic products. Fluoroquinolones such as ciprofloxacin turn topoisomerases into DNA endonucleases; aminoglycosides interrupt translation, causing production of misfolded toxic peptides; and cell wall synthesis inhibitors lead to the activation of autolysins and cell lysis. Jim Collins and colleagues at Boston University in Boston, Mass., recently reported that bactericidal antibiotics additionally lead to production of toxic reactive oxygen species.

If a cell is dormant and the targets inactive, antibiotics can no longer kill. Of course, this protection comes at a price—persisters forfeit propagation, at least until they awaken. However, if a population of cells encounters antibiotics, the persisters will be the only ones waking up.

We use the term antibiotic tolerance in order to differentiate the dormancy protection from mechanistically distinct and more familiar forms of antibiotic resistance. All resistance mechanisms essentially prevent binding of the antibiotic to the target. Resistant cells can then grow in the presence of elevated levels of antibiotic, which manifests itself as an increase in the minimal inhibitory concentration (MIC). The presence of dormant persisters has no effect on the MIC, since these cells are not growing and lack resistance mechanisms. Dormancy resolves the paradox of untreatable infections, explaining how a pathogen that has no resistance mechanisms can resist being killed by antibiotics.

In Search of the Mechanism of Persister Formation

To identify genes coding for a complex function and then decipher the underlying mechanism, microbiologists have successfully used screening

Downloaded from www.asmscience.org by
IP: 137.142.46.113
On: Wed, 18 Jan 2017 18:29:59

Lewis: From Russia with Love, Particularly for Microbiology

Kim Lewis calls himself a "garden-variety American," born in New York, but raised in Moscow after his family moved there "for idealistic reasons" when he was two. He attributes his happy childhood there to being "shielded by the grown-ups from the unpleasant reality of the police state," he says. "I left Russia because I had to live free, which I do now."

Lewis, 57, professor in the Northeastern University Department of biology and director of its Antimicrobial Discovery Center, says that his interest in biology goes back as far as he can remember. "The first book I checked out of the library at age five was about animals," he says. His mother, whose work entailed translating Russian literature into English, later gave him the collected works of German zoologist Alfred Edmund Brehm (1829–1884), including the *Life of Animals*, which made a big impression. "One of my early dreams was to discover new animals, and then I realized with regret that this was not going to happen," he says. "Little did I know that many years later microbiology would give me the opportunity not only to find new organisms, but that the majority of microbes are 'uncultured,' and waiting to be explored."

Before the Soviet Union broke apart, educators there encouraged students to specialize early. Thus, Lewis enrolled in a Moscow biology school while in the 8th grade. "Talking to other kids who were similarly obsessed with biology was one of the most pleasurable experiences I can recall," he says. By junior high school, he was reading works by the animal physiologist Konrad Lorenz, whose research on imprinting is considered a fundamental contribution. "The experiments seemed so clever—like presenting seagull chicks with a long stick with a red ball at the end emulating the seagull's beak, to which the chicks responded enthusiastically—that I was gravely concerned that I would not be able to think of my own experiments," Lewis says.

He need not have worried. In the 10th grade, he read a textbook by Professor Vladimir Skulachev of Moscow University on the emerging field of bioenergetics. After visiting the professor, he volunteered to work in his lab. Skulachev "is one of the most imaginative scientists I have met, and that, of course, had a big influence on my future choice of projects," Lewis says.

"Perhaps the strongest impression was made by reading a rather thin textbook on biochemistry that belonged to my stepfather, who was finishing medical school at the time," he continues. "In the chapter on protein synthesis, the textbook matter-of-factly explained how information is translated from DNA into proteins into functions. The essential mechanism of heredity was actually understood at the molecular level! I knew that whatever I chose to study, I would want to understand things at the level of molecules."

Lewis received his B.S. degree in biochemistry from Moscow University in 1976, and his Ph.D., also in biochemistry from Moscow University, four years later. He returned to the United States in 1987, losing his academic research position at Moscow University when he applied to emigrate.

