Healthcare industry BW

A doorman in the bacterial membrane

We are fortunate to have membranes; they separate the interior of cells from the exterior and ensure that precious substances do not leave the cell and toxic substances cannot enter. Membrane proteins do an amazing job in transporting substances from one side of the membrane to the other. This process occurs in bacteria and in humans in much the same way. Prof. Dr. Hans-Georg Koch and his team at the Institute for Biochemistry and Molecular Biology at the University of Freiburg have found out how transport channels are formed and how proteins are integrated into the membrane by way of auxiliary components.

Escherichia coli (E. coli) is a rod-shaped bacterium, just one to two micrometres in size that commonly lives in the human intestine. Moreover, it is the preferred model organism in modern bacterial research. While bacteria possess only one membrane where all processes take place, structurally more complex eukaryotic cells are characterised by the effective division of tasks: nutrient uptake and communication mainly take place in the cytoplasmic membrane, energy production in the mitochondrial membrane and signal transduction in the membranes of the endoplasmic reticulum and the nucleus, amongst other places.

Human cells are organised into interior reaction compartments enclosed by lipid membranes (organelles). Although much simpler in structure than eukaryotes, prokaryotes such as E. coli also have an internal organisation. “Bacteria were previously thought to be a bag full of enzymes in which enzymatic reactions take place by chance,” Prof. Dr. Hans-Georg Koch from the Institute for Biochemistry and Molecular Biology at the University of Freiburg says. “However, we now know that although prokaryotes have no membrane enclosed compartments, they do also have an intracellular organisation, although it is often not visible when examined by light- and electron microscopy,” Koch adds.

Not a bag full of enzymes

Prof. Dr. Hans-Georg Koch is specifically focussed on bacterial membranes.
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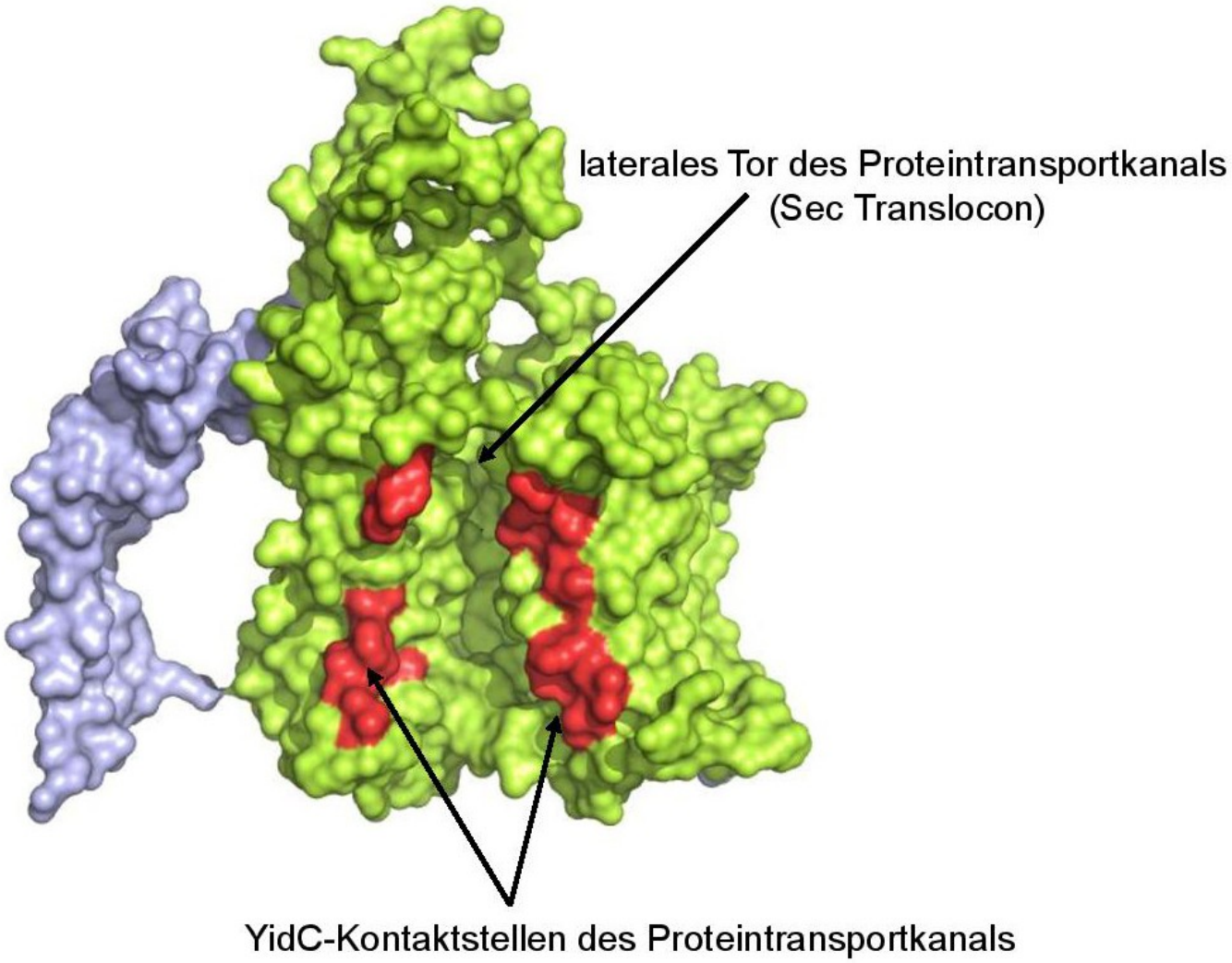
The intracellular bacterial organisation is difficult to capture using classical biological methods because most of it is below the resolution limit, as Koch points out. However, it is known that some reactions only take place in the parts of the bacterial membrane where specific enzymes and membrane proteins accumulate. “Bacteria like E. coli produce around 4,000 different proteins; around one third of these leaves the cytoplasmic space when they are incorporated into the membrane or transported across it,” Koch says.

