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Review

Role of persister cells in chronic infections: clinical relevance and perspectives on anti-persister therapies

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Certain infectious diseases caused by pathogenic bacteria are typically chronic in nature. Potentially deadly examples include tuberculosis, caused by *Mycobacterium tuberculosis*, cystic fibrosis-associated lung infections, primarily caused by *Pseudomonas aeruginosa*, and candidiasis, caused by the fungal pathogen *Candida albicans*. A hallmark of this type of illness is the recalcitrance to treatment with antibiotics, even in the face of laboratory tests showing the causative agents to be sensitive to drugs. Recent studies have attributed this treatment failure to the presence of a small, transiently multidrug-tolerant subpopulation of cells, so-called persister cells. Here, we review our current understanding of the role that persisters play in the treatment and outcome of chronic infections. In a second part, we offer a perspective on the development of anti-persister therapies based on genes and mechanisms that have been implicated in persistence over the last decade.

Introduction

Antibiotic treatment of microbial populations typically results in a biphasic killing pattern (Lewis, 2010). When the antibiotic concentration exceeds a certain threshold, only so-called 'persister' cells survive (Fig. 1). Persister cells are highly multidrug-tolerant cells that constitute a small fraction of the population. They are transiently refractory to killing, without having acquired resistance through genetic modification (Keren *et al.*, 2004a). Consequently, when the antibiotic pressure drops, the cells will give rise to a population that is as susceptible as the original one, and that again possesses a similarly small proportion of persister cells. This discriminates persister cells from resistant mutants, which exhibit stable, inheritable drug insensitivity (Fig. 2). Microscopic observation of individual bacteria grown in microfluidic devices demonstrated that persisters have a significantly reduced growth rate (Balaban *et al.*, 2004). Their indifference to the presence of antibiotics can therefore be explained by a global shutdown of processes essential for active growth, as the very processes that are targeted by antibiotics are no longer operational and hence not subject to inhibition any more. This also corroborates the non-specific nature of persister cell drug tolerance.

A full decade ago, it was discovered that persister cells are present in significant numbers in bacterial biofilms. Based both on these findings and on modelling studies, it was suggested that persisters may well represent the long-looked-for explanation for biofilm tolerance to antibiotics (Roberts & Stewart, 2005; Spoering & Lewis, 2001). Considering that biofilms are associated with numerous chronic diseases through their resilient presence in the human body, it comes as no great surprise that interest in persister cells has seriously picked up since then. A more general role in chronic infections, beyond the involvement in biofilm tolerance, was subsequently put forward for persisters (Lewis, 2007), explaining why important chronic infections such as tuberculosis and pneumonia often recur despite the application of antibiotics and the absence of detectable antibiotic resistance through laboratory testing of the causative agents. Only recently, a direct link was provided between the recalcitrance of chronic infections and persistence (LaFleur *et al.*, 2010; Mulcahy *et al.*, 2010), a major breakthrough in this research area.

Extensive reviews on the genetic basis of persistence and the methods that have been used to study the phenomenon in the past have been published elsewhere (Gefen & Balaban, 2009; Jayaraman, 2008; Lewis, 2010). In the current review, we will focus on the role of persister cells in notable chronic infections (cystic fibrosis pneumonia, candidiasis and tuberculosis) and present a perspective on anti-persister therapies based on currently known persistence mechanisms. A better understanding of the latter may contribute to the future development of persister-specific drugs and improved treatment of chronic infectious diseases.

Clinical relevance

The recalcitrant nature of chronic infections despite antibiotic treatment is puzzling given the observation that

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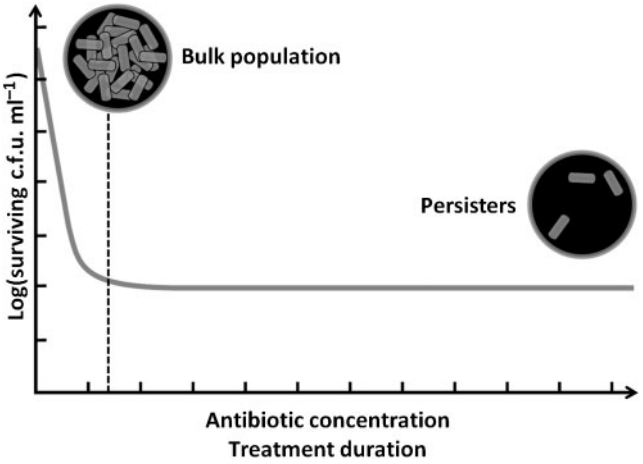

Fig. 1. Biphasic killing pattern in response to antibiotics. Viable c.f.u. counts are plotted in function of antibiotic concentration and/or treatment duration and depicted by a grey curve. Addition of increasing concentrations of antibiotics and/or increasing the treatment duration leads to an initial phase of rapid killing of the bulk population. However, beyond a certain threshold (indicated by a dashed vertical line), a killing plateau is observed as only persister cells remain viable.

laboratory testing of the causative agents often fails to identify resistance to the administered drugs. Though long suspected (Tuomanen *et al.*, 1986), accumulating evidence now convincingly points to the importance of transiently drug-tolerant persister cells as a major culprit (Lewis, 2010). These have long been overlooked because routine clinical susceptibility tests focus on the bulk of a bacterial sample, whereas persisters make up only a small part of the population. Additionally, persisters are slowly growing or non-growing cells whose formation is favoured under growth-limiting conditions (Fung *et al.*, 2010), while MIC assays are carried out with full nutrient supply and monitor visibly detectable growth resulting from actively dividing cells. Given that persisters have been observed in numerous pathogenic microbes such as *Escherichia coli*, *Candida albicans*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Dhar & McKinney, 2007), the implications of microbial persistence to antibiotics could be far-reaching.