Lewis served as a research associate at the University of Wisconsin, Madison, from 1987 to 1988, and then assistant professor of biology at the Massachusetts Institute of Technology to 1994. He spent the next three years as an associate professor of medical and research technology at the University of Maryland School of Medicine in Baltimore, and then returned to Boston, to become a research associate professor in the Tufts University biotechnology center. He joined Northeastern University there in 2001.

Lewis is now a full-fledged microbiologist, studying antimicrobial drug tolerance while also searching for novel antimicrobial drugs. "Going into microbiology was an easy choice—it uniquely allows you to remain a biologist without necessarily limiting your choices to a narrow field," he says. "Microorganisms interact as pathogens or symbionts with every living creature on the planet, and in this regard your horizons are as broad as you wish."

Persister cells are a special interest. They are dormant variants of bacteria that are highly tolerant to killing by all known antibiotics. Persister cells are largely responsible for relapsing chronic infections caused by biofilms, Lewis says. "Biofilms seemed like a perfect . . . paradox. Bacterial cells that are susceptible to antibiotics become recalcitrant when forming a biofilm, without actually acquiring any apparent resistance mechanism. Analyzing this paradox lead to the discovery of dormant persister cells in the biofilm."

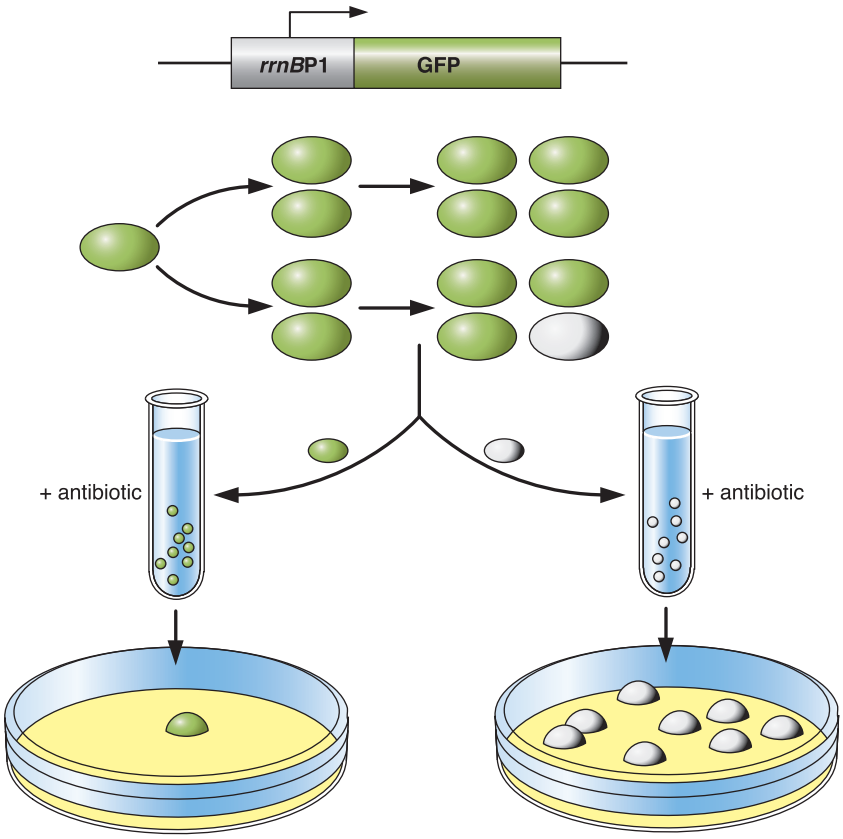
His wife, Tanya, a molecular biologist, works on transgenic animal models of human diseases at the Novartis Research Institute in Cambridge, Mass. He has two daughters, Sasha, an attorney in New York, and Maria, who works in land management in Moscow. His favorite vacation is "kayaking with my wife somewhere up north."

In his spare time, Lewis enjoys music, reading, and art. "Growing up in Russia, one acquired the typical set of 19th-century interests—reading, art, and classical music," he says. "This is not due to any refinement on my part; those were simply the only choices available. Now I also enjoy jazz and rock."

Marlene Cimons
Marlene Cimons lives and writes in Bethesda, Md.

