BACTERIAL EVOLUTION

Evolving virulence

Bacterial pathogens and their hosts participate in a constantly evolving battle of attack, defence and counter-attack. In a paper recently published in *Current Biology*, researchers from Imperial College London and the University of the West of England show how one bacterial pathogen, through the power of evolution, achieves a significant advantage over its plant host. In this example, exposure of the invading micro-organism to the defence mechanisms of the plant drives the evolution of a new virulent form of the pathogen through loss of a bacterial genomic island.

Many phytopathogenic bacteria inject virulence effector proteins into plant cells through a type III secretion process. Without these effectors, the pathogens are unable to overcome basal host defences. However, some (termed avirulence) effectors can act as molecular double agents that betray the pathogen to plant defences. In these plants, resistance to infectious disease is based on the specific recognition of bacterial effectors by the products of resistance genes. When an effector is recognized, a hypersensitive resistance reaction (HR) is triggered that results in the establishment of a plant antimicrobial response. To investigate how bacterial pathogens overcome this form of host defence, Andrew Pitman and colleagues focused on the emergence of new virulent forms of *Pseudomonas syringae* pv. *phaseolicola*, the microorganism that is responsible for the economically important halo-blight disease of the common bean. This pathogen encodes a number of effectors that generate a hypersensitive resistance reaction when they encounter a plant with the corresponding resistance gene. When the authors repeatedly passaged the pathogen through bean leaves undergoing the resistant reaction, bacterial strains were selected that lacked the corresponding avirulence effector gene (*avrPphB*), which triggers the HR defence response in plants encoding the matching R3 resistance gene. After each passage, an increasing proportion of the bacterial colonies tested were able to cause disease, indicating a strong selective pressure to lose the effector gene. These experiments also gave the authors an unprecedented opportunity to directly observe the evolution of microbial pathogenicity in host tissue.

Further analysis of the deletion event revealed that *avrPphB* is part of a 106-kb genomic island (PPHGI-1) that shares strong similarities with integrative and conjugative elements and pathogenicity islands found in other bacteria — exposure of the pathogen to the HR resulted in deletion of the entire island. Surprisingly, the loss of this island did not seem to compromise the ability of the bacterium to grow and cause disease in the plant. The authors speculate that PPHGI-1, which contains genes encoding a type IV pilus and proteins involved in photosensory and chemotatic signalling, could confer a selective advantage to the bacterium under environmental conditions more relevant to its origins in the upland regions of East and Southern Africa.

David O'Connell

ORIGINAL RESEARCH PAPER Pitman, A. R. *et al.* Exposure to host resistance mechanisms drives evolution of bacterial virulence in plants. *Curr. Biol.* **15**, 2230–2235 (2005)
FURTHER READING Alfano, J. R. & Collmer, A. R. Type III secretion system effector proteins: double agents in bacterial disease and plant defense. *Annu. Rev. Phytopathol.* **42**, 385–414 (2004)

▼ Bean pods naturally infected with halo-blight disease caused by *Pseudomonas syringae* pv. *phaseolicola*. Bacteria multiply in susceptible varieties, causing the characteristic water-soaked lesions shown here. In resistant varieties, they are restricted by the hypersensitive reaction. Image courtesy of J. Mansfield, Imperial College London, UK.

IN BRIEF

GENOMICS

Essential genes of a minimal bacterium

Glass, J. I. *et al. Proc. Natl Acad. Sci. USA* **103**, 425–430 (2006)

382 protein-coding genes are essential to sustain bacterial life, according to the latest estimate from researchers in Craig Venter's Synthetic Biology group. The ~580-kb genome of the obligate intracellular human pathogen *Mycoplasma genitalium* is the smallest of any of the organisms that can be grown in pure culture and contains just 482 protein-coding genes. *M. genitalium* was the subject of the original 'minimal genome' work by Venter and colleagues in 1999, in which global transposon mutagenesis in *M. genitalium* was used to identify the non-essential genes, and the original estimate obtained was 265–350. However, these gene disruptions were only putative. In this latest work, individual transposon mutants were isolated and characterized, and 100 protein-coding genes were deemed non-essential, according to strict criteria. None of the RNA-coding genes could be disrupted, so is the recipe for the simplest living organism add 43 RNA-coding genes to 382 protein-coding genes and stir? Watch this space to find out.

