Recycling of bacterial cell wall constituents

A bacterial cell is similar to a football. The football's leather cover is like the murein (in Latin murus means wall, protection) sacculus which surrounds the bacterial cell. The murein sacculus is stable at the same time as being flexible and dynamic. Like the football industry, whose aim is to produce as many footballs as possible, the bacterial cell is also focused on growth and proliferation. These processes are initiated by cellular enzymes that break up the cell wall material (murein), introduce new material and degrade material that is no longer needed. And all this in large amounts: about 50 per cent of murein are degraded and newly formed (turnover) per cell generation. But why so much waste? Dr. Christoph Mayer and his team from the University of Constance have shown that the cells carry out effective recycling processes.

In contrast to animal cells, the majority of bacterial cells are surrounded by a stabilising layer, known as a cell wall or murein sacculus. The cell wall determines the shape of the cell, protects it against adverse environmental influences and counteracts the high turgor pressure of the cell contents against the cell wall. When bacterial cells grow and proliferate, they incorporate large amounts of new cell material into the cell wall and degrade old cell wall material. This process is referred to as cell wall recycling. Dr. Mayer and his team are interested in obtaining detailed insights into what happens with the large quantity of degradation products and which transport vehicles are used to reintegrate them into the bacterial metabolism.

The bacterial cell wall (murein sacculus) consists of peptidoglycan, also known as murein, which is a polymer consisting of short peptide (protein) and long sugar (glycans) chains. The peptide component gives the cell wall its flexibility whereas the glycans help maintain the structure of the cell wall. It is worth noting that peptidoglycan is a huge molecule (a macromolecule) that covers the entire bacterial cell. A very special mechanism is required to break up such a macromolecule and to insert new material. The mechanism of cell wall formation and degradation must be precisely coordinated in order to guarantee the stability of the cell wall at all times. What happens to the degradation products? The researchers led by Dr. Christoph Mayer succeeded in showing that in bacteria such as intestinal Escherichia coli bacteria, a key component of the cell wall sugars (N-acetylmuramic acid (MurNAc)) is taken up and recycled.

Bacterial mutants and growth tests shed light on transport system

Dr. Christoph Mayer, molecular and microbiologist at the University of Constance.

MurNAc is a characteristic sugar that occurs in almost all bacteria. It occurs only in bacteria, and in no other organisms. MurNAc is the vital connection between the sugars and peptide chains of peptidoglycan and is characterised by an unusual, very stable ether binding. Dr. Christoph Mayer: "The earth is home to 10^{32} bacterial cells, an astronomically large number of bacteria. When the cells die, the cell wall mass needs to be reintroduced into the cycle. Like in leaves where cellulose is degraded, the bacterial cell wall, i.e. peptidoglycan, also needs to be degraded. It is not yet known how this works. Using bacteria mutants and growth tests, we have found out how the essential cell wall glycan (MurNAc) is taken up and degraded. MurNAc is released from the cell wall through enzymes, for

example lysozyme. We have discovered a transport system through which the sugar is taken up and phosphorylated before the stable ether binding can be broken up. We have discovered the enzymes that are responsible for this."

Bacterial enzymes that can destroy the bacteria's own cell wall are referred to as autolysins. Bacteria need autolysins in order to create openings when they grow and divide. New cell wall constituents can then be integrated into these openings. Once the researchers understand the proper function of the enzymes, and the steps that lead to the formation and degradation of the cell wall, they will be able to interfere with this mechanism, either by enhancing or preventing it.

Practical benefit

Dr. Christoph Mayer investigates the formation and degradation of bacterial cell walls.
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The bacterial cell wall is the most important target for antibiotics since it contains peptidoglycan that only occurs in bacteria, and not in humans. The autolysins, i.e. the cell wall destroying enzymes in bacteria, are unable to harm humans. Therefore, autolysins can be used to specifically target bacteria without leading to damaging effects in humans. Antibiotics such as penicillin affect the biosynthesis of the cell wall. Dr. Mayer: "It can also be assumed that antibiotics affect the degradation of the cell wall, thereby inducing its degradation. It could potentially be possible to develop antibiotics that activate the bacteria's cell-wall destroying autolysins. The inhibition of certain enzymes involved in the recycling of the bacterial cell wall can induce the destruction (autolysis) of bacteria."

Recently, research has shown that cell wall degradation products are important signalling substances. They activate the human immune system and can, for example, induce the germination of spores and other persistent dormant bacteria (for example those of the tuberculosis pathogen, Mycobacterium tuberculosis). Dr. Mayer: "We are working hard to gain a more detailed understanding of the signalling pathways and the regulation of autolysis in order to create the basis for developing therapeutics that are able to inhibit the reactivation of persistent pathogens."

Molecular microbiology and synthetic biology

Dr. Mayer believes that the idea of synthetic biology is based on the knowledge that things can only be comprehensively understood when they can be synthesised. The synthesis of the cell wall can only be understood in full detail when it can be controlled and initiated by the researchers. Dr. Mayer: “One of our long-term goals is to be able to use components of the synthesis apparatus to create cell-like structures (membrane vesicles) that are able to form a cell wall on their own.”

The researchers are pursuing two different approaches: bottom-up and top-down. The bottom-up approach involves the creation of a membrane vesicle, an artificial cell, which contains all the components that are required for the synthesis of the cell wall. The artificial cell is then used to find out whether it is indeed able to form a macromolecule around the membrane vesicle, and which other components are required to do so.

The top-down approach does the opposite in that it starts with existing cells. It is known that certain bacteria can switch off cell wall synthesis, thereby enabling them to evade the human immune system and survive in the human body as dormant bacteria that do not have any cell wall. The immune system only recognises the degradation products of the cell wall when the cells grow, because the growth of cells leads to the formation of degradation products. The question is, how can cells leave the dormant stage under better conditions, i.e. in which the immune system does not take the trouble to eliminate intruders. How

can they form a cell wall, which they need in order to grow and divide? Top-down approaches focus on solving this question using existing cells.

Dr. Mayer received a Heisenberg scholarship from the German Research Foundation (DFG) in 2006 in recognition of his research achievements. One project is currently being dealt with in the Chemical Biology Graduate School at the University of Constance and financially supported by the pharmaceutical company Dr. Kade Pharmazeutische Fabrik GmbH (Berlin, Konstanz).

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