As much as 30 or 40% of the cytoplasmic membrane is composed of proteins that are either embedded in or associated with a bilayer consisting of phospholipids, for example proteins of the large electron transport chain complexes that are involved in the generation of energy (i.e. formation of ATP, adenosine triphosphate). “Before a membrane protein can be embedded in a membrane, it must of course first of all enter the membrane,” Koch says.

This is achieved by other membrane proteins, i.e. so-called translocases, that assist in moving other

molecules across or into a membrane. However, the activity of these protein-conducting channels needs to be controlled in order for the cell to be able to open up and close them and in order to select substrates specific for the respective translocases. The protein substrates are selected based on their specific recognition sequences, charge and size.

Different paths for different proteins

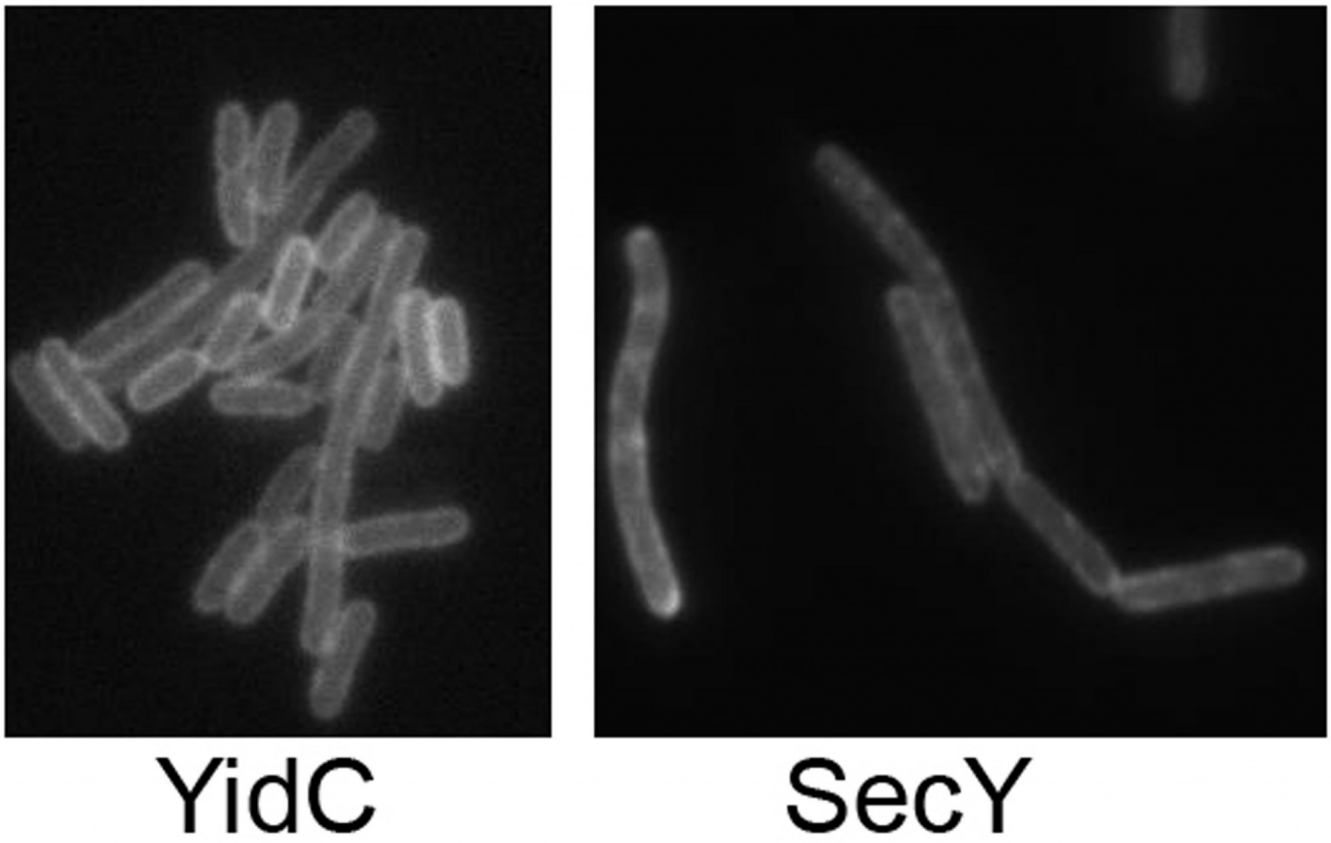


Three-dimensional structure of the Sec translocase. The helper protein YidC, a membrane insertase, binds specifically to the lateral gate of the channel (binding sites shown in red) through which the membrane protein enters the lipid layer of the membrane.

The Sec translocase is the translocase in bacteria that has been studied most closely. It facilitates the translocation of proteins across membranes or their insertion into the latter. Nascent strongly hydrophobic molecules are guided co-translationally (i.e. during their synthesis at the ribosome) to the translocon (ed. note: universally conserved protein conducting channel that orchestrates co-translational and post-translational protein transport processes) and integrated into the membrane before synthesis and three-dimensional folding is completed. Thus, membrane proteins only become functional when they have reached their final destination inside the hydrophobic lipid membrane. The coupling of protein transport and synthesis prevents hydrophobic proteins from aggregating in the aqueous cell environment and becoming useless. A protein's hydrophobicity determines whether it passes through the Sec translocase or enters the lipid phase of the membrane. A lateral gate in

the Sec complex enables – at least in theory – all molecules to enter into membranes. However, a 'doorman' located at this gate ensures that only truly hydrophobic membrane proteins are able to enter. This 'doorman', an insertase known as YidC, takes its role seriously and pushes all proteins that are too hydrophilic back through the lateral gate into the channel from where they continue their journey to the other side of the membrane. "We have shown that the YidC protein is located right at the lateral gate in the lipid phase," says Koch, going on to explain that, "if YidC detects transmembrane stretches of around 20 hydrophobic amino acid residues, the polypeptide is selected for integration into the membrane. If YidC only detects two or three amino acid residues, the protein ends up outside the cell. "

A close look at YidC