Downloaded from www.asmscience.org by
IP: 137.142.46.113
On: Wed, 18 Jan 2017 18:29:59

FIGURE 2



A strain of *E. coli* that carries a degradable GFP under the control of a ribosomal promoter can be used to physically sort persisters. In a growing population, regular cells express GFP and are green, but a cell that entered dormancy has diminished protein synthesis and becomes dim due to GFP degradation. Cell sorting then allows to separate the bright and the dim subpopulations. Antibiotic kills mostly the bright, but not the dim sorted cells.

of transposon insertion libraries in search of mutants lacking the phenotype of interest. Mapping the insertions produces a catalog of the principal genes. This straightforward approach has lead to the identification of genes coding for such properties as sporulation, virulence, and flagellum formation.

In *E. coli*, we can do even better than a transposon insertion screen. Recently, a fairly complete set of knockout strains was created by Hirotada Mori and his colleagues at the University of Nagoya (the Keyo collection). This collection is being generously shared with the scientific community. We screened the Keyo library for persister genes and found not a single mutant unable to make persisters. A number of strains showed diminished persister levels (as judged by the number of cells surviving treatment with high levels of antibiotic). Strains with diminished persister levels were primarily deleted in genes coding for global regulators (*dnaK, dnaJ, hupAB, ihfAB, dksA*).

From these findings, we concluded that persisters are not formed through a single control pathway ending in an execution mechanism. Rather, there appear to be independent, parallel mechanisms of persister formation (Fig. 1B). As if a temporary phenotype and the small fraction of cells exhibiting it were not enough of an obstacle!

When the usual molecular genetic approaches fail, there is always transcriptome analysis. Persisters isolated by cell sorting and collected by simply lysing a population of growing *E. coli* with ampicillin produced a transcriptome showing upregulation of genes whose products could potentially lead to dormancy—the toxin-antitoxin (TA) modules. Discovered originally as a plasmid maintenance mechanism, the toxins come in a variety of classes and shut down cellular functions. If a daughter cell loses a plasmid, the more labile antitoxin diminishes, releasing the toxin that either kills the cell or causes stasis.

Whole-genome sequencing showed that TA modules are present in bacterial chromosomes as well, but their function was unknown. It seemed that "toxins" are excellent candidates for persister genes. According to Ken Gerdes at the University of Newcastle in Newcastle, U.K. and his collaborators, some of the *E. coli* toxins—RelE and MazF—act as mRNA endonucleases and inhibit protein synthesis. This inhibition can be reversed by expression of their antitoxins.

We found that ectopic expression of RelE causes multidrug tolerance, emulating native persisters. Alex Neyfakh and Nora Vazquez-Laslop at the University of Illinois in Chicago, Ill., also found multidrug tolerance in cells overexpressing MazF.

Another interesting *E. coli* toxin is coded by the *hipBA* TA locus. Harris Moyed selected for high-persister strains in the early 1980s and identified a *hipA7* gain-of-function allele which increased persister production 100-fold in an exponentially

