New transporter for recycling of bacterial cell wall found

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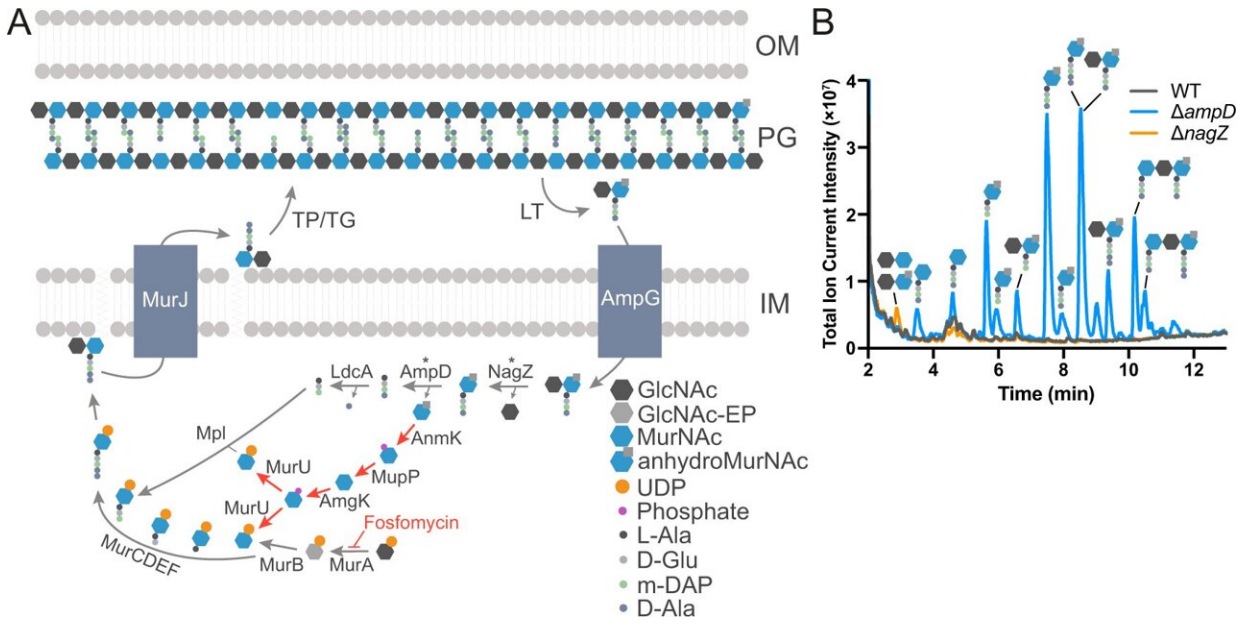

A. tumefaciens recycles muropeptides. A Schematic diagram of PG recycling pathway in E. coli. Pseudomonas MurNAc recycling pathway depicted with red arrows. EP enolpyruvate, TP transpeptidase, TG transglycosylase, LT lytic transglycosylase, OM outer membrane, PG peptidoglycan, IM inner membrane. * AmpD and NagZ activities are promiscuous: AmpD can act on muropeptides with or without GlcNAc, and NagZ can act on disaccharides with or without peptide chain7. B Total Ion Current (TIC) chromatogram showing soluble PG fragments that accumulate in the cytoplasm of A. tumefaciens WT, ΔampD and ΔnagZ strains detected using LC-MS. Credit: *Nature Communications* (2022). DOI: 10.1038/s41467-022-35607-5

A transporter which some bacteria use to recycle fragments of their cell wall has been discovered by researchers at Umeå university, Sweden. They found that the transporter controls resistance to certain kinds of cell-wall targeting antibiotics.

The transporter that Professor Felipe Cava and Ph.D. student Michael Gilmore at Umeå university have found is vital for cell wall integrity in the plant pathogen Agrobacterium tumefaciens, a convenient lab model of some human pathogens.

