The toxin-antitoxin system with a few tricks up its sleeve

Dr Clare Kirkpatrick
Antibiotic resistance is a growing concern for microbiologists and poses an increasingly serious health problem globally. Following the discovery of antibiotics, starting with the isolation of penicillin in 1928 by Alexander Fleming, their use became increasingly widespread around the end of the second world war. The number of highly antibiotic-resistant strains of bacteria has increased concomitantly ever since, leading to the emergence of MRSA, to give one example. Before doctors and scientists understood the potentially catastrophic consequences of their overuse, the use of antibiotics was largely uncontrolled until recently. In many parts of the world, it remains unregulated, and antibiotics can be bought freely over the counter. The number of new antibiotic classes being discovered has also slowed down dramatically, with almost all of the antibiotics currently in use having been discovered prior to the 1990s. Without new classes being discovered, the counter. The number of new antibiotic classes being discovered has also slowed down dramatically, with almost all of the antibiotics currently in use having been discovered prior to the 1990s.

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expression of this efflux pump, for reasons that are still unknown. HigBA was found to be involved in this process, as the toxin, HigB, decreases the expression of the efflux pump, by specifically targeting its RNA and degrading it. If the antibiotics, High, is mutated to become non-functional, the toxin is set free and the cell’s resistance to nalidixic acid is partially restored. The toxin, therefore, provides increased tolerance to this antibiotic by reducing expression of the efflux pump, and actually promotes bacterial cell growth, rather than inhibiting it in this situation. Again, this is unusual as efflux pumps would usually confer resistance, by pumping antibiotics out of the cell, rather than increasing antibiotic sensitivity.

Interestingly, the opposite is true during the DNA damage response, where bacteria with a functional HigB toxin gene show increased resistance to DNA damage-inducing agents such as mitomycin C and ciprofloxacin. This was discovered by testing the Tiph-defective mutant’s growth in the presence of a chemical library containing a wide range of antibiotics, to search for others which, like nalidixic acid, were specifically inhibitory for this mutant. Two other antibiotics with these properties were found, but surprisingly the HigB toxin did not protect against them. Because they both belonged to the family of quinolone antibiotics, that block DNA replication, these findings were also tested in cells which are mutated to constantly behave as they do in the presence of DNA damage, by mutation of a gene called LexA. This experiment showed that HigB was only inhibitory if the antibiotic did not cause DNA damage; otherwise it contributed to cell death. Indeed, this is what makes the HigBA system unique. Other TASs seem to work in concert, responding to general stress to the cell, whereas the HigBA system seems to respond specifically to stress induced by DNA damage.

The HigBA system is involved further still in the DNA damage response pathway. The LexA gene, it turned out, explained why it is even possible to produce mutants which lack a functional High G gene. Usually, in a TAS such as this one, disrupting the function of the antitoxin element of the system leads to inevitable cell death resulting from unregulated activity of the toxin, which is pretty much the point of these systems. In the HigBA system, LexA binds to the HigB G gene in the same way that High does, by performing transcriptional repression. Only in the presence of DNA damage, or when both LexA and High are deleted, is the toxin fully derepressed and allowed to kill the cell.

The influence of HigBA on the cell cycle

Although HigBA certainly is involved in the DNA damage response, that does not apply to its sole function, with the system also having a role in regulating the cell cycle. The cell cycle is the highly complex ‘program’, involving a large number of molecular pathways, which cells use in order to carry out the processes of growth, DNA replication and cell division in the appropriate order and at the appropriate time. While looking at the RNA being targeted by the High toxin, Dr Kirkpatrick and her colleagues found that in addition to the efflux pump RNA, High also targeted CtrA. CtrA is a regulatory protein involved in controlling the cell’s transition into the DNA replication stage of the cell cycle, necessary for subsequent cell division. Its function is to maintain the cells in the swimming stage of the cell cycle (in which they do not replicate their DNA) and clearance of CtrA out of the cell allows the DNA replication stage of the cell cycle to start. They found that when the antitoxin was defective, there were fewer CtrA-dependent swimming cells in the population. So when the High toxin levels are higher, its action against CtrA allows the cells to proceed more quickly to the DNA replication and division stages of the cell cycle. Again, this makes the HigBA system unique among TASs in its ability to fine-tune the cell cycle, and it may further contribute to the DNA damage response since actively replicating cells are more sensitive to DNA damaging agents. HigB is highly specific both in its activation conditions and its response, ‘explains Clare. ‘It is dedicated exclusively to the DNA damage response in these bacteria and attacks a small set of essential targets in the cell, leading to inevitable cell death.’

‘Discovery of new pathways within bacteria that could provide a source of new drug targets, as well as new molecules that interfere with them, is a challenge that can more easily be met in an academic setting without the requirement to generate profits for shareholders.’