ANTI-INFECTIVES

Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase

Robicsek, A. *et al. Nature Med.* **12**, 83–88 (2006)

In a recent paper in *Nature Medicine*, researchers report a worrying development — the discovery of a naturally occuring plasmid-borne single gene variant of an aminoglycoside acetyltransferase that can reduce the activity of not only the aminoglycosides but also the fluoroquinolone ciprofloxacin. Bacterial resistance to antibiotics is acquired by chromosomal mutations or horizontal gene transfer, and the mechanism of resistance can involve the efflux of the antibiotic or modification of the antibiotic target, both of which can affect a broad spectrum of drug classes, or enzymatic drug modification, which is usually specific for a single drug class. Until now, resistance to the fluoroquinolones has comprised drug efflux or mutations in DNA gyrase or topo IV, their target enzymes. Given that the fluoroquinolones are entirely synthetic compounds, the discovery of an inactivating enzyme is surprising.

ENVIRONMENTAL MICROBIOLOGY

Slugs: potential vectors of *Escherichia coli* O157

Sproston, E. L. *et al. Appl. Environ. Microbiol.* **72**, 144–149 (2006)

Although outbreaks of *Escherichia coli* O157 are most often associated with meat and dairy products, vegetables such as lettuce and radish sprouts have been implicated as potential sources of infection. Additionally, results have indicated that invertebrates can act as vectors in *E. coli* O157 transmission. In this work, grey field slugs, a frequent contaminant of salad vegetables, were collected from farmland that was known to be grazed by sheep shedding *E. coli* O157 in faeces. The prevalence of *E. coli* O157-positive slugs was low, at less than 1%, but PCR and VNTR analysis confirmed that the slug isolates were the same as isolates from sheep faeces. *In vitro* analysis with a commensal *E. coli* strain showed that the bacteria could be transferred to an agar plate by wiping the slug across the plate, and that *E. coli* excreted in slug faeces survived for several days.

BACTERIAL PATHOGENESIS

A sweet attachment

The pathogenic bacterium *Vibrio cholerae* successfully colonizes contrasting environments such as the human small intestine and environmental water sources. Now, a new study shows that *V. cholerae* uses a remarkably simple strategy to colonize these disparate habitats. Thomas Kirn, Brooke Jude and Ronald Taylor report in *Nature* that *V. cholerae* uses a single protein to attach to sugars that are commonly found on the surfaces of both zooplankton and human epithelial cells.

To identify proteins involved in the intestinal colonization of *V. cholerae*, the authors screened a panel of *V. cholerae* mutants for defective epithelial-cell attachment. From this analysis, a putative chitin-binding protein, called *N*-acetylglucosamine-binding protein A (GbpA), was selected. Although the chitin polymer is not found on intestinal epithelium, a chitin monomer

BACTERIAL PATHOGENESIS

Rickettsia's admission ticket

As an obligate intracellular bacterium, *Rickettsia conorii* must enter its target cells to replicate and survive, but the mechanism by which this occurs has not been determined until now. Reporting in *Cell*, Pascale Cossart and colleagues show that internalization of *R. conorii* is mediated by its interaction with the protein Ku70 at the surface of host cells and that rickettsial outer-membrane protein B (OmpB) is a ligand for Ku70.

The authors set out to identify host proteins that bind *R. conorii*. Using a 'pull-down' assay, in which lysates from mammalian cell lines were incubated with whole bacteria, *R. conorii* was found to be associated with several proteins, including Ku70 and Ku86 (which are components of DNA-dependent protein kinase), PARP (which can interact with Ku70–Ku86 heterodimers) and β-actin. The cell-surface proteins of these mammalian cell lines were then labelled and incubated with whole bacteria to determine which protein(s) might function as a receptor(s) for *R. conorii*. A 70–75-kDa plasma-membrane protein was found to interact with *R. conorii* but not with several other intracellular bacteria, and it was identified as Ku70, by western blotting.