Bacteria are enclosed by a protective exoskeleton, the cell wall. The cell wall is essential for bacteria since it determines their shape and allows them to protect themselves, and many of our best antibiotics target the proteins that build and remodel this structure. As bacteria grow and divide, they remodel their cell wall, which results in the release of cell wall fragments known as muropeptides. The released muropeptides can go to the environment, where they can have a far-reaching impact in interspecies interactions, or be transported back into the cell for recycling. To recycle muropeptides, some bacteria use a membrane transporter called AmpG. However, many bacteria don't have this transporter, and so it is not known how or if they recycle their cell wall.

When bacteria encounter a cell-well targeting antibiotic such as penicillin, excess muropeptides are released. Some bacteria detect these excess muropeptides during recycling, and use this as a signal to produce enzymes called β-lactamases which break down the antibiotic. However, not all bacteria do this, and muropeptide recycling is not well understood.

"We were really interested in studying cell wall recycling because its purpose is poorly understood. The bacterium E. coli for example produces multiple proteins to recycle its cell wall, but blocking recycling has no effect on its ability to thrive. To understand this process better,

we wanted to study alternative bacteria which do things differently," says Michael Gilmore, first author on the study.

Studying the bacterium Agrobacterium tumefaciens, a bacteria which causes Crown Gall disease in plants, the researchers identified a new transporter which takes over the role of AmpG in this and related bacteria. Screening for genes which are required for growth when new cell wall synthesis is reduced by the antibiotic Fosfomycin, they found that a transporter belonging to the ATP-binding cassette, ABC, family seemed to be become essential for the bacteria to survive. When they deleted the transporter, they found that muropeptides accumulated in the cells' growth medium, while no muropeptides were present inside the cells. The newly found transporter is named YejBEF-YepA.

When the researchers tested the resistance of the transporter mutant to β-lactam antibiotics such as ampicillin, they found that it became extremely sensitive. As expected, this corresponded with a decrease in the activity of a β-lactamase enzyme. Remarkably however, the transporter mutant became even more ampicillin sensitive than a strain which completely lacked the β-lactamase, meaning that there must be more going on. To investigate this further, the researchers tried stressing the recycling defective strain by growing it in a low-osmolarity medium where the support provided by the cell wall becomes very important. The bacteria grew very poorly, and displayed swelling and lysis, meaning that the integrity of the cell wall was greatly reduced.

"This transporter showed up in our screen and represented an ideal candidate for a new transporter of muropeptides. We expected to see a drop in antibiotic resistance due to lower β-lactamase expression, but how sick the bacteria became when deleted the transporter was very surprising," says Michael Gilmore.

Studying the cell wall chemistry in more detail, the researchers found

that the thickness of the cell wall in the transporter mutant was much lower than normal, which corresponded with a similar decrease in cell wall precursor molecules. Also, the cell wall was less crosslinked that normal, and relied more on an unusual type of crosslinking enzyme. They concluded that the loss of recycled cell wall material must be a major cause of the antibiotic sensitivity and cell wall defects seen in bacteria missing the transporter.

Interestingly, the transporter has actually been reported before, as being important for Sinorhizobium meliloti to form a plant symbiosis, and mammalian pathogen Brucella melitensis to resist antimicrobial peptides. However, it is not until now that its function as a cell wall recycling transporter has been revealed. It could therefore represent a promising target for antibiotics or adjuvants in certain human pathogens, but is also relevant to agriculture in either plant pathogens or symbionts.

"It seems that a general peptide transporter YejBEF has been co-opted to transport muropeptides by the evolution of a new subunit, YepA. Interestingly, the transporter is present in many other bacteria including human pathogens like Brucella and Ochrobactrum, and this work is just the start in characterizing its potential role in, for example, infection," concludes Professor Cava, senior author of the study.

The findings are published in the journal *Nature Communications*.

More information: Michael C. Gilmore et al, Peptidoglycan recycling mediated by an ABC transporter in the plant pathogen Agrobacterium tumefaciens, *Nature Communications* (2022). DOI: 10.1038/s41467-022-35607-5

Provided by Umea University