Levin & Rozen (2006) were the first to come up with a sound theoretical basis for postulating the clinical importance of persisters in chronic infections. Using a mathematical model that incorporates pharmacokinetics and pharmacodynamics of antibiotics and bacteria, persister cells were predicted to reduce the efficacy of chemotherapy, prolonging infection duration and possibly leading to treatment failure. Furthermore, persisters could provide a reservoir of viable bacteria that can acquire resistance by random mutation or horizontal gene transfer, thereby promoting the generation of antibiotic-resistant mutants. Experimental evidence directly supporting these predictions is essentially non-existent because the *in vivo* models required for such studies have not yet been developed. However, recent studies provide strong indications for the importance of persister cells in recurrent infectious

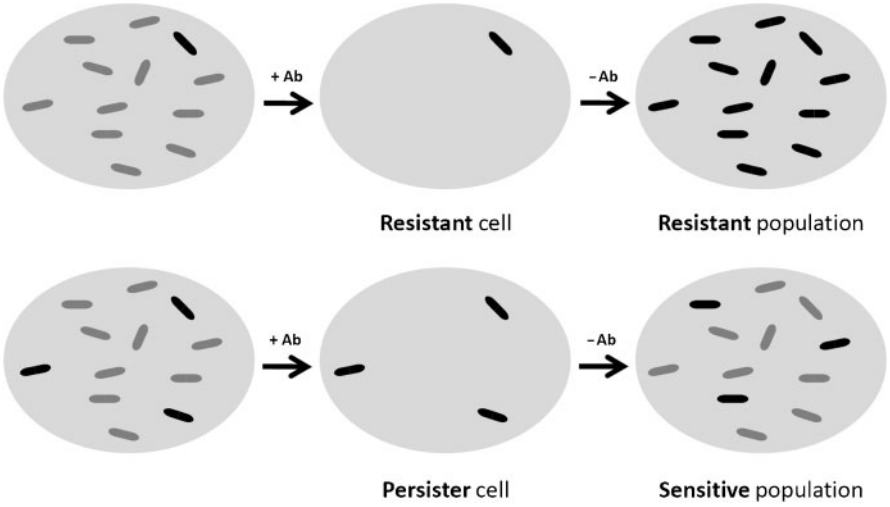

Fig. 2. Antibiotic resistance versus persistence. A microbial population (confined by a light-grey ellipse) initially consists of mainly antibiotic-sensitive cells (dark-grey). (top panel) In addition, the population may also contain *resistant* cells (black), resulting from a permanent change at the genetic level. After antibiotic treatment (+Ab), only resistant cells remain. Upon regrowth (−Ab), the entire population is composed of resistant individuals. (bottom panel) Alternatively, the population may contain *persister* cells (black), resulting from a reversible phenotypic switch to a tolerant state. After antibiotic treatment, only persister cells remain. Upon regrowth, the population will exhibit the same sensitivity as the original population.

diseases. In the following paragraphs, we discuss three major chronic infections in the context of persistence.

Cystic fibrosis pneumonia

Cystic fibrosis (CF) is the most common fatal hereditary disease among Caucasians and is characterized by an imbalance in chloride and sodium levels in epithelial tissues in the lungs and intestines (Boucher, 2001). Even though this causes a number of severe disease symptoms, 85–90 % of CF patients will ultimately succumb to the consequences of a bacterial infection, with *P. aeruginosa* the leading cause of death (Gibson *et al.*, 2003; Lyczak *et al.*, 2002). The establishment of a permanent chronic infection is the result of a phenotypic switch by the bacterium to a mucoid form (Harrison, 2007) and results in chronic inflammation, continuously damaging the lungs and eventually leading to respiratory failure.

P. aeruginosa cells in CF lungs are extremely tolerant to antibiotics during the course of the chronic infection, and the reason for this has long remained unclear. Resistance-conferring mutations do occur, probably selected for by intensive antibiotic treatments. However, isolates of *P. aeruginosa* strains do not always develop resistance (Burns *et al.*, 1999; Gibson *et al.*, 2003; Gilligan, 2006; Mulcahy *et al.*, 2010). Lewis (2007) suggested that the recalcitrant nature of *P. aeruginosa* infections in CF lungs is largely the consequence of the presence of a drug-tolerant subpopulation of persister cells. To provide evidence for this assumption, Mulcahy *et al.* (2010) reasoned that if persister cells are important for the recalcitrance of chronic *P. aeruginosa* infections, there would be a selection for high persistence (*hip*) mutants in the CF lung because of the periodical administration of antibiotics. Analysis of 35 longitudinal clinical isolates of a single CF patient, isolated between the ages of 8 and 96 months (Smith *et al.*, 2006), indeed revealed a dramatic 100-fold increase in persistence against several antibiotics for the latest isolates compared to isolates from the onset of the chronic infection. Expanding this study to a larger group of patients, pairs of clonal early and late clinical *P. aeruginosa* isolates from 14 CF patients were analysed for their persistence phenotype. Ten of the 14 late isolates displayed a *hip* phenotype in comparison to the corresponding early isolates. This study provides for the first time evidence of a direct relationship between the presence of bacterial persister cells and the recalcitrant nature of chronic infections (Mulcahy *et al.*, 2010).

Candidiasis

The causative agent of chronic infections can also be eukaryotic. In particular, the infections caused by the pathogen *C. albicans* are a serious threat to human health today. There is an increasing incidence of candidiasis in hospital environments, likely a result of *Candida* biofilms formed on medical indwelling devices such as catheters, heart valve replacements and prostheses (Chandra *et al.*, 2005; Emerson & Camesano, 2004; Ramage *et al.*, 2006). These infections can be life-threatening, especially in immunocompromised patients.

The structure and formation of eukaryotic biofilms is far less well studied than is the case for bacterial biofilms. However, their importance in chronic infections is becoming increasingly clear, motivating more researchers to tackle the issue of fungal biofilms. LaFleur *et al.* (2006) reported for the first time the presence of a population of antifungal-tolerant cells within a *Candida* biofilm, much resembling the bacterial persister cells found in *P. aeruginosa* and *E. coli* planktonic and biofilm populations. Contrary to bacterial populations, planktonic *Candida* populations do not produce persister cells refractory to the lethal action of antibiotics. Similarly to the *in vivo* selection of *P. aeruginosa hip* mutants in CF lungs (Mulcahy *et al.*, 2010), LaFleur *et al.* (2010) were able to demonstrate that *in vivo* selection for *Candida hip* mutants occurs in patients with oral candidiasis. For this study, the persistence phenotype of 150 clinical isolates obtained from cancer patients, who were at high risk for candidiasis because of chemotherapy treatment, was determined. The samples were taken on a weekly basis from a total of 22 patients. The persister levels in the patient group with chronic carriage (>8 weeks) were significantly higher than the persister levels in the group that displayed transient carriage (<8 weeks) of *Candida* species. The results clearly established a link between persistence and chronic *C. albicans* infections (LaFleur *et al.*, 2010).

Tuberculosis

Human tuberculosis is one of the most notorious examples of a chronic bacterial infection, claiming 1.6 million lives on a yearly basis (WHO, 2009). Estimates are that one in every three people carries the main causative agent, *M. tuberculosis*, in a latent form, which in 5–10 % of infected people will resuscitate and cause chronic disease at some time in the future, resulting in severe lung damage (Bloom & Murray, 1992). Even though most of the *M. tuberculosis* cells involved in an acute infection are effectively killed within 14 days of antibiotic treatment, complete eradication of the bacteria is rarely achieved and is believed to result from the presence of a subpopulation of dormant, drug-tolerant cells (Hu & Coates, 2003; Wayne & Sohaskey, 2001; Zhang, 2005), their tolerance presumably not due to drug-specific mechanisms (Wallis *et al.*, 1999).