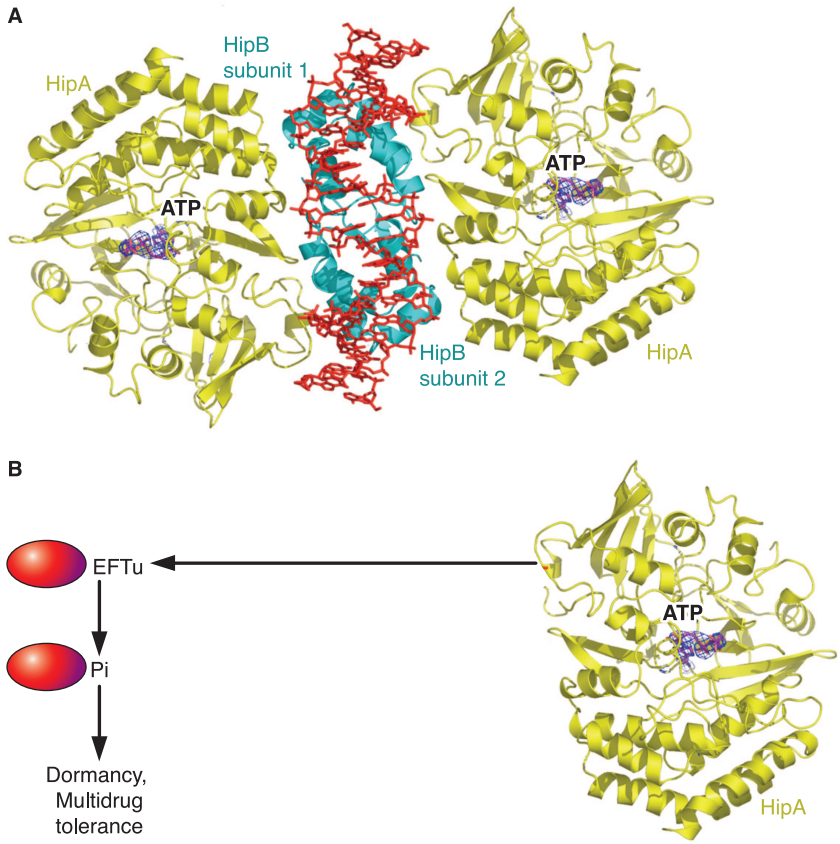
Downloaded from www.asmscience.org by
IP: 137.142.46.113
On: Wed, 18 Jan 2017 18:29:59

growing culture. Eugene Koonin and Kira Makarova at the National Institutes of Health in Bethesda, Md., noticed that HipA belongs to the phosphatidylinositol family of protein kinases, and in collaboration with Dick Brennan and Maria Schumacher, we found that HipA is a serine protein kinase which phosphorylates and inhibits elongation factor EF-Tu (Fig. 3). Overexpression of HipA produces drug tolerance.

It appears that different toxins can cause dormancy by inhibiting protein synthesis through independent mechanisms. However, knocking out the toxins does not produce a discernible phenotype, probably because there is a high degree of redundancy. There are now 15 identified TA modules in *E. coli*, and 80 in *M. tuberculosis*, and these numbers continue to increase.

Although persisters can form stochastically, there is also a deterministic component. For example, increasing cell density in a growing culture results in a sharp increase in persister levels, reaching around 1% in *E. coli* in stationary state. The component responsible for this rise in unknown. We reasoned that a given TA module under conditions when it is highly expressed will act as a deterministic component of persister formation. In search of such an inducible TA module, we turned to the SOS response.

FIGURE 3



The HipA toxin of *E. coli* forms a complex with the antitoxin HipB, and both act as repressors, binding to the promoter region of the *hipBA* locus (A). When released from HipB, the HipA kinase phosphorylates elongation factor Ef-Tu, inhibiting protein synthesis and causing dormancy.

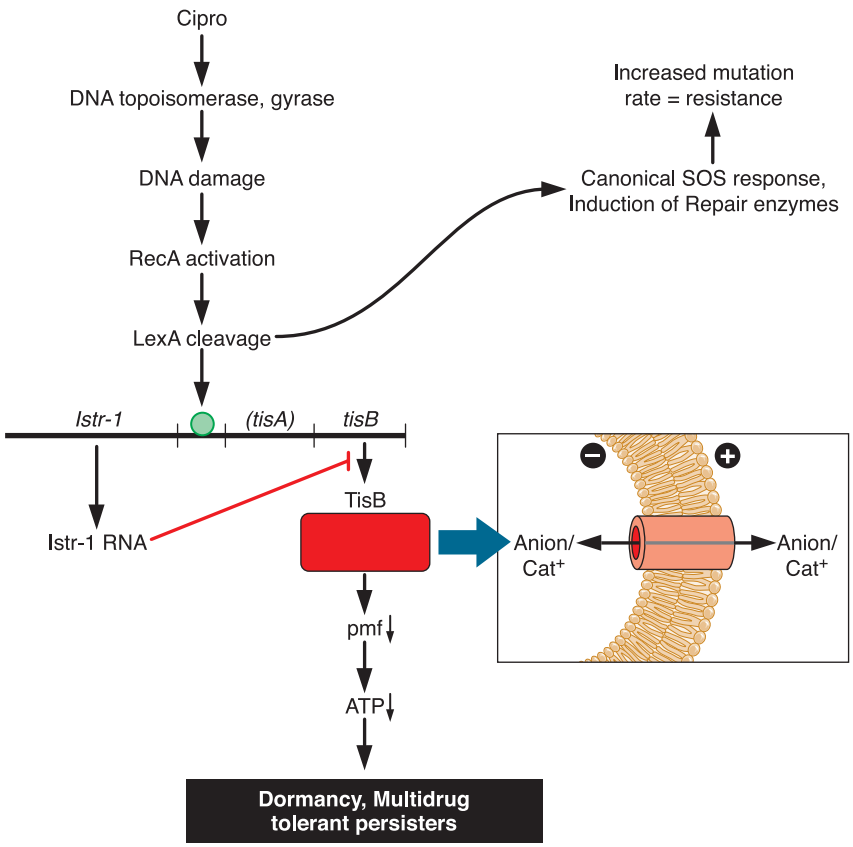
Stress Response, a Key Component of Persister Formation

