Dissecting How Mtb Makes Its Wall, Buffering Endosomal pH, and Discovery of Ribocil

Each month, Chemistry & Biology Select highlights a selection of research reports from the recent literature. These highlights are a snapshot of interesting research done across the field of chemical biology. Our November 2015 selection includes an insight into non-overlapping biosynthetic pathways that lead to formation of Mycobacterium tuberculosis peptidoglycan, a new method to not only measure but also buffer the endosomal pH using nanoparticles, and a demonstration that non-coding RNAs can be a target for antibiotic discovery.

Many Roads Lead to the TB PG

When it comes to tuberculosis (TB), the World Health Organization (WHO) is pretty clear: this infectious disease kills more people than HIV/AIDS and is one of top 5 killers of women between the age of 20 and 59. WHO also highlights that the number of reported cases of multi-drug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) is growing at an alarming rate. - TB, however, is not a new disease: it’s been the foe of humanity for millennia, and so prominent in the public life around the turn of 20th century that it inspired numerous works of art while at the same time wiping out many of prominent artists, writers, philosophers, public figures, and millions of others.

Today, TB diagnosis is no longer a certain death sentence, but the disease is still very serious, especially in immunocompromised individuals, and the emergence of MDR-TB and XDR-TB make further research into the biology of TB and its causative agent, Mycobacterium tuberculosis (Mtb), exceptionally important. There are many aspects of Mycobacteria, Mtb included, that make them unique in the bacterial world; for example, Mtb, like many other bacteria, has a peptidoglycan (PG) cell wall, but there are so many differences in how Escherichia coli, one of the favorite model organisms in microbiology, makes its PG and Mtb that mechanistic and structural insights obtained using E. coli often don’t apply to Mtb. To address this, Kieser et al. take a closer look at what exactly happens in Mtb by focusing on three enzymes known to be important for PG biosynthesis: PonA1 and PonA2, responsible for glycan polymerization and peptide cross-linking, and LdtB, a major L,D-transpeptidase in Mtb that cross-links the PG peptides. The authors employ transposon mutagenesis combined with high-throughput sequencing to map out all the genes in the Mtb genome linked to genes coding for PonA1, PonA2, and LdtB, and see that the three genes have different connections and roles in distinct genetic networks. The most interesting observation Kieser et al. made is that each network displays different antibiotic tolerance, which suggests that dissecting the exact roles each enzyme plays in PG biosynthesis and Mtb physiology might lead to opportunities to fine-tune antibiotic cocktails and optimize treatment strategies to deliver a more effective punch to Mtb. The work also highlights that despite similarity between biosynthetic enzymes, as in the case of PonA1 and PonA2, we should not assume functional redundancy.


Measuring and Buffering Endosomal pH

Ask any biology student what the physiological pH is and you are likely to have 7.4 offered as an answer. This pH value is actually the pH of blood and commonly referred to as the physiological pH. The actual pH values, however, vary from tissue to tissue, from cell type to cell type, and within a cell, from one cellular compartment to the next; for example, endosomes, organelles involved in transporting cargo from the outside of the cell to the lysosome or other locations, in the process known as endocytosis, go through several stages, each marked by a specific shift in the endosomal pH value. These changes in pH are programmed and structured in a way that ensures efficient delivery of the cargo to its final destination.
In general, pH is not hard to measure, and in some cases, you can get a good accuracy read by using nothing fancier than a bit of pH indicator paper that changes color depending on the pH value of the solution to which it has been exposed. However, measuring pH inside an endosome is not as straightforward.

Although the method is conceptually similar to using pH indicator paper because it depends on detecting a change in color, it is often difficult to implement in a targeted way as the dyes used are often deposited not only in endosomes but in other cellular compartments. Recently, Wang et al. developed a method that overcomes these obstacles by using ultra-pH-sensitive (UPS) nanoparticles. These particles are made of poly(ethylene oxide) (PEO) and an ionizable random copolymer block that defines their physicochemical properties, such as the pKa value. The copolymers assemble into micelles at higher pH values and dissociate once the pH in the micelles falls below a threshold pH; this makes the copolymers very sensitive to pH changes and potentially useful as pH indicators. More importantly for the purpose of measuring endosomal pH, UPS nanoparticles are taken up by and stay in endosomes throughout their intracellular journey. What Wang et al. demonstrate is that UPS nanoparticles have a large buffer capacity and can act as “proton sponges” to soak up excess protons in specific pH ranges that are functionally relevant: 6.0-6.5 in early endosomes, 5.0-5.5 in late endosomes, and 4.0-4.5 in lysosomes. Wang et al. provide several examples of how UPS nanoparticles can be used to get deeper insight into physiology and metabolism and how they are affected by pH transitions.


A Light at the End of a Tunnel

Everything is not well in the world of antibiotic drug discovery and development. Many would actually say that “not well” is putting mildly, because the discovery of new antibiotics has been deprioritized by many pharmaceutical companies. At the same time as R&D efforts have been slowing down, the rate at which bacteria develop resistance to existing agents has been increasing, leading to big headlines with a word “superbug” in them.

In order to tackle the crisis, we not only need to continue to invest in antibiotic research but also to continue to question our assumptions about what constitutes a viable antibacterial target and a likely antibacterial agent. Recent work by Howe et al. takes antibiotic discovery into a new direction. The authors, who are all members of Merck Research Laboratories, a good sign that antibiotic drug discovery can still thrive in big pharma, focus on investigating what it would take to be able to target bacterial riboflavin riboswitches. Riboswitches are regions within messenger RNAs that do not carry any coding information, but they do play an important role in the regulation of gene expression. They bind to small molecules, most commonly different metabolites, which leads to specific conformational changes within the RNA and modulates the expression of a given gene. In the case of riboflavin riboswitches, these bind flavin mononucleotide (FMN) and can be inhibited by related riboflavin derivatives. However, due to significant side effects from the lack of selectivity, riboflavin derivatives are not viable antibiotic candidates. Howe et al. screen a collection of approximately 57,000 compounds with known antibacterial activity and identify a compound they name ribocil, which binds the FMN riboswitch. The structural analysis shows that ribocil assumes a similar binding pose in the FMN riboswitch as a natural ligand. Ribocil is specific for FMN riboswitches and has antibacterial effects that can be reversed by the addition of exogenous riboflavin. The work opens future opportunities in the exploration of riboswitch targeting as a broader antibiotic strategy and it is remarkable as an example of how shifting the focus from target based to phenotypic screening, or from protein-centric view of targets to an RNA-centric perspective, is needed to shift the discussion in antibiotic discovery and development from lamenting past glory to rejoicing in the future.