Antibiotic tolerance in bacteria: how to regain susceptibility?

Bacteria use a plethora of mechanisms to evade killing by antibiotics. Resistance is the best documented mechanism. Here, genetic changes in the bacterial DNA result in antibiotic insensitivity. As a consequence, resistant bacteria are able to grow in the presence of the antibiotic. Another, less known, mechanism, allowing bacterial populations to evade eradication by antibiotics, is the formation of persister cells. Indeed, bacterial populations contain a subfraction of cells, called persisters, that are transiently antibiotic-tolerant. These persisters do not grow, but they can withstand otherwise lethal concentrations of antibiotics. When the antibiotic pressure drops, persister cells that exit the persister state are able to recolonize the environment (Fig. 1). Importantly, this new population is antibiotic-sensitive, but will again harbor a subfraction of persister cells. This phenomenon complicates complete bacterial clearance when considering a bacterial infection. While the immune system of healthy persons is capable of eradicating persister cells, this is not necessarily the case in immunocompromised patients or when the bacteria occupy niches difficult to reach by the immune system. Indeed, the presence of persister cells has been linked with the recalcitrance of several chronic infections, such as recurrent lung infections in cystic fibrosis patients or chronic urinary tract infections. In addition, persisters constitute a pool from which resistant mutants can emerge. Despite their clinical importance, there is no effective treatment available to date to clear persister cells present at the site of infection.

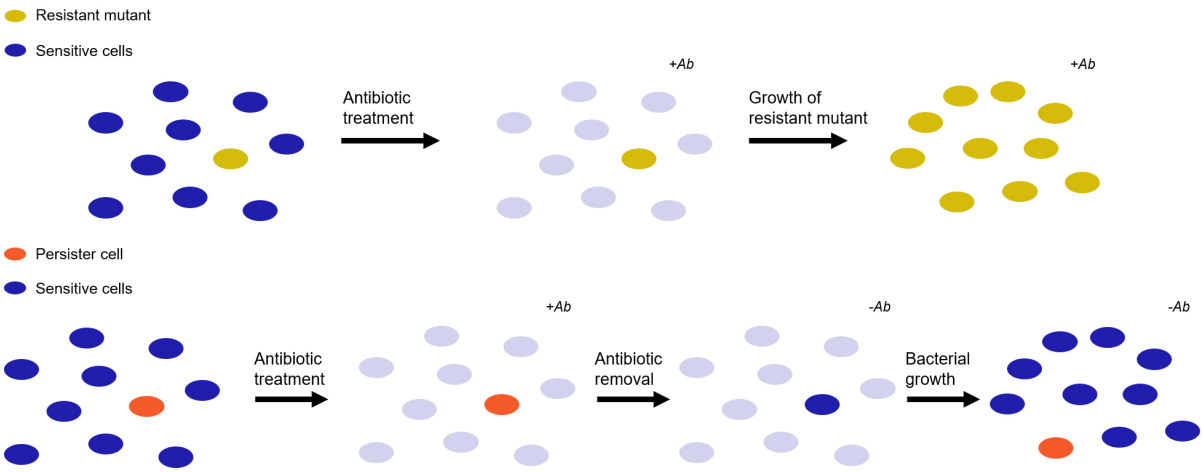

Fig. 1. Difference between resistance and persistence. In contrast to persister cells, resistant mutants can grow in the presence of the antibiotic. When the antibiotic is removed from the environment, persisters that leave the persister state can regrow and form a new bacterial population.

The antibiotic tolerant state of persister cells results from physiological differences with their sensitive kin. Several processes have been shown to lead to the induction of the persister state,

such as energy depletion, decreased activity of macromolecular processes such as protein synthesis, reduced antibiotic uptake and repair of antibiotic-induced damage. In contrast, molecular mechanisms underlying exit from the persister state are largely uncharted. One of the most promising strategies to eliminate persisters is by triggering persister state exit right before antibiotic treatment, as this exit leads to antibiotic susceptibility. To learn more about persistence, we used a model system by expressing the small toxic peptide HokB to induce persistence. Next, we investigated how these persisters are formed, and how they escape from the persister state.

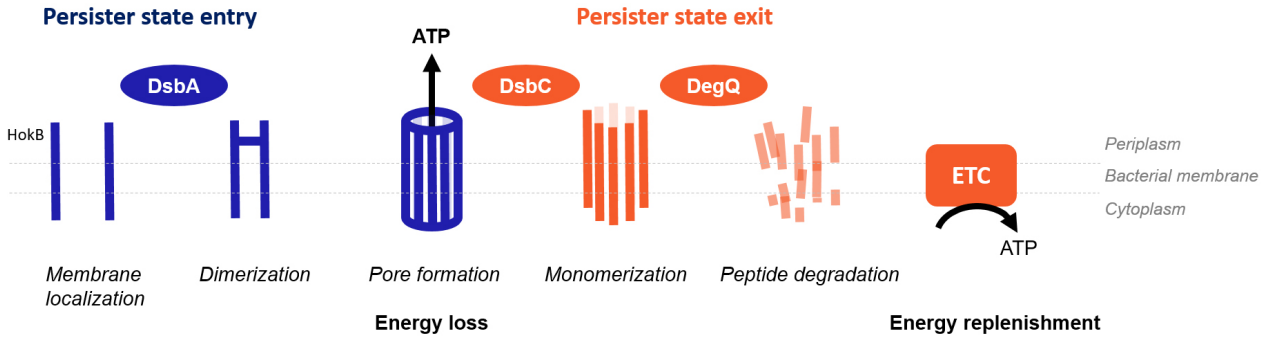

Fig. 2. Model for persister state entry and exit of persisters induced by the HokB peptide. HokB peptides insert into the bacterial membrane, after which the oxidoreductase DsbA forms peptide dimers. These dimers form a pore, through which intracellular ATP leaves the cytoplasm concomitantly with the induction of the persister state. In order to exit the persister state, DsbC removes the disulfide bridge, monomerizing HokB which is subsequently degraded by the protease DegQ. Ultimately, the electron transport chain (ETC) replenishes cellular energy levels.

HokB is a small peptide toxin that localizes in the bacterial membrane. We demonstrated that HokB peptides are stabilized via the formation of a disulfide bridge between two HokB peptides, which is mediated by the periplasmic oxidoreductase DsbA. HokB dimers form a pore in the bacterial inner membrane, resulting in the leakage of ATP and, at the same time, the formation of the persister cell. Persister state exit is orchestrated by the monomerization of these peptide dimers, mediated by the periplasmic oxidoreductase DsbC. After monomerization, the periplasmic protease DegQ degrades the HokB peptides. Eventually, the electron transport chain replenishes cellular energy levels (Fig. 2). Combined, the HokB-persister pathway is one of the best-resolved persistence pathways to date. A detailed understanding of awakening mechanisms could open up novel avenues for the treatment of bacterial infections.

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Publication

HokB Monomerization and Membrane Repolarization Control Persister Awakening
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