The significance of dormant tubercle bacteria to recalcitrance and recurrence of tuberculosis remains unclear. In addition, the term dormancy is often operationally defined in *M. tuberculosis* research (Chao & Rubin, 2010; Zhang, 2004). The consensus refers to a stable but reversible, non-replicating state, with the necessity of an external stimulus for resuscitation (i.e. culturability) of 'truly' dormant cells a matter of debate. Interestingly, a recent report states that *M. tuberculosis* is also capable of forming complex biofilms

containing drug-tolerant cells that share important features with persister cells of other species (Ojha *et al.*, 2008). First, a small fraction of biofilm cells are insensitive even to antibiotics that target non-replicating bacteria, at drug concentrations of up to 100 times the MIC. Second, the presence of *M. tuberculosis* biofilm persisters, reflected by a plateau in the killing curve, becomes apparent only after an initial period of rapid cell death, giving rise to the biphasic killing pattern that is typical of persistence to antibiotics.

Previously, a correlation was observed between increased phenotypic tolerance of clinical isolates of *M. tuberculosis* and prolonged drug exposure *in vivo* (Wallis *et al.*, 1999). Building on the *P. aeruginosa* and *C. albicans* case studies described above, we anticipate that much more may be learned about the importance of mycobacterial persistence as defined in this review by analysis of longitudinal isolates from patients with chronic or recurrent *M. tuberculosis* infections. Importantly, a lot of effort has been devoted to the development of animal models for the *in vivo* study of tuberculosis, with the different variants reproducing specific aspects of human disease (Chao & Rubin, 2010). In the future, this could prove to be a huge advantage over current model organisms for persistence research, which are limited to *in vitro* experiments due to the lack of availability of a validated *in vivo* system.

Perspectives on anti-persister therapies

The dawning importance of persister cells in real-life settings has encouraged theoreticists and experimentalists alike to tackle the problem. Based on mathematical modelling of persistence, several authors have simulated the effect of varying antibiotic treatment protocols on bacterial survival. For example, De Leenheer & Cogan (2009) suggested that, due to the presence of persister cells, a bacterial population can be most effectively sterilized through periodic dosing of antibiotics. The optimal protocol entails specific treatment and withdrawal durations, dependent on bacterial kinetics. However, the practical utility of these predictions is limited by the need to experimentally determine model parameters, a highly challenging task in *in vivo* situations. Their value therefore seems to lie in guiding experimental design, more than in improving the outcome of chronic infections.

The experimentalist's approach to confront persistence is to unravel the underlying molecular mechanisms by identifying their genetic basis. These genes can then be used as drug targets in the development of specific anti-persister drugs. Hypothetically, several ways can be envisaged for drugs to defeat persistence. First, through direct killing of persister cells. This could be accomplished by an antibiotic that is also, or solely, active against dormant cells. The feasibility of finding such a molecule is underlined by a recent screening effort that resulted in the identification of compounds that almost exclusively kill non-replicating *M. tuberculosis* cells (Bryk *et al.*, 2008). A second option would be to resuscitate dormant persisters in order to sensitize them to the lethal action of conventional antibiotics. Several examples of stimuli promoting exit from dormancy have been described (Dworkin & Shah, 2010), although the existence of a persister-specific signal remains to be demonstrated. A third alternative is to prevent or decrease the formation of persister cells. Most persistence genes that have been identified to date are likely involved in persister formation and rational drug design aimed at these targets can therefore be expected to prevent rather than cure persistence.

Below, we will briefly discuss some of the mechanisms and genes that have been implicated in persistence and that may serve as targets for anti-persister drugs in the foreseeable future. An exhaustive overview of currently known persistence genes is given in Table 1.

Toxin–antitoxin modules

Toxin–antitoxin (TA) loci are primarily known as plasmid-borne operons that promote plasmid inheritance by the expression of a stable toxin, which is counteracted by an unstable antitoxin (Van Melderen, 2010). Genomic copies of TA modules have been found, but no clear function has been assigned to them yet. The discovery of the involvement of the predicted TA module *hipBA* in persistence (Moyed & Bertrand, 1983) encouraged researchers to explore the role of other TA modules. For many of the genomic TA modules in *E. coli*, a role in persistence was shown through deletion or overexpression of the corresponding genes (see Table 1). A second line of evidence supporting the involvement of TA modules in persistence comes from the expression profile of persister cells; TA modules were found to be highly overexpressed in persister cells compared to the rest of the population (Shah *et al.*, 2006). Below, the two most prominent TA loci involved in persistence, *hipBA* and *tisAB*, are discussed briefly.

hipBA. The *hip* locus was the first genetic locus described to be involved in persistence (Moyed & Bertrand, 1983; Scherrer & Moyed, 1988). Mutant allele *hipA7* increases the persister fraction from 10^{-6} to 10^{-2} without altering the MIC. Further investigation of the *hip* locus revealed that it consists of two genes, *hipA* and *hipB* (Black *et al.*, 1991; Moyed & Broderick, 1986). Biochemical and structural studies have shown that the toxin HipA is a member of the phosphatidylinositol 3/4-kinase superfamily and can phosphorylate the translation factor EF-Tu (Schumacher *et al.*, 2009). This kinase activity is required for the induction of the dormant state of the persister cells (Correia *et al.*, 2006). The *hipB* gene product is a small Cro-like protein with a helix–turn–helix DNA-binding domain. It functions as a repressor for the *hipBA* operon by binding to four operator sites on the promoter region of *hipBA* and inhibits HipA activity through covalently binding to the toxin (Black *et al.*, 1991, 1994;

Table 1. Exhaustive overview of currently known persistence genes

White fields are mutants for which the MIC values are not different from those for the wild-type and are true persistence mutants. Light-grey fields indicate that MIC values were not determined, therefore resistance cannot be ruled out. Dark-grey fields indicate that the MIC value is different from that for the wild-type, indicating that resistance may be involved.

