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Science writer **Meriel Jones** takes a look at some recent papers in SGM journals which highlight new and exciting developments in microbiological research.

Getting into shape

Pul, Ü., Lux, B., Wurm, R. & Wagner, R. (2008). Effect of upstream curvature and transcription factors H-NS and LRP on the efficiency of *Escherichia coli* rRNA promoters P1 and P2 – a phasing analysis. *Microbiology* **154**, 2546–2558.

The double helix structure of DNA is one of the great icons of the 20th century. However, DNA has additional structural features. As well as the exact sequence of the nucleotide bases, the shape of the DNA molecule is important. Each comparatively large DNA molecule folds to fit into the cell, but regions remain available for use at a moment's notice, controlled by proteins and DNA structure. The DNA bases ahead of every gene are important for regulating when each is switched on or off by proteins binding at a region called the promoter. DNA curvature and regulatory proteins act together to regulate the efficiency with which genes are used, but many of the exact details are unknown.

Researchers in Düsseldorf, Germany, have been studying how DNA and proteins interact at two promoters in *Escherichia coli*. The P1 and P2 promoters both regulate production of a structural RNA molecule required as part of the protein synthesis machinery. The DNA upstream of P1 is curved, while there is a complete lack of curvature upstream of P2. To test the relative importance of DNA structure and proteins, the researchers changed the DNA bases in the promoter region to vary the amount of curvature. Regardless of curvature, the gene could always be switched on, but the level of activity was highest when the inside of the curved section of DNA and the start of the gene were in the same plane.

Proteins called transcription factors are normally involved in regulating gene activity through modulating interactions with the RNA synthesis system. The researchers went on to test the effect of three *E. coli* transcription factors called H-NS, LRP and FIS in conjunction with the curved DNA molecules. FIS activates P1 promoters while LRP and H-NS inhibit transcription from them. The subtle effects seen with the differently curved molecules differed between P1 and P2, indicating that these two promoters are regulated differently, with P1 being more strongly inhibited.

From these results it is evident that the combined effects of DNA curvature and several transcription factors cause the different levels of activity of each gene, but much more work is needed before accurate predictions can be made for every gene.

Bugs and colon cancer

Allen, T.D., Moore, D.R., Wang, X., Casu, V., May, R., Lerner, M.R., Houchen, C., Brackett, D.J. & Huycke, M.M. (2008). Dichotomous metabolism in *Enterococcus faecalis* induced by haematin starvation modulates colonic gene expression. *J Med Microbiol* **57**, 1193–1204.

The colon is home to around 10^{11} bacteria per gram of contents. For many years, scientists have argued about whether the presence of this vast microbial horde is always in the best interests of its human host. Specifically, the idea that some bacteria are involved in sporadic colorectal cancer has been around, but unproven, for decades. The major difficulties with making any progress are that most of the microbes have not been identified and the interaction between bacteria and the surface of the colon has many unknown features.

To make progress, scientists in Oklahoma, USA, have concentrated on bacteria that are known to damage DNA since this could start the genetic changes required for cancer. The damage is caused by highly reactive forms of oxygen known as superoxide, hydroxyl radicals and hydrogen peroxide which diffuse from some bacteria when they are stressed and into the surrounding animal or human tissues.

The authors have been experimenting with *Enterococcus faecalis*, a minor inhabitant of the colon but one that can cause DNA damage and promote chromosomal instability by releasing reactive oxygen species. They wanted to know how the colon responded to this threat. They have discovered that, although the colon tissue looks perfectly normal under the microscope, the activity of several genes altered rapidly once the bacteria released oxidizing chemicals. Looking in more detail, the researchers proved that *E. faecalis* could activate the NF-κB signalling pathway in white blood cells – this signal can promote tumours.

After checking 5,000 genes, the researchers identified 42 that increased or decreased in expression. Several were involved in the immune response, while 9 were implicated in stress responses and a further 10 in control of whether the cell should divide or die. Looking into the roles of these genes in more detail brought out the message that *E. faecalis* affects a single response network that contains NF-κB signalling and several genes that have been implicated in cancer biology.

The argument about the role of bacteria in colon cancer has thus moved forward with implication of specific mechanisms through which *E. faecalis* can increase the susceptibility of colon cells to DNA damage.