The canonical SOS response is triggered by DNA damage and induces expression of DNA repair enzymes and protective proteins. Damage is recognized by the RecA protein, which in turn causes self-cleavage of the LexA repressor occupying an operator sequence upstream from SOS genes (Fig. 4). The Lex-box operator is present upstream of several TA modules. Knocking out one of these modules, TisAB, causes a sharp decrease in persister formation in a culture treated with ciprofloxacin, an antibiotic that leads to DNA damage. This suggests that most persister formation is TisB dependent under conditions of SOS response. Overexpression of the TisB toxin causes high levels of tolerance not only to fluoroquinolones, but also to unrelated antibiotics—β-lactams and aminoglycosides. Thus, an unexpected side-effect of ciprofloxacin is production of multidrug-tolerant cells resistant to killing by all antibiotics, probably contributing to recalcitrance of infections. Indeed, we did not suspect that treatment with one antibiotic can promote production of cells tolerant to all antibiotic therapy.

TisB seems an unlikely candidate for a persister protein since it is a typical antimicrobial peptide. TisB is 29 amino acids long, hydrophobic, positively charged, and acts by disrupting the membrane potential, leading to a drop in ATP and, when artificially overexpressed, to cell death, according to Cecilia Unoson of Uppsala University in Uppsala, Sweden, and her collaborators. Ap-

Downloaded from www.asmscience.org by
IP: 137.142.46.113
On: Wed, 18 Jan 2017 18:29:59

FIGURE 4



The common antibiotic ciprofloxacin causes DNA damage by converting its targets, DNA gyrase and topoisomerase, into endonucleases. This activates the canonical SOS response, leading to increased expression of DNA repair enzymes. At the same time, the LexA repressor that regulates expression of all SOS genes also controls transcription of the TisAB toxin/antitoxin module. The TisB toxin is an antimicrobial peptide, which binds to the membrane, causing a decrease in proton motive force and ATP. This produces a systems shutdown, blocking antibiotic targets, which ensures multidrug tolerance.

parently, mild overexpression of the protein during the SOS response decreases the proton motive force enough to cause dormancy but not death. ATP depletion seems like a perfect switch for a cellular systems shutdown.

The experiments with TisB pinpoint with considerable confidence a mechanism of persister formation, ending a 60-year quest. It seems likely that TisB is only the first in what will undoubtedly be a large number of mechanisms for persister formation. Apart from setting a precedent, the process of elucidating the role of TisB may also serve as a key for discovering these additional mechanisms—finding conditions when a particular candidate is expressed.

Dormancy and the Curious Case of Antimicrobial Peptides

Antimicrobial peptides are widely distributed among bacteria, fungi, plants, and animals. In humans, antimicrobial peptides are an important component of the innate immune response.

Among plants, antimicrobial peptides produced by *Medicago* legumes were identified as the long-sought factors causing terminal differentiation of their symbionts, nitrogen-fixing rhizobia, according to Peter Mergaert at Centre National de la Recherche Scientifique in Gif-sur-Yvette, France, and his collaborators. At high concentrations, the *Medicago* peptides cause membrane disruption and death of bacteria. At low physiological concentrations, bacterial cells stop dividing and enter into an apparent irreversible dormancy. The supply of nutrients by the plant is apparently sufficient to allow these cells to continue to fix nitrogen.