Organism	Gene affected*	Type of mutation†	Fold change‡	Gene function/ mechanism	Reference PubMed ID
Mutations causing a decreased persister fraction					
Escherichia coli	*hipBA*	Knockout	↓ $10–10^2$	TA locus	15576765
Escherichia coli	*glpD*	Deletion	↓ 22–55	Phospholipid synthesis	16816185
Escherichia coli	*glpABC*	Deletion	↓ NS	Phospholipid synthesis	16816185
Escherichia coli	*plsB*	PlsB with higher K_m	↓ NS	Phospholipid synthesis	16816185
Escherichia coli	*dnaK*	Δ	↓ 11–111	Protein folding	18519731
Escherichia coli	*dksA*	Δ	↓ 6–40	Stringent response	18519731
Escherichia coli	*apaH*	Δ	↓ 2	Purine metabolism	18519731
Escherichia coli	*surA*	Δ	↓ 2.5	Protein folding	18519731
Escherichia coli	*ygfA*	Δ	↓ 3–82	Folate biosynthesis	18519731
Escherichia coli	*yigB*	Δ	↓ 10–30	Unknown	18519731
Escherichia coli	*hupB*	Δ	↓ NS	DNA binding in many processes	18519731
Escherichia coli	*hupAB*=HU null strain	Δ	↓ 40	DNA binding in many processes	18519731
Escherichia coli	*yafQ*	Δ	↓ 760–2400 in biofilm	TA locus	19307375
Escherichia coli	*tisAB*	Δ	↓ 10–1000	TA locus	20186264
Escherichia coli	*istR-2*	Δ	↓ 10–1000	TA locus	20186264
Escherichia coli	*lexA3*	Point mutation (no DNA repair)	↓ NS	SOS response	11822773
Escherichia coli	*recA13*	Point mutation (no DNA repair)	↓ NS	SOS response	11822773
Escherichia coli	*lexA3*	Point mutation (no DNA repair)	↓ NS	SOS response	20011100
Escherichia coli	*hhA*	Δ	↓ 4	Toxin	19909729§
Escherichia coli	*hokA*	Δ	↓ 3	Toxin	19909729§
Escherichia coli	*cspD*	Δ	↓ 2	Toxin	19909729§
Escherichia coli	*mqsR*	Δ	↓ 6	TA locus	19909729§
Escherichia coli	*mqsRA*	Δ	↓ 7	TA locus	19909729§
Escherichia coli	*ybfM*	Δ	↓ 30	Putative outer-membrane porin	19909729§
Escherichia coli	*hfq*	IPTG induced expression	↓ 2	RNA chaperone	19909729§
Escherichia coli	*recA*	Δ	↓ 40	DNA repair	20011100
Escherichia coli	*recB*	Δ	↓ 35–103	DNA repair	20011100
Escherichia coli	*xerC*	Δ	↓ 7	DNA repair	20011100
Escherichia coli	*xerD*	Δ	↓ 9	DNA repair	20011100
Escherichia coli	*relA spoT hipA7*	Δ	Loss of *hipA7* phenotype	Stringent response/TA locus	14622409
Escherichia coli	*relA hipA7*	Δ	↓ $10–10^2$	Stringent response/TA locus	14622409
Escherichia coli	*relA spoT*	Δ	↓ NS	Stringent response	14622409
Escherichia coli	*phoU*	Δ	↓ 2–10	Phosphate metabolism	17420206
Escherichia coli	*sucB*	Δ	↓ NS	Energy metabolism	20041955
Escherichia coli	*ubiF*	Δ	↓ NS	Energy metabolism	20041955
Mycobacterium tuberculosis	*phoY2*	Δ	↓ 10–30	Phosphate metabolism	20360062
Mycobacterium tuberculosis	*relE2*	Δ	↓ 7–8	TA locus	20061486

Table 1. cont.

Organism	Gene affected*	Type of mutation†	Fold change‡	Gene function/ mechanism	Reference PubMed ID
Mycobacterium tuberculosis	*relE3*	Δ	↓ 4–9	TA locus	20061486
Pseudomonas aeruginosa	*relA*	Δ	↓ 10	Stringent response	16625057
Pseudomonas aeruginosa	*relA spoT*	Δ	↓ 10	Stringent response	16625057
Pseudomonas aeruginosa	*rpoS*	Δ	↓ 40–70	Alternative sigma factor	15621433
Pseudomonas aeruginosa	*lasR*	Δ	↓ 42	Quorum sensing	19645822
Pseudomonas aeruginosa	*lasI*	Δ	↓ 48	Quorum sensing	19645822
Pseudomonas aeruginosa	*dinG*	Knockout	↓ 16	DNA replication?	19508279
Pseudomonas aeruginosa	*edpA*	Knockout	↓ 10–1000	Unknown	
Pseudomonas aeruginosa	*spuC*	Knockout	↓ 4	Putrescine and spermidine utilization	19508279
Pseudomonas aeruginosa	*PA14_17880*	Knockout	↓ 3	Fatty acid and phospholipid metabolism	19508279
Pseudomonas aeruginosa	*fosA*	+	↓ 2–5	Fosfomycin resistance	21212150
Pseudomonas aeruginosa	*glpT*	Δ	↓ 2	Fosfomycin resistance	21212150
Mutations causing an increased persister fraction					
Escherichia coli	*hipA7*	Point mutations: G22S and D291A	↑ 10^3	TA locus	6348026
Escherichia coli	*hipA*	IPTG induced expression	↑ 10	TA locus	9835528
Escherichia coli	Intergenic region *aldB-yiaW*	Δ	↑ 10^2	Non-coding DNA	15668009
Escherichia coli	*glpD*	Arabinose induced expression	↑ 4–10	Phospholipid synthesis	16816185
Escherichia coli	*ygfA*	+	↑ NS	Folate biosynthesis	18519731
Escherichia coli	*yigB*	+	↑ NS	Unknown	18519731
Escherichia coli	*ihfB*	Δ	↑ NS	DNA binding in many processes	18519731
Escherichia coli	*ihfAB*=IHF null strain	Δ	↑ NS	DNA binding in many processes	18519731
Escherichia coli	*yafQ*	+	↑ 3300–10^3	TA locus	19307375
Escherichia coli	*istR-1*	Δ	↑ 10–100	TA locus	20186264
Escherichia coli	*relE*	+	↑ 10–10^4	TA locus	15576765
Escherichia coli	*dnaJ*	+	↑ 10^2–10^3	Toxin	16672603
Escherichia coli	*pmrC* (*S. typhimurium*)	+	↑ 10^2–10^3	Toxin	16672603
Escherichia coli	*mazF/chpAI*	+	↑ 10^2–10^5	TA locus	16672603
Escherichia coli	*hhA*	IPTG induced expression	↑ NS	Toxin	19909729§
Escherichia coli	*hokA*	IPTG induced expression	↑ NS	Toxin	19909729§
Escherichia coli	*cspD*	IPTG induced expression	↑ NS	Toxin	19909729§
Escherichia coli	*mqsR*	+	↑ 12–16	TA locus	19909729§
Escherichia coli	*oppA*	+	↑ 12	Periplasmic oligopeptide-transport protein	19909729§
Escherichia coli	*ybfM*	+	↑ 28	Putative outer-membrane porin	19909729§
Escherichia coli	*hfq*	Knockout	↑ 11	RNA chaperone	19909729§
Mycobacterium tuberculosis	*relE2*	+	↑ 2–13	TA locus	20061486
Mycobacterium tuberculosis	*relE3*	+	↑ 15	TA locus	20061486
Pseudomonas aeruginosa	*spoT*	Knockout	↑ 30–42	Stringent response	16625057
Pseudomonas aeruginosa	*dksA*	Knockout	↑ 125	Stringent response	16625057
Pseudomonas aeruginosa	*rpoN*	Knockout	↑ 15	Alternative sigma factor	17261620

Table 1. cont.

