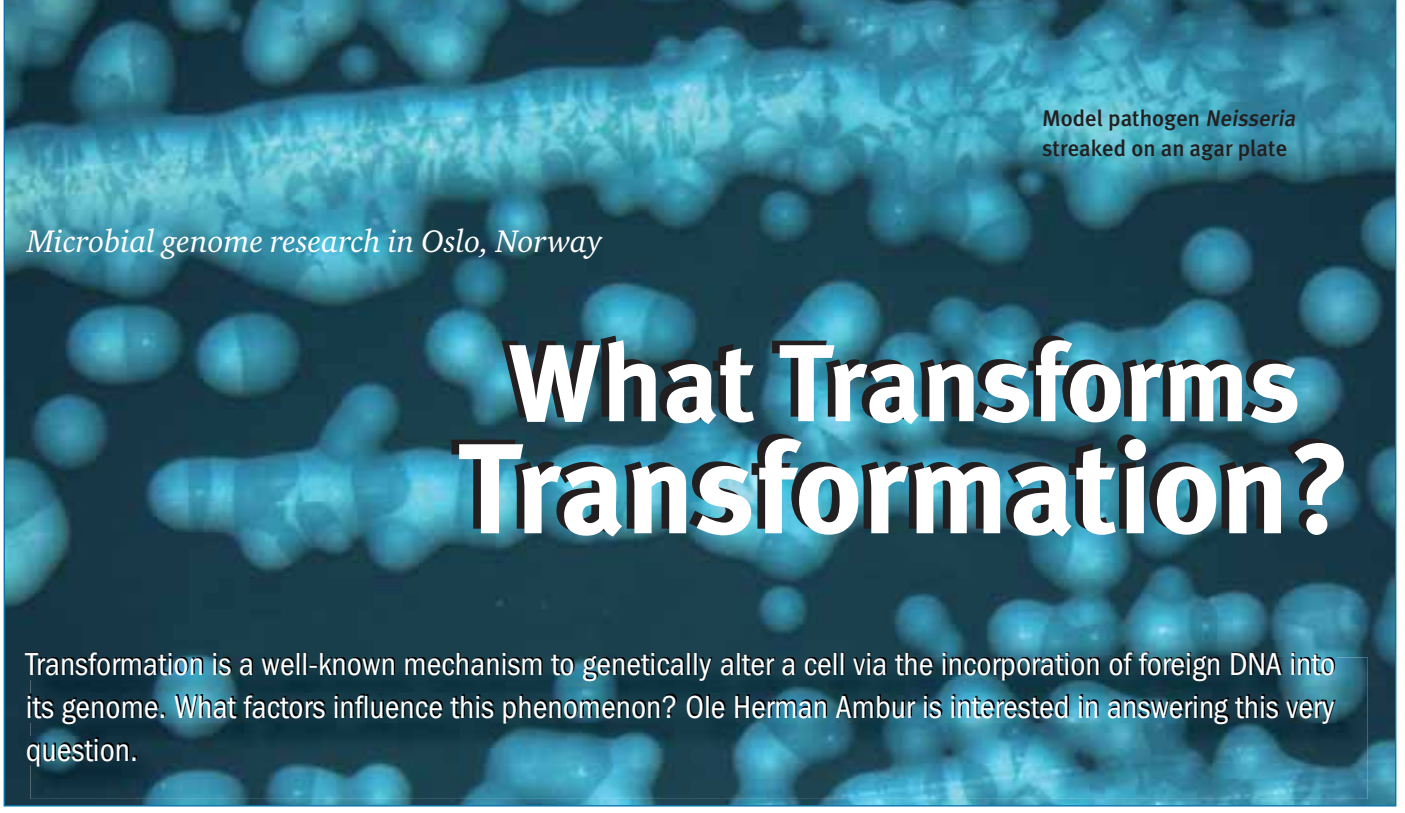

Model pathogen *Neisseria* streaked on an agar plate

Microbial genome research in Oslo, Norway

What Transforms Transformation?

Transformation is a well-known mechanism to genetically alter a cell via the incorporation of foreign DNA into its genome. What factors influence this phenomenon? Ole Herman Ambur is interested in answering this very question.

Transformation is a complicated business. Even though it occurs naturally in some bacteria, others first need to be 'convinced' by artificial means. For transformation to happen at all, bacteria should be in a ready state of 'competence'. There then follows a multiple set of interactions, beginning with the binding and uptake of DNA, right up to its incorporation into the bacterial genome. All of this has to be well-orchestrated. A team of Norwegian scientists has now shown that certain factors can influence transformation positively and negatively at different stages of the process (*PLoS ONE,* 7(7): e39742). *Lab Times* contacted lead author Ole Herman Ambur at the University of Oslo for the full story.

Pathogen to the rescue

At the centre of Ambur's research is *Neisseria meningitidis* or *meningococcus,* a bacterium more famously known for its role as a dreaded pathogen. It is the culprit behind meningitis, an infection of the membrane surrounding the brain and spinal cord, as well as meningococcemia, a life threatening, bloodstream infection. Naturally, we were interested in knowing why he and his colleagues chose this particular organism for their study. Ambur explains, "*Neisseria meningitidis* is an ideal model organism for the study of transformation due to its small – 2.2 Mb – and dynamic genome. We have particularly been interested in the evolution of a small repeat, the DNA uptake sequence (DUS), which is required for transformation and is found in thousands of copies throughout the genome."