The authors next examined whether the interaction between *R. conorii* and Ku70 is crucial for infection of non-phagocytic cells (which could not otherwise internalize bacteria). Using fluorescence microscopy, Ku70 at the surface of mammalian cell lines was found to be recruited to sites of bacterial entry. Entry to these cells, but not adhesion, was blocked in the presence of a monoclonal antibody specific for an extracellular region of Ku70, showing that Ku70 is involved in internalization. Furthermore, entry was inhibited to a similar extent when Ku70 expression was reduced, confirming that this protein has an important role in

N-acetylglucosamine (GlcNAc) is a component of many intestinal-surface-bound lipids and glycoproteins. The authors therefore reasoned that GbpA might facilitate *V. cholerae* intestinal colonization by binding to GlcNAc. By comparing the binding efficiency of wild-type and *ΔgbpA V. cholerae* strains using *in vitro* attachment assays, they determined that GlcNAc is indeed a GbpA ligand. *V. cholerae* also required GbpA expression to bind efficiently to a crustacean exoskeleton that resembles the chitinous structures on the surfaces of zooplankton. These findings indicate that GbpA has an important role in the colonization strategies of *V. cholerae* in both its aquatic habitat and in the human intestinal tract.

The authors used a mouse model of cholera infection to establish that GbpA was an important *V. cholerae* virulence factor that is required for efficient *in vivo* colonization of the gastrointestinal tract. And GbpA also showed promise as a vaccine candidate. When mice were simultaneously inoculated with *V. cholerae* and GbpA hyperimmune antiserum, the animals that received the antiserum showed a significant survival advantage.

Perhaps the most intriguing finding from these studies is that GbpA is not attached to the bacterial surface, but is exported from the cell through the extracellular protein secretion apparatus. How a secreted protein mediates bacterial attachment remains a puzzle. The authors speculate that GbpA might bind to GlcNAc after exiting the cell and then attach to the surface of the bacteria. Alternatively, there might be a bacterial-surface-associated form of the protein which as yet defies detection. Finally, perhaps secreted GbpA coats GlcNAc surfaces in the *V. cholerae* habitat, 'reserving seats' for future *V. cholerae* occupancy. In this way, *V. cholerae* could outmanoeuvre competing bacterial species.

Shannon Amoils

ORIGINAL RESEARCH PAPER Kirn, T. J., Jude, B. A. & Taylor, R. K. A colonization factor links *Vibrio cholerae* environmental survival and human infection. *Nature* **438**, 863–866 (2005)
WEB SITE:
Ronald Taylor's laboratory:
http://www.dartmouth.edu/~rktlab

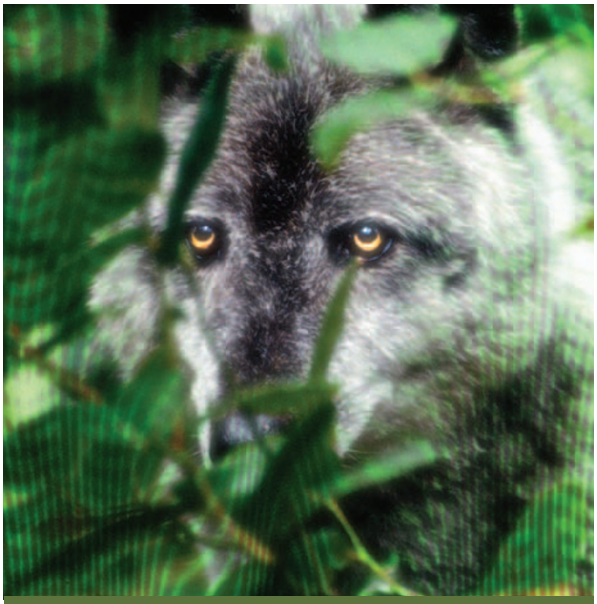
internalization. *R. conorii* internalization was also shown to depend on cholesterol, which is abundant in lipid rafts, and the authors found that a considerable proportion of plasma-membrane Ku70 is present in lipid-raft-containing membrane fractions and that the association of Ku70 with these lipid microdomains is important for *R. conorii* internalization.

To determine the bacterial ligand(s) for Ku70, *R. conorii* protein extracts were incubated with human Ku70. A single 150-kDa protein was found to be associated with Ku70 and was identified by mass-spectrometric analysis as OmpB, the most abundant surface protein of rickettsiae.