Organism	Gene affected*	Type of mutation†	Fold change‡	Gene function/ mechanism	Reference PubMed ID
Pseudomonas aeruginosa	*rpoS*	+	↑: restores *lasR* phenotype	Alternative sigma factor	19645822
Pseudomonas aeruginosa	*lasR*	+	↑ 5	Quorum sensing	19645822
Pseudomonas aeruginosa	*PA4115*	Δ	↑ 20–48	Cadaverine synthesis	20855735
Pseudomonas aeruginosa	*PA14_13680*	Knockout	↑ 3	Unknown	19508279
Pseudomonas aeruginosa	*algR*	Knockout	↑ 3	Two-component regulator	19508279
Pseudomonas aeruginosa	*ycgM*	Knockout	↑ 9	Unknown	19508279
Pseudomonas aeruginosa	*pilH*	Knockout	↑ 17	Global regulator	19508279
Pseudomonas aeruginosa	*pheA*	Knockout	↑ 18	Phenylalanine biosynthesis and metabolism	19508279
Pseudomonas aeruginosa	*yfiR*	Δ	↑ NS	Putative signalling peptide	20300602
Streptococcus pneumoniae	*vcnS*	Knockout	↑ NS	Two-component regulator	10376600

*For more details on the strain characteristics, see references.
†Δ, Inactivation of the gene(s); +, overexpression.
‡↓, Decreased fraction; ↑, increased fraction; NS, numerical fold change not specified.
§According to personal communication with the authors, regrowth was not observed after 6 h of treatment.

Schumacher *et al.*, 2009). Upon exceeding a threshold level, unbound HipA triggers growth arrest and thus entry into the persistent state (Rotem *et al.*, 2010). Detailed knowledge of the crystal structure of HipA and the binding mechanism with HipB make this a very interesting target for the rational design of an anti-persistence therapy.

***tisAB*.** The SOS response is a stress response mechanism that has previously been implicated in persistence (Debbia *et al.*, 2001; Dörr *et al.*, 2009; Keren *et al.*, 2004b) and is induced by DNA damage, such as that caused by fluoroquinolone antibiotics. Because several TA modules are under the control of the SOS response, the role in persistence of one of these loci, *tisAB/istR*, was recently studied in more detail (Dörr *et al.*, 2010). TisB toxicity is inhibited by an antisense RNA antitoxin, IstR-1. TisA contains the binding site for this antisense RNA molecule (Vogel *et al.*, 2004). A Δ*tisAB* mutant displays a significantly decreased number of persister cells after treatment with ciprofloxacin, and the persister level increases equally in a Δ*istR* deletion mutant, which is correlated with an overexpression of the toxin TisB. A persistence mechanism mediated by a nucleic acid rather than proteins may offer novel perspectives for designing therapeutics, e.g. through the application of synthetic nucleic acid analogues (Karkare & Bhatnagar, 2006).

While HipA and TisB represent the best-studied persistence proteins, it is important to note that their potential as drug targets is restricted by a limited phylogenetic distribution. For example, neither *hipA* nor *tisB* is present in the genomes of *M. tuberculosis*, *P. aeruginosa* or *S. aureus* (M. Fauvart, unpublished).

Extracellular factors and surface modifications

Quorum sensing (QS) molecules are well-known extracellular factors that mediate cell–cell-communication and affect many bacterial processes. A recent study showed that cell-free spent medium induces persistence in *P. aeruginosa* strains, but not in *E. coli* or *S. aureus* (Möker *et al.*, 2010). Specifically, this effect could be attributed to the *P. aeruginosa* QS molecules pyocyanin and 3-OC12-homoserine lactone. LasR and LasI mutant strains of *P. aeruginosa*, which are disabled in their QS response, also display an aberrant persistence phenotype (Kayama *et al.*, 2009). Interestingly, *lasR* is one of the most frequently mutated genes in late isolates from *P. aeruginosa*-infected CF lungs (Smith *et al.*, 2006), possibly explaining the observed *hip* phenotype of these strains (Mulcahy *et al.*, 2010). Because of its crucial role in regulating clinically relevant processes such as biofilm formation and virulence, inhibition of QS as a non-lethal means of fighting infectious disease has been pursued for many years (Janssens *et al.*, 2008). It will therefore be interesting to find out whether such inhibitors also affect persistence.

The characterization of a mutant strain identified in our screening for *P. aeruginosa* persistence genes (De Groote *et al.*, 2009) revealed the involvement of surface-associated components in persistence (V. N. De Groote and others, unpublished). Mutation of *edpA* (for gene involved in the synthesis of an extracellular determinant of persistence; gene locus PA5002 in *P. aeruginosa* PAO1) resulted in a decrease in persisters of 10–1600-fold depending on medium composition. The *edpA* gene, conserved among *Pseudomonas* species though not in other genera, is located in a large gene cluster in which many known LPS synthesis genes are located. The mutant's defect in persistence is

likely modulated by an extracellular component, as complementation of the *edpA* mutant's low persistence phenotype was achieved by co-culturing the strain with the wild-type strain. EdpA is a member of the PIG-L superfamily of proteins, which all possess *N*-deacetylase activity on the *N*-acetylglucosamine moiety of their substrate. Currently, the native substrate of EdpA is unknown. Because of its predicted enzymic function, EdpA may serve as an attractive drug target, for example by *in vitro* screening for specific inhibitors of EdpA using small molecule libraries.

Importantly, these cases strongly suggest the possibility of inhibiting persister formation without the need for drugs to be taken up by the bacteria. This is especially relevant for Gram-negative pathogens with notoriously impermeable cell membranes, such as *P. aeruginosa* (Strateva & Yordanov, 2009).

Global regulators

Numerous global regulators have been linked to persistence (see Table 1 for details). For example, both in *E. coli* and in *P. aeruginosa*, these include *dksA*, *relA* and *spoT*, genes associated with the stringent response (Hansen *et al.*, 2008; Korch *et al.*, 2003; Viducic *et al.*, 2006). However, because of their simultaneous involvement in additional processes, their suitability as candidate drug targets remains to be determined.