Ambur and co. are also seasoned workers in this field. "At our institute we have more than fifty years' experience in the study of transformation in *N. meningitidis* and we have achieved high transformation rates, which is a great advantage for these types of studies," he elaborates. "Also, since the bacterium is a feared human pathogen, it is important to fully understand its lifestyle in order to devise strategies for intervention," he adds.

Restricting transformation

The group began their studies of transformation mediators with the restriction enzyme *Nla*IV. Restriction enzymes work in combination with a methylase enzyme (together constituting a restriction modification system or RMS) and introduce double strand breaks in foreign DNA, entering the bacterial cell. There are 22 RMS known in meningococci and the *Nla*IV RMS belongs to the top two of the most commonly occurring systems. Furthermore, there are 1,873 sites where the enzyme can 'cut' the *N. meningitidis* genome, making it a very interesting candidate for this study.

To analyse its effect, Ambur and co. used three versions of one plasmid that varied in the number of *Nla*IV sites (8, 1 and 0). These were tested for their ability to transform wild type *N. meningitidis* and a mutant form that did not express the restriction enzyme. They saw that the presence of *Nla*IV sites has a negative influence on plasmid transformation, meaning that fewer *Nla*IV sites equal higher transformation success.

Homology goes a long way

The researchers then looked at how the presence of homologous DNA influences the process of transformation. Earlier observations, made during restriction analysis, had told them that a 'continuous stretch of homologous DNA' is more efficient than 'homologous DNA interrupted by heterology'. They now wanted to know if the amount of homologous sequence present in the transforming DNA could also affect transformation efficacy. They employed 'three versions of a test plasmid, in which the homologous sequence was altered' and found that already small deletions or interruptions in the homologous sequence had a strong effect on transformation frequency.

Next, the investigators set their sights on the DNA Uptake Sequence (DUS), which is thought to aid species conservation 'by limiting DNA import to homologous alleles and contributing to sexual isolation'. It is

Photos (2): Ambur

Ready to transform: Ole Herman Ambur has his favourite pathogen, *Neisseria meningitidis*, under control.

generally considered to be a positive transformation mediator. The team again tested different versions of a plasmid; this time the DUS was inserted in differing orientations (forward/reverse), locations (three distinct sites) and numbers. The results caused great excitement as they "documented for the first time that variable placement of DUS relative to the homologous region in the donor DNA" affects transformation.

It was, in fact, earlier work on the DUS that first got the scientists interested in this project. "We were interested in learning more about the positive and negative drivers of transformation in order to study the specific activities of the DUS. Serendipitously, we noticed that one of our plasmids outperformed similar constructs and we identified that a small deletion was causing this effect. This deletion, as described in the paper, had removed three *Nla*IV restriction sites and encouraged us to fully characterise the influence of this restriction system on transformation," says Ole Herman Ambur.

Transforming roadblocks into success

He goes on to share his experiences of working on this project, "We worked on this particular project for five years, and some of the plasmid constructs have been employed in the lab for nearly ten years. Working with the same type of plasmids has been advantageous for us since that enabled the detection of subtle aberrant behaviours."

However, it was not always smooth sailing for the scientists and it took a lot of hard work and team effort to achieve success. Ambur narrates the trials involved, "One of the greatest challenges for us during this study was to achieve day-to-day reproducibility in the transformation frequencies. We were four scientists working in the laboratory with this study and we spent our time standardising each step in the experimental protocol. One important realisation was that there is no such thing as an ultimate transformation frequency for an individual strain transformed with a particular plasmid, but that the bacterial suspensions from each day was allowed to vary slightly and that this affected the transformation frequencies of that day. Since we observed the behaviours of a large set of slightly divergent plasmids, and that they displayed reproducible relative differences each day, we were able to detect and follow subtle phenotypic differences."

In the near future, Ambur plans to explore these observations in greater detail. "We plan to fully characterise the DUS-specific transformation pathway by making selected gene knock-outs and continue to make new plasmid constructs that may shed light on this highly coordinated and complex process. We are also very interested in studying transformation in an evolutionary perspective through genomics and hopefully establish a platform for experimental evolution of transformation," he explains.

Biochemistry in Cambridge...

Ambur's scientific journey began in Oslo, Norway and has come full circle with his return to the country. He clarifies, "I did my undergraduate degree in biology and philosophy at the University of Oslo before my studies at the University of Cambridge, where I earned my PhD from the Department of Biochemistry in 2003. My degree was on the molecular mechanisms responsible for vancomycin resistance in enterococci."

After completing his doctoral research, he kept a lookout for interesting projects that he could work on back home. "I have always nurtured an interest in microbiology and when an opportunity opened in my home country to study meningococci I jumped at it and started my postdoc there. Following the postdoc I have worked as a scientist in the same laboratory and I am currently supervising PhD and master students in the fascinating biology of meningococci and particularly in the transformation phenomenon," he tells us.

...microbiology and fly fishing in Oslo

Besides his active contribution to meaningful research, Ole Herman Ambur has also perfected the art of striking a good work-life balance. "These days I spend my free time together with my partner running after our very active and adorable two-year-old daughter. She has a keen interest in insects, so maybe she will choose biology one day herself. I love to spend time in the forests around Oslo and I hope to qualify as a mushroom inspector this autumn. I am a keen fly-fisherman and both in science and fishing I sometimes experience moments when everything is harmonious and perfect. Then I know that all the hard work at the bench or practising casting has paid off, and I have readied myself for new challenges," he concludes elegantly.

As we take leave, we wish him and his team many such 'transforming' results in the coming future.

Latika Bhonsle