This is the first report of a receptor–ligand pair that mediates the internalization of a rickettsial species. Improving our understanding of these complex host–pathogen interactions could lead to the development of new and effective treatments for rickettsial diseases.

Davina Dadley-Moore

ORIGINAL RESEARCH PAPER Martinez, J. J., Seveau, S., Veiga, E., Matsuyama, S. & Cossart, P. Ku70, a component of DNA-dependent protein kinase, is a mammalian receptor for *Rickettsia conorii*. *Cell* **123**, 1013–1023 (2005)



DIAGNOSTICS

Hidden no more?

Although global prevalence of leprosy has decreased approximately 90% over the last fifteen years thanks to the leprosy elimination programme of the WHO, over half a million new cases are still detected every year. This might be due to the long incubation period of the disease (2–10 years), complicated by the fact that there is currently no diagnostic test that detects infection and multiplying *Mycobacterium leprae* before the onset of clinical signs. Now, in the January issue of *Infection and Immunity*, Stewart T. Cole and colleagues report on their use of bioinformatics and comparative genomics to identify potential protein antigens that could be used for diagnostic purposes.

Using this approach, three classes of proteins were defined: class I proteins are restricted to *M. leprae*, class II proteins have orthologues in species other than mycobacteria, and class III proteins are exported or surface-exposed proteins. Stewart T. Cole and co-workers selected twelve *M. leprae* genes (two class I, four class II and six class III), cloned them in *Escherichia coli* and purified their protein products. Of these 12 proteins, six were detected in *M. leprae* cell extracts by immunoblotting.

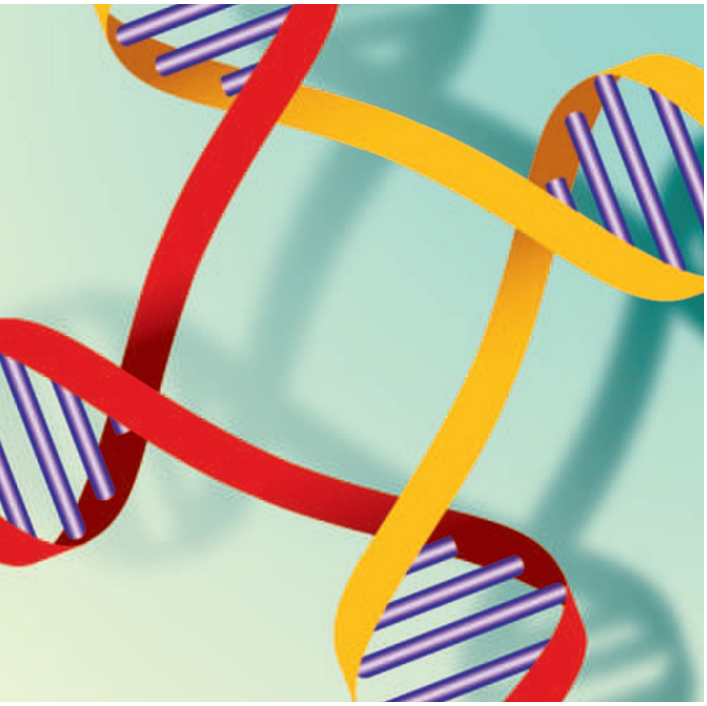
To test the immunogenicity of the recombinant proteins in infected humans, they measured the reactivity of serum samples to the recombinant protein, as well as the IFN-γ response in T-cell restimulation assays on whole blood samples. Two class II proteins, ML0308 and ML2498, were shown to have marked humoral and cellular immunogenicity. They could therefore be promising candidates for the diagnosis of both the tuberculoid and lepromatous forms of leprosy.

Annie Tremp

ORIGINAL RESEARCH PAPER Aráoz, R. *et al.* Antigen discovery: a postgenomic approach to leprosy diagnosis. *Infect. Immun.* **74**, 175–182 (2006)
FURTHER READING Engers, H. & Morel, C. M. Focus: leprosy. *Nature Rev. Microbiol.* **1**, 94–95 (2003)

ANTI-INFECTIVES

Holliday key to novel antibacterials?



New research from Anca Segall's lab has indicated that DNA repair could be a viable target for new antibacterial agents.

