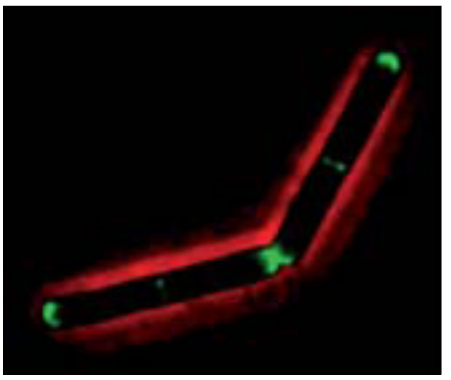
Bacterial cell division in Newcastle, UK

A 'Potential' Driver of Cellular Architecture

The need for novel antibiotic formulations with broad spectra has led to a burgeoning focus on bacterial cell biology. As Leendert Hamoen's team at the University of Newcastle uncovers a potent cellular feature that modulates localisation of membrane proteins in bacteria, does their research offer attractive prompts to the pharma industry?



Cell division protein MinD before the addition of ionophore CCCP that dissipates the proton motive force...

Nattō has held a permanent place in Japanese cuisine since the prehistoric ages and, despite its 'viscid' or 'nebaneba' texture, the soya bean dish continues to be the favourite for a Japanese breakfast. The dish is thought to produce an antibiotic effect besides being loaded with essential vitamins and anti-oxidants. A little further into its making and we learn that the dish is, in fact, soya bean fermented with bacteria.

Resolving the scope of B. subtilis

Quoting Louis Pasteur, "Intervention in the antagonism between bacteria can offer the greatest hopes for therapeutics," products of one bacterial species are ideal candidates for antibiotic formulations against different other species. In *Nattō,* the antibiotic properties are derived from *Bacillus subtilis,* a Gram-positive rhizobacterium that is included during the preparation. It is not surprising that microbiologists have to-date identified and characterised over two-dozen antibiotics produced in *B. subtilis.* The organism has become popular for research, not only as a source of antibiotics but also as a paradigm for Gram-positive bacteria, and offers derivable solutions to fundamental questions.

Leendert Hamoen (left) and his team

Leendert Hamoen reflects on his past, when he started his scientific career nearly two decades ago and shares with *Lab Times* his questions on bacterial cell biology, many that he has successfully cracked and others that are on his agenda.

Genetic competence, a property of some eubacteria, is the natural ability of a cell to take up DNA by homologous recombination in an effort to transform itself. *B. subtilis* represents a model organism of this property, which is unfurled under nutritional deprivation. When Leendert Hamoen stepped into his PhD at the University of Groningen, The Netherlands, the primary question that he put forth was 'how does *B. subtilis* acquire genetic competence?' In the following years, he was involved in dissecting the complex gene regulatory process of competence development, which, in *B. subtilis,* is a 'bistable response' viz. the transformation of only a sub-population of cells in an isogenic culture.

Leendert Hamoen's preliminary work won him an EMBO fellowship and took him to Oxford where he worked with Jeff Errington, one of the pioneers in Bacillus cell biology. His interests widened to the study of localisation of bacterial proteins, particularly those that assembled at the cell poles and participated in bacterial cell division. *B. subtilis* soon became his favourite model to address his questions. "*B. subtilis* is safe to handle. By virtue of its genetic competence, it is also possible to combine mutations with ease. One can conveniently generate knockouts by feeding the cells with DNA that contain the preferred gene deletion," he elaborates. He explains that natural genetic competence is restricted to some species of bacteria; *S. pneumonia* besides *B. subtilis* can do it, but for example *E. coli* does not develop such an efficient natural recombination system.

Cracking a taunting question

His study in Oxford steered Leendert Hamoen's focus to DivIVA, a bacterial protein that localises to cell division sites and cell poles. To work out the molecular mechanism of polar targeting of DivIVA, he proceeded to look at other proteins whose localisation was regulated by DivIVA. This was when Hamoen stumbled upon an anomalous observation that posed a mighty question, on which he was to ponder for many years and that eventually led to his latest research paper (*PNAS* 107(27): 12281-6). "Most cell division proteins are anchored to the membrane. When I tested the localisation of DivIVA-GFP, I found that the protein clearly localised to the membrane. On the other hand, when I tried to reproduce the membrane localisation of GFP-MinD, another membrane-targeted protein and a binding partner of DivIVA, all I was left with was a blurred field. There was no intact staining," Hamoen recollects the confounded moment. "Eventually, I could only think of poly-lysine-coated glass slides as a source of the problem.