Finding TisB-producing persisters and *Medicago* peptides inducing dormancy in rhizobia raises an intriguing question regarding conventional antimicrobial peptides known as "killer toxins." Might exported antimicrobial peptides produced by bacteria, such as lantibiotics, play an (additional) role of a community sedative? Bacteria are protected from their own antimicrobial peptides primarily through efflux; when these peptides are released in the environment, they may play a dual role—killing susceptible neighboring species while inducing dormancy in the producing colony. Like typical antibiotics, antimicrobial peptides are usually induced upon entering the stationary state, when cells would benefit from increased tolerance to threats.

Clinical Significance of Persister Cells

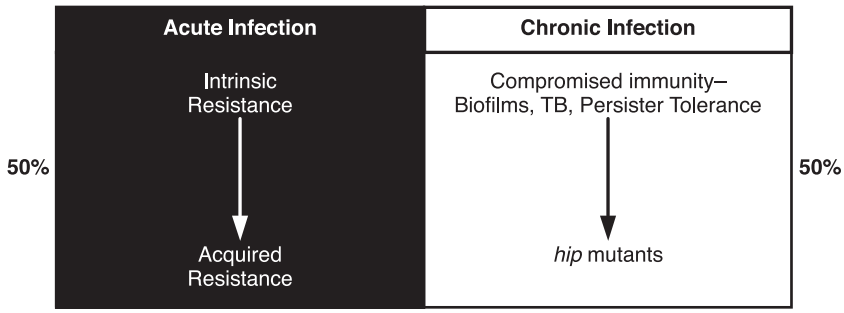
It appears that there are two faces of the pathogen threat—resistance in acute infections, and persister tolerance in chronic infections (Fig. 5). There are many parallels between these two mechanistically distinct protective mechanisms—intrinsic resistance/intrinsic ability to

Downloaded from www.asmscience.org by
IP: 137.142.46.113
On: Wed, 18 Jan 2017 18:29:59

produce tolerant persisters; resistant/tolerant (*hip*) mutants; and induction of both types by stress responses.

We have discussed the role of biofilms in chronic infections, and it seems that the primary role of a biofilm is to provide a protective habitat for persisters by shielding them from the immune system. Persisters have broader significance than in the context of biofilm infections, and these cells are likely to play a critical role in recalcitrance to therapy whenever the immune response is limited. Such cases would include disseminated infections in immunocompromised patients undergoing cancer chemotherapy or infected with HIV.

FIGURE 5



Resistant pathogens play a major role in acute infections, and a variety of acquired mechanisms lead to multidrug resistance. In the parallel world of chronic infections, tolerant persister cells survive the attack of antibiotics and repopulate the infection, causing a relapse. Lengthy antibiotic treatment favors selection of high-persister mutants, further complicating therapy.

Persisters are also likely to play an important role in immunocompetent individuals in cases where the pathogen is located at sites poorly accessible by components of the immune system. These include the central nervous system, where pathogens such as *Meningococcus* and *Neisseria* cause debilitating meningitis and brain abscesses, and the gastrointestinal tract, where hard-to-eradicate *Helicobacter pylori* cause gastroduodenal ulcers and gastric carcinoma.

Tuberculosis is perhaps the most prominent case of a chronic infection by a pathogen evading the immune system. The acute infection may resolve spontaneously or as a result of antimicrobial therapy, but the pathogen often remains in a "latent" form. It is estimated that 1 in 3 people carry latent *M. tuberculosis*, and 10% of carriers develop an acute infection at some stage in their lives. Virtually nothing is known about this latent form that serves as the main reservoir of tuberculosis. One simple possibility is that the latent form of the pathogen is persisters.

