Study provides insight into bacterial resilience and antibiotic targets

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Researchers at UC San Francisco and Stanford University have performed the first comprehensive survey of the central genes and proteins essential to bacterial life. The study, which combined a new variant of CRISPR gene-editing technology with automated cell imaging, generated new understanding of the fundamental gene networks that make bacteria so resilient to environmental stress and—increasingly—to antibacterial drugs. The research also demonstrated a practical approach to identifying the mechanism of action of potential new antibiotic compounds, which the researchers hope can be harnessed to aid the design of better drugs to fight a growing epidemic of antibiotic resistance.

Most of the core aspects of complex life, such as how cells copy their DNA, reproduce, and make key proteins and membranes, are based on the same genes and protein machinery found in simple, single-celled bacteria. But even in bacteria, how all these proteins work together to power life is only partly understood. In the new study, a team led by UCSF cell biologist Carol Gross, PhD, and Stanford bioengineers K.C. Huang, PhD, and Stanley Qi, PhD, used their combined expertise in microbiology, cellular imaging, and genetic engineering to develop a new approach to understanding what makes bacteria tick.

"Previously, genetic study of the most essential genes for life was very challenging," said Gross, a professor of cell and tissue biology and of microbiology and immunology in UCSF's School of Dentistry. Geneticists often learn about a gene's function by experimentally

switching off a gene and observing what happens to the cell in what is called a "knockout" experiment, Gross said. "The problem with studying the most fundamental genes, though, is that you can't knock them out – the cells would just die."

The new findings—published online in the journal *Cell* on May 26, 2016—relied on a new technique that allowed the researchers to instead generate "knockdowns" of each gene of interest. Unlike a knockout's binary on-off switch, a knockdown experiment essentially places a volume knob on each gene to gently turn down how much protein a cell makes. This way the researchers could turn down an essential gene's activity just enough to examine its importance in a cell's daily activities, but not enough to kill the cell outright.

The technique, called CRISPR interference (CRISPRi), was recently developed by Qi, now an assistant professor of bioengineering and of chemical and systems biology in Stanford's Schools of Engineering and Medicine, when he was a Systems Biology Fellow at UCSF. Qi's CRISPRi technology is quite different from the CRISPR-Cas9 techniques that are increasingly used by genetic engineers as a simple tool for cutting and splicing DNA: Instead of modifying DNA, CRISPRi precisely tunes cells' production of specific proteins.

The researchers used CRISPRi to systematically knock down the production of each of 258 essential proteins in the bacterium Bacillus subtilis, one gene at a time, and then observed how the cellular machinery performed in this weakened state using high-throughput, computer-controlled microscopy developed by Huang's lab.

For the vast majority of essential proteins, the researchers found, a complete loss of the protein produced major disruptions to the cells' integrity: deforming their normal shape or causing them to burst open and sabotaging cell division or simply halting growth altogether. By

contrast, using CRISPRi to partially deprive the cells of these proteins produced subtler changes, and revealed that the essential proteins fell into two classes: those that changed cell shape through direct control of the bacterial cell wall, and modulators that affected cell shape through indirect mechanisms.

"These findings reveal a new set of failure modes that can be targeted by antibiotics and demonstrate how cells have evolved to couple their systems together to avoid these fates," said Huang, a professor of bioengineering and of microbiology and immunology in Stanford's schools of Engineering and Medicine.

Genetic redundancies and fail-safes are key to bacterial resilience

The team also subjected each knockdown to more than 100 different stresses, such as dosing them with antibiotics or varying their nutrient supply. By analyzing nearly 30,000 combinations of essential protein knockdowns and environmental stressors, the team characterized the importance of the different essential proteins for coping with particular environmental stressors, and observed a number of key principles of bacterial resilience. They also showed that the technique has the potential to be used to identify the biological mechanisms of new antibiotic compounds.

To test their approach as a platform for drug discovery, the researchers demonstrated that the knockdown of a particular enzyme important for building bacterial cell walls made cells uniquely susceptible to an antibiotic whose mode of action was previously unknown. Such experiments, the team said, highlight the power of studying all essential genes at once, an approach they say could be an efficient way to characterize targets of other antibiotic drugs, which is a major

bottleneck in the transfer of drugs from the lab to the clinic.

Other experiments illustrated that bacterial cells have evolved many redundancies—such as producing more of each critical protein than they need as a rainy-day supply for times of starvation. The researchers learned that bacteria also have backups for many essential proteins, a fail-safe mechanism that allows them to better withstand genetic mutations or pharmacological attacks.

For example, one experiment focused on three proteins known to play critical roles in creating bacterial cells' protective outer layer, a vital process that is targeted by several of the most effective current antibiotics.

"We turned down the first protein from full blast to zero, and the cells were fine," Gross said. "We did the same for the second protein and still things were fine. We had to knock down all three proteins before the cell died. So while the process is essential, each protein was not."

The team went on to discover dozens of pairs of proteins with seemingly unrelated functions that provide similar levels of resilience to environmental stresses, suggesting that cells have redundant backup systems for dealing with disruptions to key systems.

"In a way, these experiments allowed us to reverse engineer evolution by observing its results across every living process," Huang said. "Our findings suggest that cells are optimized to survive adversity. It makes sense given that often during bacterial evolution, nutrients would have been in short supply and environmental conditions harsh. Therefore the essential genes and proteins would have evolved so that cells survive in times of scarcity."

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