A global regulator that might be promising as a persistence drug target is *phoU*, a negative regulator of the *pst* operon that is responsible for phosphate uptake (Lamarche *et al.*, 2008). An *E. coli phoU* mutant is more susceptible to a variety of antibiotics, probably because the cell is in a highly active state, which makes it more vulnerable to stress. In addition, stationary-phase cultures of a *phoU* mutant are completely sterilized after prolonged treatment with either ampicillin or norfloxacin, suggesting that no persisters are present (Li & Zhang, 2007). The *phoU* gene is highly conserved among bacteria, with homologues present in *M. tuberculosis*, *P. aeruginosa* and *S. aureus*. Interestingly, a *M. tuberculosis* mutant affected in the corresponding *phoY2* gene also displays low persistence, *in vitro* as well as *in vivo* (Shi & Zhang, 2010). This could promote PhoU/PhoY2 to a broad-spectrum drug target in the battle against persistence.

Antibiotic resistance mechanisms

Great emphasis is often placed on the distinction between antibiotic resistance and persister tolerance (Lewis, 2010). Surprisingly then, a recent study revealed the involvement of antibiotic resistance mechanisms in persistence. Knockout mutations of *fosA* and *glpT*, both resulting in increased resistance against fosfomycin, gave rise to decreased persistence (De Groote *et al.*, 2011). GlpT was originally identified as a glycerol-3-phosphate (G3P) transporter in *E. coli*, but is also able to transport glycerol 2-phosphate (G2P) and fosfomycin into the bacterial cell, illustrating its relatively low substrate specificity (Law *et al.*, 2009). As the observations were made in the absence of fosfomycin, one or more other substrates transported by GlpT are likely to be involved in persistence. G2P, G3P or P_i are possible candidates, but considering the broad substrate specificity of GlpT, other phosphates or phosphonates could also be possible inducers of persistence. Interestingly, preliminary results indicate that adding fosfomcyin to growing cultures of *P. aeruginosa* causes an increase in persistence (V. De Groote, unpublished), providing further evidence for crosstalk between resistance and persistence mechanisms.

Intriguingly, G3P, imported by the fosfomycin transporter GlpT, was previously implicated in persistence. Screening of an *E. coli* overexpression library led to the discovery of *glpD* and *plsB* as persistence genes (Spoering *et al.*, 2006). GlpD is a G3P dehydrogenase involved in the catabolism of glycerol. PlsB converts G3P to 1-acyl-G3P, an essential precursor of the phospholipid biosynthesis pathway (Lin, 1976). Possibly, these enzymes affect persister formation or resuscitation by modulating cellular G3P levels (Spoering *et al.*, 2006). If and how these findings are connected to fosfomycin resistance remains to be determined.

In addition to supplying candidate persister drug targets, the above also serves as a caveat: selective pressure exerted by antibiotic treatment may inadvertently also affect persister levels.

Conclusions and perspectives

Chronic infectious diseases are a major challenge in present-day global health care. The spread of multidrug-resistant strains and the lack of novel antibiotics for the foreseeable future mean that this is likely to remain the case for some time to come. The problem of antibiotic resistance is compounded by the presence of persister cells, which, although formed in small numbers and only transiently so, are virtually impossible to kill with conventional drugs. Their importance in treatment failure is increasingly being recognized, fuelling efforts to develop specific anti-persister drugs. This will undoubtedly enhance our understanding of microbial infections and, hopefully, result in better treatment options in the future.

Persistence research may also benefit other areas of health care. Indeed, cancer cells often exhibit a heterogeneous response to drug treatment in which a minority of cells are unaffected, sometimes referred to as 'fractional killing' and requiring multiple rounds of chemotherapy for complete curing (Chabner, 2006). Recently, this observation was attributed to the presence of reversibly drug-tolerant cells, also called persisters (Sharma *et al.*, 2010). Moreover, as has been shown for bacterial persister cells (Rotem *et al.*, 2010), the cause for this heterogeneity may lie in natural cell-to-cell variations of protein levels, rather than in differences in genotype or cell cycle state (Spencer *et al.*,

2009). It is not unlikely that insights gained by studying either of these seemingly radically different model systems will ultimately lead to advances in controlling both.

In spite of the major progress that has been realized in the study of persistence in the last decade, several obstacles remain before clinical applications can be realized. For instance, little is known about the functional conservation of persistence mechanisms in distantly related bacteria, a prerequisite for designing broad-spectrum anti-persister drugs. As indicated above, this is not merely a question of performing the relevant experiments, given the limited phylogenetic distribution of most validated persistence genes identified to date. Another matter of concern is the possible interplay between antibiotic resistance and persistence mechanisms. Although evidence for the generality of such crosstalk is yet to emerge, caution is clearly warranted. Once a candidate drug has been identified, animal models are required for determining *in vivo* activity and for carrying out preclinical tests. With the exception of *M. tuberculosis*, no validated models for studying persistence *in vivo* have been described, representing a major challenge for the near future.

In conclusion, many questions concerning persistence to antibiotics are still left unanswered. However, realizing the importance of persistence in chronic infections will surely invigorate research into the matter, and as our fundamental understanding of the process improves, so will ideas for subverting it emerge.

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References

Balaban, N. Q., Merrin, J., Chait, R., Kowalik, L. & Leibler, S. (2004). Bacterial persistence as a phenotypic switch. *Science* **305**, 1622–1625.

Black, D. S., Kelly, A. J., Mardis, M. J. & Moyed, H. S. (1991). Structure and organization of *hip*, an operon that affects lethality due to inhibition of peptidoglycan or DNA synthesis. *J Bacteriol* **173**, 5732–5739.

Black, D. S., Irwin, B. & Moyed, H. S. (1994). Autoregulation of *hip*, an operon that affects lethality due to inhibition of peptidoglycan or DNA synthesis. *J Bacteriol* **176**, 4081–4091.

Bloom, B. R. & Murray, C. J. (1992). Tuberculosis: commentary on a reemergent killer. *Science* **257**, 1055–1064.

Boucher, R. C. (2001). Pathogenesis of cystic fibrosis airways disease. *Trans Am Clin Climatol Assoc* **112**, 99–107.

Bryk, R., Gold, B., Venugopal, A., Singh, J., Samy, R., Pupek, K., Cao, H., Popescu, C., Gurney, M. & Hotha, S. (2008). Selective killing of nonreplicating mycobacteria. *Cell Host Microbe* **3**, 137–145.