Persisters in Chronic Infections

Persisters are a plausible candidate for explaining the recalcitrance of some chronic infections. But plausibility is not causality, and exploring the involvement of persisters in disease is not trivial. Introducing isolated persisters into an animal and examining antibiotic tolerance will not work, since persisters will revive and turn into regular cells. A helpful clue comes from experiments aimed at selecting for high-persister mutants in vitro. A culture is treated with a high concentration of a bactericidal antibiotic, surviving persisters are reinoculated, and the selection is repeated. After several cycles, the culture is enriched for high-persister mutants. This approach is useful for identifying candidate persister genes. But this is also the procedure used in antimicrobial therapy of chronic infections—periodic application of high doses of bactericidal antibiotics. If this leads to the selection of *hip* mutants, it would mean that persisters provide a selective advantage to the pathogen, and are indeed causally connected to recalcitrance of the disease. In this sense the experiment has already been done, and we simply need to look at the result.

The most dramatic case of chronic infection comes from cystic fibrosis, when patients are periodically treated with high doses of antibiotics over the course of decades. We took advantage of the longitudinal isolates of *P. aeruginosa* collected from a single CF patient over the course of many years by Jane Burns and colleagues at Seattle Children's Hospital, and measured the level of persisters by adding high doses of ofloxacin and determining the number of surviving cells. This experiment showed that persister levels increased dramatically, by 100-fold, in late isolates of this clonal lineage. Similar increases in persister levels were observed in late isolates from most CF patients examined. Importantly, in approximately half of the cases, the pathogen showed no increase in MIC to antibiotics used to treat this infection. This experiment suggests that the major culprit respon-

Downloaded from www.asmscience.org by
IP: 137.142.46.113
On: Wed, 18 Jan 2017 18:29:59

sible for the incurable infection is the presence of persister cells.

A similar study examined the role of persisters in a recalcitrant infection of *Candida albicans*. This fungal pathogen also produces persisters, though they are probably analogous rather than homologous to their bacterial counterparts. In cancer patients undergoing chemotherapy, the immune system is compromised, and the opportunistic pathogen *C. albicans* forms a characteristic white biofilm known as oral thrush, or oropharyngeal candidiasis. Patients are treated daily with topical chlorhexidine, and after 3 weeks the infection may resolve, but it often remains recalcitrant to treatment. *C. albicans* strains were collected from patients and tested for survival to high doses of amphotericin B. Invariably, all isolates from nonresolving infection appeared to be high-persister mutants. The two cases examined so far come from completely unrelated microorganisms and suggest a general role for *hip* strains and persister cells in recalcitrant chronic infections.

Prospects for Persister Eradication

The incidence of chronic infections in the developed world is comparable to acute infections, but we do not have a single agent capable of effectively eliminating dormant cells. In part, this is due to the FDA requirement to test new drugs against exponentially growing pathogens. Most antibiotics would fail if tested against stationary cells, and all will fail if tested against persisters. While resistance is a serious problem, we do have a very large arsenal of antibiotics to treat pathogens in acute infections. Knowing that persisters are largely responsible for recalcitrance of chronic infections is an important first step to developing therapeutic agents, but the road will not be easy—developing an antibiotic even in an "easy" case of a narrow-spectrum compound acting against exponentially growing cells is extremely difficult. Only three novel compounds have been developed over the past 30 years–linezolid (oxazolidinone), daptomycin (peptidolipid), and synercid (Quinupristin/dalfopristin). All three act only against gram-positive species.

Multidrug-resistant, gram-negative pathogens such as *P. aeruginosa, Burkholderia cepacia, Acinetobacter baumannii*, and *Klebsiella pneumoniae* are becoming major problems, with no new drugs in sight. An overreliance on the major source of antibiotics—cultivable bacteria—reduced the pace of discovery to a trickle by the early 1960s. Gram-negative pathogens have a formidable penetration barrier made of two membranes and transenvelope MDR pumps that export amphipathic compounds across this barrier. Synthetic drugs are almost invariably extruded by the MDRs. Add these difficulties to the need to kill dormant cells which form by redundant mechanisms, and the prospect of eradicating a chronic infection appears bleak.