Normally poly-lysine is used to attach cells on glass slides but I knew from old

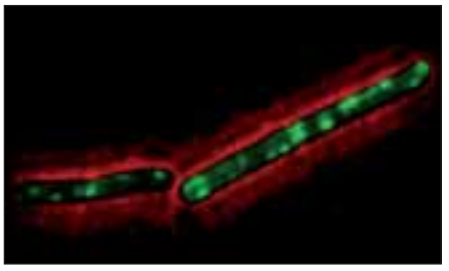
work that poly-lysine could penetrate and dissipate membrane potential," he continues. It was this striking observation that stirred Leendert Hamoen's curiosity and brought him to conclude that it has "something to do with the membrane", a proton motive force (pmf) that was dissipated or an unknown cause for leakage of the membrane. It was not until six years later, in June 2010, that Leendert Hamoen successfully published his findings in *PNAS, 'Membrane potential is important for bacterial cell division'*.

Drug-sensitive proteins

With a Career Development Fellowship from the Wellcome Trust, Leendert Hamoen established an independent group in 2008 at Newcastle University in the Centre for Bacterial Cell Biology. This came in handy to explore his research interests and, together with post-doc Henrik Strahl, Hamoen sequentially resolved his questions of half-a-decade. To start off, they tested localisation defects of over 20 different bacterial proteins upon dissipation of the pmf by a specific proton-ionophore binding drug. Nine of the twenty proteins exhibited aberrant localisation following treatment. Given that the pmf is composed of both the transmembrane proton gradient (ΔpH) and the transmembrane electric potential (Δψ) and that the drug affected both these components, they then worked down to that component of the pmf that specifically regulated protein localisation. Nigericin and valinomycin are commercially available antibiotics that have been shown to specifically dissipate ΔpH and Δψ, respectively. The duo discovered that all nine proteins sensitive to pmf, were also sensitive to valinomycin and, hence, to Δψ, whereas the dissipation of ΔpH by nigericin had no influence on the localisation pattern. Subsequently, they were also able to pin down the Δψ-dependent response to amphipathic helices in MinD, the protein that had initially won Leendert Hamoen's attention.

Dissipating the proton motive force

At this point, I am tempted to raise a question on the normal stomach and gastrointestinal flora. Do they maintain their membrane potential in adverse conditions, such as changes in acidity? Leendert Hamoen has a quick answer, "I believe that even under extreme conditions bacteria can maintain their membrane potential, though there are surprising reports that cells grow without membrane potential. Even anaerobic strains generate a charge over the membrane, after which we suppose that protein localisation is normal." It is lucid that his results hold manifold implications both in the drug industry and in eukaryotic research. There is already some evidence for the involvement of membrane potential in localisation of proteins in yeast (*EMBO J.* 26: 1-8).



...dislocalisation of MinD after CCCP addition

Unfinished tasks

Why is membrane potential at all important for protein localisation, when there are other means of tethering proteins to the membrane? This is one major question that intrigues Leendert Hamoen.

"Membrane work is challenging but doable", mentions Leendert Hamoen as he introduces another of his group's interests. In a couple of recent papers (*EMBO J.* 28: 2272-82; *EMBO J.* 29: 1988-2001), the Hamoen group have been involved in deciphering the crystal structure of DivIVA and have also shown that the protein recognises negative membrane curvature (the inner side of membranes). The property of direct sensing of negative membrane curvature is not clearly understood, though there have been studies on binding of BAR domains in eukaryotic proteins to positive curvatures. Hamoen hopes to resolve the mechanism of curvature-sensing but underscores the limitations of current methods of generating membrane curvatures *in vitro*. "DivIVA binds to the negative curvature of membranes. It's hard to load such a sticky protein into liposomes for our work *in vitro*. It is experimentally challenging but we will work it out," he completes.

Leendert Hamoen heads a small but young and vibrant group and looks hopefully at his agenda. He emphasises the need to characterise cell division proteins and I would nod in accord; indeed, it is one crucial step in the development of potent antibiotics.

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