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Microbiology: Diversity breeds tolerance

David G. Russell

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A gene has been identified that underpins the capacity of mycobacterial cells to divide to produce physiologically different daughter cells. This finding has implications for drug treatment of tuberculosis. See Letter **p.153**

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Infection with *Mycobacterium tuberculosis*, the mycobacterial species responsible for tuberculosis, is one of the leading causes of death from infectious disease, and a frequent cause of death [1] for people with HIV. The ability of *M. tuberculosis* to cope with a range of environmental extremes, as well as its capacity to overcome many host immune defences and drug regimens, is central to its success as a pathogen. One tactic used by *M. tuberculosis* to survive such challenges is the generation of heterogeneity — a process whereby bacterial cells divide to produce daughter cells that are genetically identical but exhibit physiological differences. Heterogeneity within a bacterial population ensures that subsets of bacteria are better equipped to survive a wide range of environmental stresses. On page 153, Rego *et al*.[2] detail the genetic basis of a process that generates diversity and variable drug sensitivity in a replicating population of mycobacterial cells.

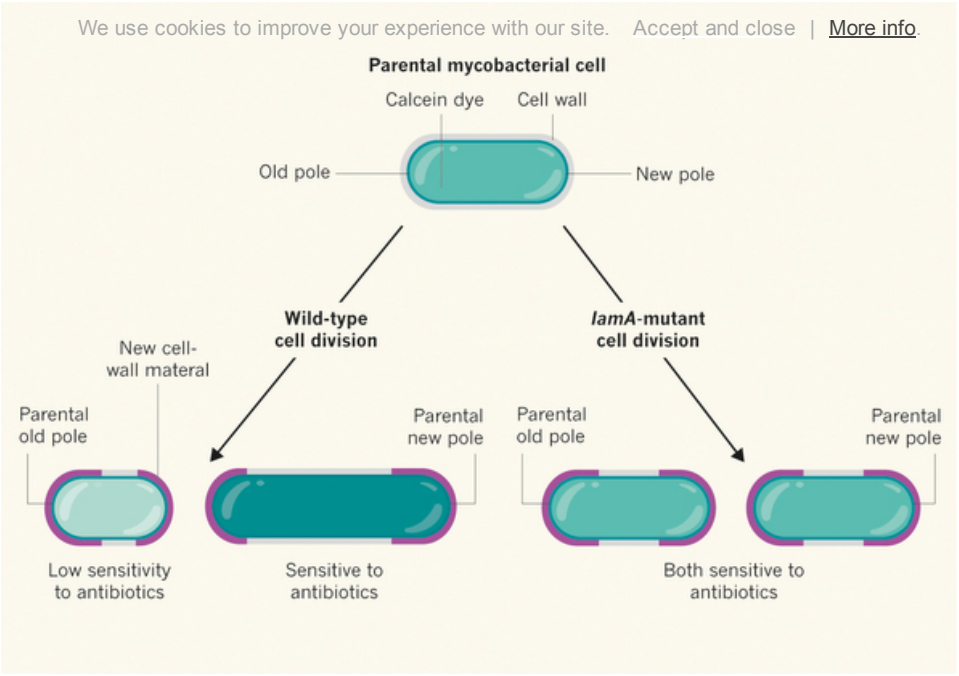
Bacteria in the *M. tuberculosis* population of an infected individual might have differential drug sensitivity, which can arise through three mechanisms[3]. 'Persistent' and 'resistant' bacteria arise from two of the three mechanisms, and these types of bacterium are the least sensitive to antibiotics. Persisters are present in extremely low numbers in all bacterial populations examined thus far[4]. They are usually non-replicating cells that have low metabolic activity and can survive exposure to antibiotic concentrations that kill the other cells in the bacterial population. Resistant bacteria have heritable traits derived from one or more mutations in genes whose products are required for maximum antibiotic efficacy. The acquisition of such mutations can lead to the generation of stable, drug-resistant bacterial strains.

A third type of reduced drug sensitivity can occur because some bacteria in a population have the capacity to survive transient exposure to antibiotic concentrations that would usually be lethal. This phenomenon, known as phenotypic tolerance, is promoted by the heterogeneity of a bacterial population. Moreover, this form of drug insensitivity is arguably the greatest hurdle to effective chemotherapy. In contrast to persistence and resistance, phenotypic tolerance involves a substantial proportion of bacteria in a given population exhibiting low sensitivity to drug exposure[3].

Heterogeneity — including differences in cell size and growth rate — has been documented in replicating populations of *M. tuberculosis* previously[5, 6]. Rego and colleagues sought to investigate the molecular basis of this heterogeneity by studying a related mycobacterial species called *Mycobacterium smegmatis*, using a fluorescent dye called calcein to monitor heterogeneity.

Rego *et al*. observed that calcein was homogeneously distributed in a parental cell, but was acquired unevenly by the two daughter cells upon division. The ends of a bacterial cell are known as the old and new poles (Fig. 1). The authors noted that more calcein was distributed to the daughter cell that inherited the parental cell's new pole. Intriguingly, Rego and colleagues observed that these highly fluorescent daughter cells had increased sensitivity to the antibiotic rifampicin, which is used in tuberculosis treatment, compared with the daughter cells that had low fluorescence and inherited the parental cell's old pole.

