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ods can provide more precise information on biological systems. It also clearly shows how multiple experimental techniques provide complementary information, allowing inferences to be drawn that could not be derived from results based on a single technique. The new results give hope that by the end of this decade, most of the mysteries of nitrogenase will be unveiled to enable the design of

benign catalysts for the conversion of atmospheric N_2 to biologically accessible forms of nitrogen.

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MICROBIOLOGY

Antioxidant Strategies to Tolerate Antibiotics

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In living organisms, aerobic metabolism produces toxic reactive oxygen species (ROS) (1). Life can thus be seen as a balance between metabolic rate and a cell's ability to detoxify ROS. This understanding has led to intense public interest and increased consumption of dietary antioxidants. Although the effectiveness of antioxidant supplements is not yet established, there is no doubt that eukaryotic and prokaryotic cells have developed efficient endogenous antioxidant mechanisms (1, 2). On pages 982 and 986 of this issue, Nguyen *et al.* (3) and Shatalin *et al.* (4) describe two such mechanisms that confer antibiotic tolerance in bacteria.

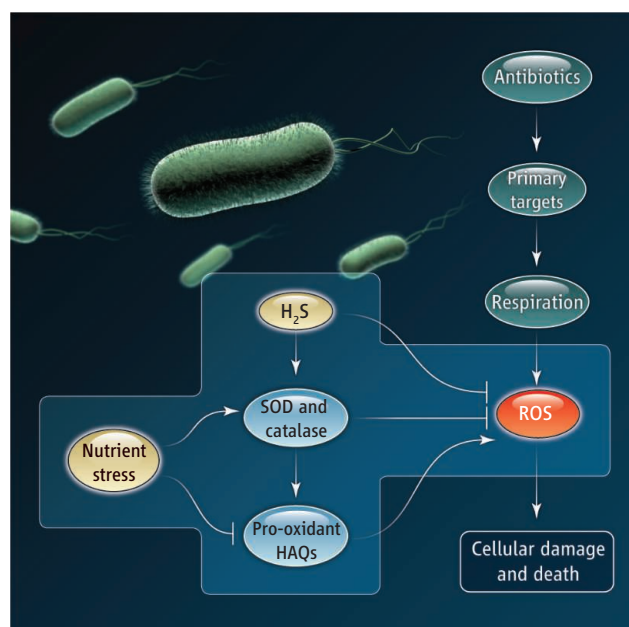
It has been proposed that bactericidal antibiotics can induce cellular death through a common oxidative damage mechanism that relies on the production of ROS (see the figure). Through their various primary targets, antibiotics can activate cellular respiration, which leads to the formation of superoxide and the release of iron from iron-sulfur clusters (5–7). Free iron then activates a chemical reaction (the Fenton reaction) to produce ROS in the form of hydroxyl radicals (OH^\bullet). These radicals can cause cellular death by damaging proteins, lipids, and DNA (1, 5), or can cause mutations leading to the development of antibiotic resistance (8).

Bacteria respond to ROS by up-regulating antioxidant enzymes, including superoxide dismutase (SOD) and catalase (1). They also produce small antioxidant molecules such as

ascorbic acid and glutathione (2). Further, some bacteria generate nitric oxide (NO), which can induce antibiotic tolerance by blocking the Fenton reaction and stimulating antioxidant enzyme action (9).

Nguyen *et al.* and Shatalin *et al.* present two convergent strategies used by bacteria to combat ROS that is produced as a result of antibiotic treatment. Nguyen *et al.* describe an antioxidant mechanism by which the starvation-signaling stringent response in *Pseudomonas aeruginosa* and *Escherichia coli* leads to antibiotic tolerance in response to nutrient limitation. The stringent response modulates the transcription of bacterial genes, diverting resources from growth to nutrient synthesis to promote survival until nutrient conditions in the environment improve. Nguyen *et al.* found that mutant bacteria deficient in the stringent response exhibited tolerance to a wide range of antibiotics (including ofloxacin, meropenem, colistin, and gentamicin) by increasing antioxidant enzyme production and blocking the production of pro-oxidant molecules, thus reducing toxic OH^\bullet . These mutant bacteria also were more susceptible to ofloxacin in a mouse infection model. In a biofilm, bacteria

Bacteria use two convergent strategies to combat toxic reactive oxygen species produced in response to antibiotic treatment.



Bacterial antioxidant strategies for antibiotic tolerance. A proposed mechanism of antibiotic-induced cellular death involves increasing the production of reactive oxygen species (ROS), whereas two overlapping tolerance mechanisms block ROS formation. SOD, superoxide dismutase; HAQs, 4-hydroxy-2-alkylquinolines.

grow in aggregates and may thus have limited access to nutrients. The study by Nguyen *et al.* provides insights into why such bacteria can be so difficult to eradicate.

