UNDER THE LENS

Tracking antibiotic mechanisms

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This month's Under the Lens discusses the use of single-molecule tracking to study the effects and response of bacterial cells exposed to antibiotics.

Antibiotics are cornerstones of modern public health, yet the rapid emergence of bacterial resistance to even our most powerful antibiotics threatens to return us to an age in which a simple cut could threaten human life. To respond robustly to this challenge, we need to grasp the mechanisms of action (MOAs) of these precious molecules.

One powerful MOA exploited by many antibiotics is to interfere with the bacterial nucleoid — the large tangle of bacterial chromosome with proteins and RNA. To observe the effects of antibiotics on the nucleoid, one can use single-molecule tracking (SMT), which has previously illuminated the cellular function of many proteins^{1,2}. SMT involves locating a single fluorescent molecule with high precision; each molecule appears as a spot spread across a few pixels of a microscope's camera owing to the diffraction from the microscope optics. The precise position of the molecule is found by fitting the shape of the fluorescence spot to a known distribution. The locations of the same molecule in successive images allow for tracking of its motion, thus reporting on the processes underlying its movement. Here, we discuss two studies that use SMT to investigate the effects and response of bacterial cells exposed to antibiotics.

One such antibiotic group involves polycationic antimicrobial peptides (AMPs), a part of the defence of many organisms, including humans. Much of the AMP potency is thought to arise from the penetration of the bacterial outer membrane. However, their cationic nature may also interfere with polyanionic intracellular components such as DNA, RNA and ribosomes, which are implicated in electrostatic interactions that 'lubricate' the bacterial cytoplasm by facilitating protein diffusion. Once inside, polycationic AMPs such as the human LL-37 polypeptide³ can rapidly disturb the balance of electrostatic interactions with lethal effects to the cell. Using SMT, Zhu et al.⁴ showed that chromosomal loci and ribosomes become much less mobile in response to exposure to LL-37, suggesting the formation of non-covalent electrostatic interactions connecting a large meshwork composed of the nucleoid and ribosomes. A slowdown of fluorescently labelled proteins throughout the cell showed a major impact of this meshwork on intracellular diffusion. The spatial distribution of ribosomes also revealed that LL-37 caused the nucleoid to expand and become more permeable to ribosomes. Together, these results suggest a model in which cationic AMPs form electrostatic interactions with negatively charged polymers to form a rigid network of pseudo-crosslinks, which irreversibly block processes vital to cell survival.

Nucleoid damage also occurs owing to exposure to antibiotics, reactive oxygen species, hydrolytic processes and ionizing radiation; as a result, bacteria have developed sophisticated mechanisms to respond to DNA damage. In the case of double-stranded breaks (DSBs), bacteria employ homologous recombination as a repair mechanism. By examining fluorescent fusions of key proteins in early homologous recombination, termed presynapsis, Röscher et al.⁵ used SMT to study how presynaptic proteins respond to DSBs caused by exposure to the antibiotic mitomycin C. The first protein

recruited to damage sites is RecN, which resembles proteins involved in structural maintenance of the chromosome. Induction of DSBs caused RecN molecules to switch from a highly diffusive state to a state involving nucleoid searching and binding, an interaction mode already seen for other DNA-binding proteins such as RNA polymerases. Following induction of DSBs, the spatial distribution of less mobile RecN molecules moved from the nucleoid periphery to its interior. RecN also tended to form a single focus in each cell at any given time, irrespective of the mitomycin concentration, suggesting the possible existence of a new mechanism that coordinates RecN assembly.

These recent studies highlight the power of SMT as a new imaging tool in understanding molecular processes in bacteria. By providing us with unprecedented single-molecule and super-resolution information on both the effects of antibiotic MOAs and the bacterial response, we now have a greater chance of keeping pace with the ever more complex molecular mechanisms employed by bacteria to resist our attempts to control them.

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Competing interests

A.N.K. is a shareholder and consultant in Oxford Nanoimaging, a company that designs, manufactures and supports single-molecule fluorescence microscopes.

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