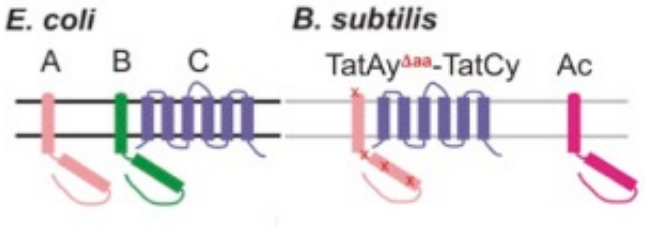
A Tat ménage a trois – when it comes to Tat secretion two Tat's are essential, but the third one makes it that much better

The membrane is the cellular barrier that keeps the inside of a cell separate from the outside, but in order to survive molecules and proteins do need to cross this barrier. Water and small molecules can do this through osmosis and diffusion, however larger molecules, ions and proteins need to be actively transported. Cells have developed molecular transporters that make the smallest possible pores in the membrane to secrete proteins as strings of amino acids.



However, active proteins need to be properly folded and sometimes include co-factors; this is usually done on the outside of the membrane. This study looks at the Twin-Arginine Translocation (Tat) system. Tat is unique because it breaks the basic principle: the smaller the pore size the better. Instead, Tat translocates large, globular proteins (fully folded and with co-factors) probably resulting in large translocation pores. Exactly how it all works still baffles scientists. *E. coli* bacteria are most extensively studied and therefore used as the primary model system. It is thus inferred that information gained in *E. coli* is true for all bacteria. The basic Tat machine in *E. coli* is made up of three proteins TatA, TatB and TatC. In the study presented here we examined the Tat system in a second model organism *Bacillus subtilis*. *B. subtilis* does not have a TatB protein, hence its system is only made up of TatA and TatC, but it has doubled up the Tat operons. It has genes for two separate TatA-TatC pairs (TatAyCy and TatAdCd) that are switched on in different conditions and a gene for a third TatA (TatAc) expressed on its own. TatAc has been somewhat of an enigma, as although it is always switched on, no function or role was so far identified. The main experimental outcome of this study was recognized when we examined TatAy-TatCy under low salt conditions where it is switched on and secretes the essential protein EfeB. We then used a collection of TatAy and TatCy single amino acid mutants; these mutations were small and in most cases didn't change the activity of the whole translocation machine, secreting EfeB and allowing the bacteria to grow normally. However, in some instances single amino acid mutations resulted in mild, severe and very severe growth phenotypes, thereby inferring these amino acid residues were vital for the activity of the whole TatAyCy translocation machine. Importantly we noticed that if one took the gene for TatAc out of the genome and examined growth phenotypes of the TatAy mutants some of these phenotypes actually worsened. TatAc on its own was not able to replace TatAy, but in the presence of a mutated TatAy, TatAc was able to functionally assist the mutated TatAy proteins. Therefore, we were able to show that TatAc supports TatAy in translocation. Whole

genome sequencing has become easier and cheaper and we are now able to investigate numerous whole bacterial genomes. These genomic studies have shown that, although in *E. coli* the Tat machine is made up of three Tat proteins, in most bacteria there are only two – TatA and TatC. Therefore the situation in *E. coli* is unique for a subset of bacteria. *E. coli* TatA and TatB are very similar and TatB is a more specialised copy of TatA. Our work presented here suggests that the TatAc protein in *B. subtilis* is an evolutionary intermediate between *E. coli* TatA and TatB – and shows that although information gained from *E. coli* studies are invaluable, they don't always give you the full picture. And in the case of the universal Tat system, two proteins (TatA and TatC) are essential and the third TatA-like protein is a nice extra.

Publication

A Tat ménage à trois--The role of Bacillus subtilis TatAc in twin-arginine protein translocation.
Goosens VJ, De-San-Eustaquio-Campillo A, Carballido-López R, van Dijl JM
Biochim Biophys Acta. 2015 Oct