Shatalin *et al.* examined the role of endogenously produced hydrogen sulfide (H_2S) gas in bacteria. They found that Gram-negative and Gram-positive bacteria could be sensitized to a wide array of antibiotics by deleting or inhibiting enzymes that produce H_2S , indicating that the gas confers antibiotic tolerance. H_2S elevated the antioxidant capacity

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of bacteria by suppressing the Fenton reaction and stimulating SOD and catalase production. Interestingly, the authors also show that H₂S can act as a diffusible protective agent in bacterial populations. Further, cells deficient in H₂S produced increased amounts of NO, and the two gases can act synergistically to induce antibiotic tolerance, demonstrating some redundancy in these protective mechanisms.

The antibiotic tolerance mechanisms presented in these two studies have several strong similarities. The most obvious common aspect is that the stringent response and H₂S both induce tolerance by elevating the production of antioxidant enzymes. These effects can be explained, in part, by considering that nutrient limitation and the production of toxic H₂S are forms of cellular stress. One possibility is that these mechanisms may act as low-level stress conditions that activate antioxidant responses, priming bacterial cells to counteract the more lethal oxidative stress induced by antibiotics—thus confirming the adage, “that which does not kill you only makes you stronger.”

The benefit of oxidative stress hormesis has been demonstrated in yeast, worms, and flies (10), and it is likely, as shown by Nguyen *et al.*, Shatalin *et al.*, and related work on low-level antibiotic stress (11, 12), that a similar mechanism functions in bacteria.

The treatment of bacterial infections is becoming more difficult because of a decline in the current arsenal of useful antibiotics, the development of antibiotic resistance, and the slow rate of new drug development (13). This situation is further aggravated by biofilms and other tolerant bacteria that underlie chronic and recurrent infections. This is particularly problematic with implantable devices such as prosthetic hips, which often require surgical removal to eliminate the infection.

Potential of currently available antibiotics presents a cost-effective option to overcome these challenges. Both Nguyen *et al.* and Shatalin *et al.* identify critical aspects of bacterial biology that could be commandeered as part of new potentiation strategies. For example, each study indicates that it may

be worthwhile to target bacterial antioxidant enzymes and associated pathways as a means to enhance the killing efficacy of bactericidal antibiotics. This could have a great impact on clinical practice and patient outcomes.

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ASTRONOMY

Analyzing Solar Cycles

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Since observational records began about 300 years ago, and very likely for millions of years before that, the Sun has displayed cyclically varying magnetic activity (1). Approximately every 11 years, a maximum of activity is reached, with a large number of sunspots (see the figure, panel A) present on the solar surface, strong x-ray emission from the corona, and a peak in the number of flares and coronal mass ejections. The latter cause mid- and low-latitude aurorae, disrupt radio communications, perturb navigation systems and radars, produce electric power outages, and can pose radiation hazards for astronauts and aircraft crew.

Solar cycle activity maxima are separated by minima during which only a few or no sunspots are present on the solar surface and other indicators of solar activity are equally muted (see the figure, panel B). Minima have lasted typically 2 to 3 years in the 20th century. Consequently, as solar activity decreased to near-minimum levels in 2005–2006, most solar astronomers expected that the Sun would be

bubbling with activity again by 2007 or 2008. However, the Sun did not restart displaying appreciable activity until 2010. Also, the rise in activity has been slow relative to most other cycles during the last century.

Surprised by this unexpectedly long minimum, the solar physics community reacted in various ways. Interpretations ranged from a lull before the storm, with the next cycle to be particularly strong, to the beginning of a grand minimum, a multidecadal episode of almost nonexistent solar activity. Such a prolonged period of quiescence last occurred in the 17th century, when almost no sunspots were visible for around 60 years—the so-called Maunder minimum (2). Which, if any, of these scenarios is correct? In particular, are we heading for a grand minimum?

Predictions of solar activity have been notoriously wayward in the past, with similar scatter of predicted behavior also true for the maximum of cycle 23, as little as 5 or 6 years before it was reached (1, 3). The best record is produced by empirical methods relying on precursors, but even they give reasonably accurate predictions of its maximum only after a cycle is well under way.

To estimate the future of solar magnetic

Does the recent longer-than-usual minimum in sunspot activity indicate that we are heading for an extended period of solar inactivity?

activity beyond the next cycle, we must therefore take guidance from its past. During the past 70 years or so, the Sun has been in a grand maximum, a period of strong activity cycles, which by chance coincided with the space age and the great variety of data that it has provided. In the 19th century, the cycle minima were similarly long and quiet as the one we have just left. Also, the slow start of the present cycle—cycle 24—suggests (according to a rule named after the Swiss solar physicist Max Waldmeier) that it will be relatively weak, peaking at a yearly averaged sunspot number value of 60 to 100 (1, 3), compared with 120 in cycle 23 and even larger values in four of the five cycles before that. There is similarity between the present cycle and the beginning of solar cycle 14 (see the figure, panel C). Cycle 14, the weakest cycle of the 20th century, peaked in 1905 at a yearly averaged sunspot number of 63.5. The sunspot number averaged over the first 9 months of 2011 is 45.5 (solid orange circle in panel B). Although this is low relative to the past nine cycles, it still exceeds 20, the amplitude of the two last cycles preceding the Maunder minimum (4). This speaks against, but does not rule out, a grand min-

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