Burns, J. L., Van Dalfsen, J. M., Shawar, R. M., Otto, K. L., Garber, R. L., Quan, J. M., Montgomery, A. B., Albers, G. M., Ramsey, B. W. & Smith, A. L. (1999). Effect of chronic intermittent administration of inhaled tobramycin on respiratory microbial flora in patients with cystic fibrosis. *J Infect Dis* **179**, 1190–1196.

Chabner, B. A. (2006). Clinical strategies for cancer treatment: the role of drugs. In *Cancer Chemotherapy and Biotherapy: Principles and Practices*, 4th edn, pp. 1–14. Edited by B. A. Chabner & D. L. Longo. Philadelphia, PA: Lippincott Williams & Wilkins.

Chandra, J., Patel, J. D., Li, J., Zhou, G., Mukherjee, P. K., McCormick, T. S., Anderson, J. M. & Ghannoum, M. A. (2005). Modification of surface properties of biomaterials influences the ability of *Candida albicans* to form biofilms. *Appl Environ Microbiol* **71**, 8795–8801.

Chao, M. C. & Rubin, E. J. (2010). Letting sleeping *dos* lie: does dormancy play a role in tuberculosis? *Annu Rev Microbiol* **64**, 293–311.

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

Debbia, E. A., Roveta, S., Schito, A. M., Gualco, L. & Marchese, A. (2001). Antibiotic persistence: the role of spontaneous DNA repair response. *Microb Drug Resist* **7**, 335–342.

De Groote, V. N., Verstraeten, N., Fauvart, M., Kint, C. I., Verbeeck, A. M., Beullens, S., Cornelis, P. & Michiels, J. (2009). Novel persistence genes in *Pseudomonas aeruginosa* identified by high-throughput screening. *FEMS Microbiol Lett* **297**, 73–79.

De Groote, V. N., Fauvart, M., Kint, C. I., Verstraeten, N., Jans, A., Cornelis, P. & Michiels, J. (2011). *Pseudomonas aeruginosa* fosfomycin resistance mechanisms affect non-inherited fluoroquinolone tolerance. *J Med Microbiol* **60**, 329–336.

De Leenheer, P. & Cogan, N. G. (2009). Failure of antibiotic treatment in microbial populations. *J Math Biol* **59**, 563–579.

Dhar, N. & McKinney, J. D. (2007). Microbial phenotypic heterogeneity and antibiotic tolerance. *Curr Opin Microbiol* **10**, 30–38.

Dörr, T., Lewis, K. & Vulić, M. (2009). SOS response induces persistence to fluoroquinolones in *Escherichia coli*. *PLoS Genet* **5**, e1000760.

Dörr, T., Vulić, M. & Lewis, K. (2010). Ciprofloxacin causes persister formation by inducing the TisB toxin in *Escherichia coli*. *PLoS Biol* **8**, e1000317.

Dworkin, J. & Shah, I. M. (2010). Exit from dormancy in microbial organisms. *Nat Rev Microbiol* **8**, 890–896.

Emerson, R. J., IV & Camesano, T. A. (2004). Nanoscale investigation of pathogenic microbial adhesion to a biomaterial. *Appl Environ Microbiol* **70**, 6012–6022.

Fung, D. K., Chan, E. W., Chin, M. L. & Chan, R. C. (2010). Delineation of a bacterial starvation stress response network which can mediate antibiotic tolerance development. *Antimicrob Agents Chemother* **54**, 1082–1093.

Gefen, O. & Balaban, N. Q. (2009). The importance of being persistent: heterogeneity of bacterial populations under antibiotic stress. *FEMS Microbiol Rev* **33**, 704–717.

Gibson, R. L., Burns, J. L. & Ramsey, B. W. (2003). Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am J Respir Crit Care Med* **168**, 918–951.

Gilligan, P. H. (2006). Is there value in susceptibility testing of *Pseudomonas aeruginosa* causing chronic infection in patients with cystic fibrosis? *Expert Rev Anti Infect Ther* **4**, 711–715.

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

Harrison, F. (2007). Microbial ecology of the cystic fibrosis lung. *Microbiology* **153**, 917–923.

Hu, Y. & Coates, A. R. M. (2003). Tuberculosis. In *Dormancy and Low-Growth States in Microbial Disease*, pp. 181–208. Edited by A. R. M. Coates. New York: Cambridge University Press.

Janssens, J. C., De Keersmaecker, S. C., De Vos, D. E. & Vanderleyden, J. (2008). Small molecules for interference with cell-cell-communication systems in Gram-negative bacteria. *Curr Med Chem* **15**, 2144–2156.

Jayaraman, R. (2008). Bacterial persistence: some new insights into an old phenomenon. *J Biosci* **33**, 795–805.

Karkare, S. & Bhatnagar, D. (2006). Promising nucleic acid analogs and mimics: characteristic features and applications of PNA, LNA, and morpholino. *Appl Microbiol Biotechnol* **71**, 575–586.

Kayama, S., Murakami, K., Ono, T., Ushimaru, M., Yamamoto, A., Hirota, K. & Miyake, Y. (2009). The role of *rpoS* gene and quorum-sensing system in ofloxacin tolerance in *Pseudomonas aeruginosa*. *FEMS Microbiol Lett* **298**, 184–192.

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

Keren, I., Shah, D., Spoering, A., Kaldalu, N. & Lewis, K. (2004b). Specialized persister cells and the mechanism of multidrug tolerance in *Escherichia coli*. *J Bacteriol* **186**, 8172–8180.

Korch, S. B., Henderson, T. A. & Hill, T. M. (2003). Characterization of the *hipA7* allele of *Escherichia coli* and evidence that high persistence is governed by (p)ppGpp synthesis. *Mol Microbiol* **50**, 1199–1213.

LaFleur, M. D., Kumamoto, C. A. & Lewis, K. (2006). *Candida albicans* biofilms produce antifungal-tolerant persister cells. *Antimicrob Agents Chemother* **50**, 3839–3846.

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

Lamarche, M. G., Wanner, B. L., Crépin, S. & Harel, J. (2008). The phosphate regulon and bacterial virulence: a regulatory network connecting phosphate homeostasis and pathogenesis. *FEMS Microbiol Rev* **32**, 461–473.

Law, C. J., Enkavi, G., Wang, D. N. & Tajkhorshid, E. (2009). Structural basis of substrate selectivity in the glycerol-3-phosphate: phosphate antiporter GlpT. *Biophys J* **97**, 1346–1353.

Levin, B. R. & Rozen, D. E. (2006). Non-inherited antibiotic resistance. *Nat Rev Microbiol* **4**, 556–562.