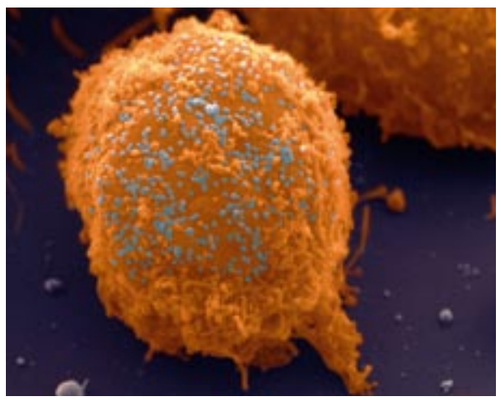
Novel bone disease mycobacterium

Bang, D., Herlin, T., Stegger, M., Andersen, A.B., Torkko, P., Tortoli, E. & Thomsen, V.O. (2008). *Mycobacterium arosiense* sp. nov., a slowly growing, scotochromogenic species causing osteomyelitis in an immunocompromised child. *Int J Syst Evol Microbiol* **58**, 2398–2402.

The most well known *Mycobacterium* species is *M. tuberculosis*, infections of which can result in the disease tuberculosis. Closely related species that cause different, rarer diseases can now be distinguished using molecular genetic and chemical analyses. Several have been identified as the cause of infections in children's bones, although most of these infections have fortunately not been severe. However, treatments with antibiotics to kill the bacteria are quite prolonged and the disease can recur. One recent serious infection in a 7-year-old girl, who had inherited a problem with her immune system, was caused by what turned out to be a novel species of bacteria. She responded well to therapy with a mixture of antibiotics for over a year, with no signs of recurrence of the infection after a further 18 months. However, she probably remains at life-long risk from non-tuberculous mycobacteria.

Staff working at the International Reference Laboratory of Mycobacteriology and National Center for Antimicrobials and Infection Control at Copenhagen in Denmark, along with colleagues at other laboratories in Denmark, Finland and Italy, studied the bacteria in detail because their features did not correspond exactly to any known species. The yellow-coloured bacterial cells grew slowly and looked like typical clinically significant mycobacteria under the microscope. However, more detailed tests that recorded the sequence of several genes from the bacteria showed some differences from all known species. In addition, some results from growth on various laboratory media and an analysis of the fatty and mycolic acids from the cells matched with several different species.

Putting all the information together, the researchers have convincing evidence for a new species that is closely related to *M. intracellulare*, but very clearly different from it. They have named it *M. arosiense* after the Latin name of Aarhus, the city in Denmark where it was first identified.



▲ Coloured scanning electron micrograph of a T-cell (orange) infected with HIV viruses (blue). *Eye of Science / Science Photo Library*

HIV by numbers

Hogue, I.B., Bajaria, S.H., Fallert, B.A., Qin, S., Reinhart, T.A. & Kirscher, D.E. (2008). The dual role of dendritic cells in the immune response to human immunodeficiency virus type 1 infection. *J Gen Virol* **89**, 2228–2239.

Trying to understand exactly why HIV-1 invariably causes a lethal infection has highlighted our lack of understanding of many of the complex interactions in the human immune system. Cells have dual roles, as targets for disruption by HIV-1 and as essential components in an immune response to counter the infection. Hogue *et al.* have tried a new approach to this problem by creating a mathematical model to describe how HIV-1 and cells interact in a human lymph node.

In the decades since HIV-1 was identified, much data have been recorded on how the numbers of viral particles and different types of human cells change throughout an infection. Myeloid dendritic cells (DCs) in the lymph nodes activate T-cells to defend against HIV-1. One type of T-cell, CD8+, has the main protective role against HIV-1 infections through killing infected cells and releasing antiviral factors. Another T-cell type, CD4+, is also important in the body's defences, but is attacked by the virus. DCs can thus promote immunity as a link between the virus and T-cell defences, but are also used by HIV-1 to boost infection. It is difficult to study DCs directly because they reside within lymph nodes which cannot be examined easily. The advantage of a mathematical model in this situation is that it may predict factors that govern the infection and which are easier to test experimentally.

In the first few weeks of infection there is usually a large amount of virus in the body and substantial immune system activity. This stabilizes into a long-term chronic infection that can last years, characterized by much lower numbers of virus particles. Eventually, for unknown reasons, this stable state breaks down, CD4+ cell counts decrease, the levels of virus increase, and the patient experiences the debilitating symptoms of AIDS. All the mechanisms proposed for this change imply that DCs are of key importance, but for several different reasons.

The equations were designed using information from human patients and similar immunodeficiency viral infections in primates. The idea was to have terms for documented significant interactions between HIV-1, DCs, CD4+ and CD8+, concentrating on DCs. The model was good at simulating the numbers of each type of cell in a healthy human lymph node, and in showing how HIV-1 infection results in T-cell depletion. Using mathematical methods for uncertainty and sensitivity analysis allowed the testing of different hypotheses about the roles of DCs. The way that DCs simultaneously infect and alert CD4+ cells seemed to be crucial in establishing infection. Later on, failure of proper DC function has a greater effect on pathogenesis of the disease than the loss of CD4+. One practical outcome of this modelling is to suggest that treatments that improve DC functions could be valuable in improving the outcome of HIV-1 infections.