Left: E. coli cells in which YidC was labelled with a fluorescent dye. Right: E. coli cells in which the Sec translocase was labelled. While YidC is distributed regularly in the entire membrane, Sec translocase accumulates in particular areas.

It is known that YidC checks membrane proteins that pass through the channel whether they are destined for insertion into the membrane or not. It is also known that in addition to acting in concert with the Sec translocase, YidC can – and apparently also must – function as insertase for membrane proteins that are not integrated by way of the Sec translocase and that they depend on YidC for doing this.

YidC itself is a membrane protein with six transmembrane domains, i.e. spans the membrane six times. In addition to being present in bacteria, it is also found in mitochondria and chloroplasts. It is most likely of bacterial origin since it is not found in eukaryotic membranes such as the endoplasmic reticulum. It is also highly conserved and relatively tolerant to mutations and other modifications.

"YidC depends on a hydrophobic surface in order to be able to exert its function," Koch says. "The type of amino acid is unimportant, the only requirement is that they are hydrophobic." It seems that YidC is an essential protein; cells where YidC is turned off die. A subunit of the ATP synthase enzyme seems to depend exclusively on YidC for membrane insertion. "Cells that are unable to generate energy die," Koch says explaining the importance of YidC.

Site-directed lateral cross-linking and interactome

Koch and his team are focussing on solving the issue of quality control in membranes. What happens when a protein is folded incorrectly? Does the cell attempt to remedy the situation by trying again? It seems more likely that incorrectly folded proteins are degraded. This is not difficult in an aqueous solution as proteases (protein degrading enzymes) need water in order to cleave peptide bonds that link amino acids together in the polypeptide chain that forms the protein. But what happens with a protein in the lipid phase? If a protein manages to enter the lipid phase, it is extremely difficult to get it out again. But how does the cell manage to do this?

One of the approaches Koch uses in order to identify protein-protein interactions is known as site-directed lateral cross-linking. A cross-linker is a molecule that is able to covalently bind to a neighbouring molecule that happens to be in its vicinity by chance. "This approach helps us identify the entire interactome of a cell, i.e. all interactions between all proteins and not just the ones we are looking for," Koch says.

Further information:

Prof. Dr. Hans-Georg Koch
Institute for Biochemistry and Molecular Biology
University of Freiburg
Stefan-Meier-Str. 17
79110 Freiburg
Tel.: +49 (0)761 / 203-5250
Fax: +49 (0)761/203-5289

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Stephanie Heyl
BioRegion Freiburg

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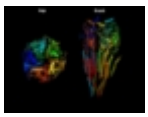

Membrane proteins


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