A possible solution may lie in the mode of action of a minor group of antibiotics that are very different from conventional target-specific compounds. These are prodrugs, used now primarily as anti-TB therapeutics. Compounds such as isoniazid (INH) or ethionamide are activated by bacteria-specific enzymes into reactive compounds. Metronidazole is the only known broad-spectrum prodrug, being activated by nitrate reductase, which is present in many bacteria. The beauty of prodrugs is that they can in principle kill dormant cells, since a reactive molecule can disrupt such cellular components as the membrane and DNA, and their covalent binding to targets produces an irreversible sink, countering efflux. Indeed, metronidazole is highly effective against gram-negative species, but its application is limited to anaerobic conditions where nitrate reductase is expressed. Known prodrugs do not sterilize an infection, but their mode of action carries the promise of developing compounds with a better fit to their activating enzyme, which may produce sufficient amounts of reactive compounds that will kill persisters.

ACKNOWLEDGMENTS

Kim Lewis is supported by grants from the National Institutes of Health, the Army Research Office, and the Bill and Melinda Gates Foundation.

Downloaded from www.asmscience.org by
IP: 137.142.46.113
On: Wed, 18 Jan 2017 18:29:59

SUGGESTED READING

Balaban, N. Q., J. Merrin, R. Chait, L. Kowalik, and S. Leibler. 2004. Bacterial persistence as a phenotypic switch. Science **305:**1622–1665.

Correia, F. F., A. D'Onofrio, T. Rejtar, L. Li, B. L. Karger, K. Makarova, E. V. Koonin, and K. Lewis. 2006. Kinase activity of overexpressed HipA is required for growth arrest and multidrug tolerance in *Escherichia coli*. J. Bacteriol. **188:**8360–8367.

Dorr, T., M. Vulic, and K. Lewis. 2010. Ciprofloxacin causes persister formation by inducing the TisB toxin in *Escherichia coli*. PLOS Biol **8:**e1000317.

Dubnau, D., and R. Losick. 2006. Bistability in bacteria. Mol. Microbiol. **61:**564–572.

Hansen, S., K. Lewis, and M. Vulić. 2008. The role of global regulators and nucleotide metabolism in antibiotic tolerance in *Escherichia coli*. Antimicrob. Agents Chemother. **52:**2718–2726.

Keren, I., N. Kaldalu, A. Spoering, Y. Wang, and K. Lewis. 2004. Persister cells and tolerance to antimicrobials. FEMS Microbiol Lett **230:**13–18.

LaFleur, M. D., Q. Qi, and K. Lewis. 2010. Patients with long-term oral carriage harbor high-persister mutants of *Candida albicans*. Antimicrob. Agents Chemother. **54:**39–44.

Lewis, K. 2007. Persister cells, dormancy and infectious disease. Nature Rev. Microbiol. **5:**48–56.

Schumacher, M. A., K. M. Piro, W. Xu, S. Hansen, K. Lewis, and R. G. Brennan. 2009. Molecular mechanisms of HipA-mediated multidrug tolerance and its neutralization by HipB. Science **323:**396–401.

I, Microbiologist

A Discovery-Based Course in Microbial Ecology and Molecular Evolution

Authors: Erin R. Sanders-Lorenz, Jeffrey H. Miller

From hypothesis to discovery, *I, Microbiologist* enables students to develop all the basic skills and experience all the wonderment of conducting a meaningful research project from start to finish—all within a one-semester laboratory course. Specifically, students learn to reconstruct the phylogeny of a unique soil-based microbial community by analyzing 16S rRNA genes. In the process, students discover new microbes, novel sequences, and previously unknown phenotypes.

Each of the text's seven units features experimental protocols and essential background information, giving students the tools and context needed to formulate hypotheses, conduct experiments, and gather data. Written assignments associated with the readings for each unit challenge students to analyze experimental data, interpret results, and evaluate conclusions. In short, this text gives students an unparalleled experience, preparing them to take on research projects in any working lab.

December 2009. Paperback.
ISBN: 978-1-55581-470-0, 420 pages est., full color throughout, illustrations, index.

List and ASM member price: $79.95

WEB: **http://estore.asm.org** • CALL: **800-546-2416** or **703-661-1593** • WRITE TO: **ASM Press, P. O. Box 605, Herndon, VA 20172, USA** • FAX: **703-661-1501**

ASM PRESS

Downloaded from www.asmscience.org by
IP: 137.142.46.113
On: Wed, 18 Jan 2017 18:29:59