Lewis, K. (2007). Persister cells, dormancy and infectious disease. *Nat Rev Microbiol* **5**, 48–56.

Lewis, K. (2010). Persister cells. *Annu Rev Microbiol* **64**, 357–372.

Li, Y. & Zhang, Y. (2007). PhoU is a persistence switch involved in persister formation and tolerance to multiple antibiotics and stresses in *Escherichia coli*. *Antimicrob Agents Chemother* **51**, 2092–2099.

Lin, E. C. (1976). Glycerol dissimilation and its regulation in bacteria. *Annu Rev Microbiol* **30**, 535–578.

Lyczak, J. B., Cannon, C. L. & Pier, G. B. (2002). Lung infections associated with cystic fibrosis. *Clin Microbiol Rev* **15**, 194–222.

Möker, N., Dean, C. R. & Tao, J. (2010). *Pseudomonas aeruginosa* increases formation of multidrug-tolerant persister cells in response to quorum-sensing signaling molecules. *J Bacteriol* **192**, 1946–1955.

Moyed, H. S. & Bertrand, K. P. (1983). *hipA*, a newly recognized gene of *Escherichia coli* K-12 that affects frequency of persistence after inhibition of murein synthesis. *J Bacteriol* **155**, 768–775.

Moyed, H. S. & Broderick, S. H. (1986). Molecular cloning and expression of *hipA*, a gene of *Escherichia coli* K-12 that affects frequency of persistence after inhibition of murein synthesis. *J Bacteriol* **166**, 399–403.

Mulcahy, L. R., Burns, J. L., Lory, S. & Lewis, K. (2010). Emergence of *Pseudomonas aeruginosa* strains producing high levels of persister cells in patients with cystic fibrosis. *J Bacteriol* **192**, 6191–6199.

Ojha, A. K., Baughn, A. D., Sambandan, D., Hsu, T., Trivelli, X., Guerardel, Y., Alahari, A., Kremer, L., Jacobs, W. R., Jr & Hatfull, G. F. (2008). Growth of *Mycobacterium tuberculosis* biofilms containing free mycolic acids and harbouring drug-tolerant bacteria. *Mol Microbiol* **69**, 164–174.

Ramage, G., Martínez, J. P. & López-Ribot, J. L. (2006). *Candida* biofilms on implanted biomaterials: a clinically significant problem. *FEMS Yeast Res* **6**, 979–986.

Roberts, M. E. & Stewart, P. S. (2005). Modelling protection from antimicrobial agents in biofilms through the formation of persister cells. *Microbiology* **151**, 75–80.

Rotem, E., Loinger, A., Ronin, I., Levin-Reisman, I., Gabay, C., Shoresh, N., Biham, O. & Balaban, N. Q. (2010). Regulation of phenotypic variability by a threshold-based mechanism underlies bacterial persistence. *Proc Natl Acad Sci U S A* **107**, 12541–12546.

Scherrer, R. & Moyed, H. S. (1988). Conditional impairment of cell division and altered lethality in *hipA* mutants of *Escherichia coli* K-12. *J Bacteriol* **170**, 3321–3326.

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

Shah, D., Zhang, Z., Khodursky, A., Kaldalu, N., Kurg, K. & Lewis, K. (2006). Persisters: a distinct physiological state of *E. coli*. *BMC Microbiol* **6**, 53.

Sharma, S. V., Lee, D. Y., Li, B., Quinlan, M. P., Takahashi, F., Maheswaran, S., McDermott, U., Azizian, N., Zou, L. & Fischbach, M. A. (2010). A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* **141**, 69–80.

Shi, W. & Zhang, Y. (2010). PhoY2 but not PhoY1 is the PhoU homologue involved in persisters in *Mycobacterium tuberculosis*. *J Antimicrob Chemother* **65**, 1237–1242.

Smith, E. E., Buckley, D. G., Wu, Z., Saenphimmachak, C., Hoffman, L. R., D'Argenio, D. A., Miller, S. I., Ramsey, B. W., Speert, D. P. & other authors (2006). Genetic adaptation by *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients. *Proc Natl Acad Sci U S A* **103**, 8487–8492.

Spencer, S. L., Gaudet, S., Albeck, J. G., Burke, J. M. & Sorger, P. K. (2009). Non-genetic origins of cell-to-cell variability in TRAIL-induced apoptosis. *Nature* **459**, 428–432.

Spoering, A. L. & Lewis, K. (2001). Biofilms and planktonic cells of *Pseudomonas aeruginosa* have similar resistance to killing by antimicrobials. *J Bacteriol* **183**, 6746–6751.

Spoering, A. L., Vulic, M. & Lewis, K. (2006). GlpD and PlsB participate in persister cell formation in *Escherichia coli*. *J Bacteriol* **188**, 5136–5144.

Strateva, T. & Yordanov, D. (2009). *Pseudomonas aeruginosa* – a phenomenon of bacterial resistance. *J Med Microbiol* **58**, 1133–1148.

Tuomanen, E., Durack, D. T. & Tomasz, A. (1986). Antibiotic tolerance among clinical isolates of bacteria. *Antimicrob Agents Chemother* **30**, 521–527.

Van Melderen, L. (2010). Toxin-antitoxin systems: why so many, what for? *Curr Opin Microbiol* **13**, 781–785.

Viducic, D., Ono, T., Murakami, K., Susilowati, H., Kayama, S., Hirota, K. & Miyake, Y. (2006). Functional analysis of *spoT*, *relA* and

dksA genes on quinolone tolerance in *Pseudomonas aeruginosa* under nongrowing condition. *Microbiol Immunol* **50**, 349–357.

Vogel, J., Argaman, L., Wagner, E. G. & Altuvia, S. (2004). The small RNA IstR inhibits synthesis of an SOS-induced toxic peptide. *Curr Biol* **14**, 2271–2276.

Wallis, R. S., Patil, S., Cheon, S. H., Edmonds, K., Phillips, M., Perkins, M. D., Joloba, M., Namale, A., Johnson, J. L. & other authors (1999). Drug tolerance in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* **43**, 2600–2606.

Wayne, L. G. & Sohaskey, C. D. (2001). Nonreplicating persistence of *Mycobacterium tuberculosis*. *Annu Rev Microbiol* **55**, 139–163.

WHO (2009). *WHO Report 2009: Global Tuberculosis Control. Epidemiology, Strategy, Financing*. Geneva, Switzerland: World Health Organization Press.

Zhang, Y. (2004). Persistent and dormant tubercle bacilli and latent tuberculosis. *Front Biosci* **9**, 1136–1156.

Zhang, Y. (2005). The magic bullets and tuberculosis drug targets. *Annu Rev Pharmacol Toxicol* **45**, 529–564.