Figure 1: Heterogeneity in bacterial-cell populations.



Mycobacterium tuberculosis, the causative agent of tuberculosis, is often insensitive to drug treatment because of heterogeneity within the bacterial population[5, 6]. Rego *et al*.[2] investigated the molecular basis of this heterogeneity, starting with the observation that a fluorescent dye, calcein, is distributed unequally between the daughter cells of the species *Mycobacterium smegmatis*. The ends of a bacterial cell are called the old pole and the new pole. Rego and colleagues observed that, in wild-type cell division, the daughter cell that inherited the parental cell's old pole had low fluorescence and exhibited low sensitivity to antibiotic treatment, whereas the daughter cell that inherited the parental cell's new pole had high fluorescence and was sensitive to antibiotics. This type of differential sensitivity to antibiotics is called phenotypic tolerance. The authors found that a mutation in the gene *lamA* resulted in cell division that produced daughter cells with similar levels of fluorescence and similar sensitivity to antibiotic treatment. Rego *et al*. observed that, in dividing wild-type cells, newly synthesized cell-wall material (purple) is not added equally to the two poles, whereas in a *lamA* mutant, the cell-wall material is added more equally.

The authors screened for genetic mutants that were defective in the generation of heterogeneous progeny by monitoring levels of calcein fluorescence. They found that the progeny of *lamA*-mutant bacteria were similarly fluorescent and similarly sensitive to antibiotic challenge. Rego *et al*. observed that, in comparison to wild-type bacteria, the *lamA*-mutant bacteria showed deposition of newly synthesized cell-wall material to both poles of the cell that was more equal. The *lamA* gene is unique to the genus *Mycobacterium*.

The authors created a version of LamA protein whose location in the cell could be monitored by a fluorescent tag. LamA was observed at the site of bacterial cell-wall division. Using other tests, the authors established that LamA is part of the protein complex known as the 'divisome' that enables cell division, and found that LamA associates with the PonA1 protein, which is known to be involved in building the cell wall during replication.

Rego and colleagues' analysis indicates that LamA is required to modify the mycobacterial cell wall in such a way that, in a single round of replication, half the daughter cells exhibit phenotypic drug tolerance. The mechanisms responsible for this differential drug sensitivity remain to be determined. This genetically encoded capacity to generate heterogeneity at each round of replication could confer a huge advantage for the maintenance of *M. tuberculosis* populations under drug pressure. The authors observed that, when treated with antibiotics, *lamA*-mutant *M. tuberculosis* cells were killed at a more uniform rate than wild-type *M. tuberculosis* cells.

“The mechanisms responsible for this differential drug sensitivity remain to be determined.”

Why would a process enabling the generation of heterogeneity in bacterial progeny through the action of LamA be found in *Mycobacterium* species? We might think of drug tolerance and drug resistance as properties emergent from modern-day antibiotic usage, but antibiotics are natural products that are particularly abundant in soil microbes. Ancestral mycobacterial species inhabiting such environmental niches would have encountered many antibiotic molecules and experienced selective pressures to acquire mechanisms of tolerance and resistance long before bacteria such as *M. tuberculosis* needed to evolve in response to modern chemotherapy.

Understanding the basis of heterogeneity might provide insight into the interplay between host immune-derived pressures and their effect on current antituberculosis treatments. Rego and colleagues' research reveals one mechanism by which heterogeneity might be generated among replicating *M. tuberculosis*, but it is certainly not the only mechanism, and the situation is undoubtedly more complex in an *in vivo* infection. Host-derived stresses and functional immune responses are known to drive an increase in heterogeneity in *M. tuberculosis*[7]. Furthermore, *M. tuberculosis* bacteria in activated host immune cells called phagocytes exhibit enhanced drug tolerance to frontline antituberculosis compounds compared with bacteria in non-activated phagocytes[8]. One could speculate that the daughter cells generated by the action of LamA that had low calcein fluorescence could be at an advantage in dealing with both host-cell-induced stress and drug pressure *in vivo*.

The pathways to drug tolerance may differ in their underlying mechanisms, but the consequences are comparable. Drug tolerance caused by heterogeneity is probably a major driver of the evolution of heritable drug resistance[9] because heterogeneity and drug tolerance promote bacterial survival and thereby expand the window of opportunity for the acquisition of mutations that reduce antibiotic efficacy.

Although the prospects for developing successful treatments for tuberculosis remain daunting, understanding the biological basis of drug tolerance might open new avenues through which antibiotics could be used in combination with compounds that block the capacity of *M. tuberculosis* to promote heterogeneity or mobilize its drug-tolerance programs[10]. Drug-discovery approaches need to move beyond the landscape of target-based screening and embrace a discovery process that incorporates and tests the complex biology of bacterial heterogeneity and drug tolerance that occurs *in vivo*.

Notes

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Author information

Affiliations

David G. Russell is in the Department of Microbiology and Immunology, Cornell University, Ithaca, New York 14853, USA.

Corresponding author

Correspondence to: David G. Russell

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