Previous work by the Segall lab had isolated Holliday junction (HJ)-trapping peptides — hexapeptides that bind as dimers to HJs and prevent their resolution. HJs are crucial intermediates in many DNA transactions, including repair of collapsed replication forks, DNA damage repair, homologous recombination and site-specific recombination reactions catalysed by tyrosine recombinases. In this work, Carl Gunderson and Anca Segall set out to characterize the antibacterial activity of three HJ-trapping hexapeptides.

Minimum inhibitory concentration (MIC) and growth-curve assays were initially used to assess the effects of the different peptides on bacterial growth. One peptide had no effect on bacterial growth, and the two most potent peptides were those that had been shown in earlier *in vitro* work to inhibit not only lambda integrase, a tyrosine recombinase, but also the unrelated *Escherichia coli* RecG helicase and RuvABC HJ resolvase complex, indicating that the peptides are specific for HJs rather than for their resolving enzymes. These peptides were shown to have broad-spectrum bactericidal activity, with greater activity against Gram-positive species than against Gram-negatives; the authors suggest that this might be due to difficulties crossing the Gram-negative membrane. Further analysis showed that cells can recover from periods of peptide treatment, which, combined with results obtained using *acrAB*-deletion mutants, led the authors to speculate that an efflux mechanism could be in operation.

Once the general effects on growth had been analysed, Gunderson and Segall moved on to look at the cellular effects of treatment with the two bactericidal peptides, using microscopy, flow

BACTERIAL PHYSIOLOGY

Fishing for success

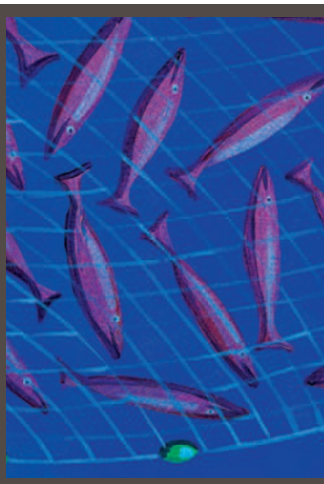
Jean-Pierre Claverys and colleagues have uncovered the mechanism behind a phenomenon that has remained a mystery in pneumococcal research for more than 30 years.

Recently, Claverys and colleagues examined the connection between *Streptococcus pneumoniae* competence, sibling fratricide and virulence. The fratricide pathway they elucidated involves a novel, competence-induced bacteriocin, CibAB, as the trigger for the lysis of non-competent pneumococci by three cell-wall hydrolases — LytA, LytC and CpbD. Lysis is followed by the release of DNA and other cellular components from the non-competent cells, including cytoplasmic virulence factors such as pneumolysin. Competent cells are resistant to the CibAB trigger owing to a cytoplasmic immunity component, CibC. Now, while investigating the 'pneumococcal clumping' phenomenon first reported more than 30 years ago, Claverys and co-workers have discovered strong similarities with competence-induced lysis.

Tomasz and Zanati observed the clumping phenomenon — strong and immediate clumping of cells that have been pelleted by centrifugation and resuspended in dilute acid — only with competent cells. However, when Håvarstein *et al.* recreated this experiment, they discovered that in fact a mixture of competent and non-competent cells is required for clumping. Intriguingly, the authors also found that either LytA or LytC, and CpbD were required for clumping, indicating the involvement of cell lysis and DNA release. More detailed analysis of CpbD pinpointed the protein domain (the putative amidase domain, CHAP) that contains the activity required for clumping and also, the authors speculate, competence-induced lysis.

So, what is the relationship between the clumping phenomenon and competence-induced fratricide? Although there are similarities, such as the requirement for a mixture of competent and non-competent cells and the involvement of cell-wall hydrolases, there are also differences, such as the immunity components: CibC is the immunity component for fratricide and, in this latest study, ComM was shown to confer immunity against clumping. Additionally, the triggers for the two processes are different, with CibAB triggering fratricide, whereas CpbD is thought to trigger clumping. The authors therefore suggest that the clumping phenomenon is a form of fratricide and that the different genetic requirements reflect the different experimental environments used and the resultant difference in cell–cell contacts.

Finally, in the original work, Tomasz and Zanati speculated that clumping was caused by an agglutinin on the surface of competent pneumococci. Håvarstein *et al.*, however, found evidence that the aggregation requires the presence of DNA in the medium.



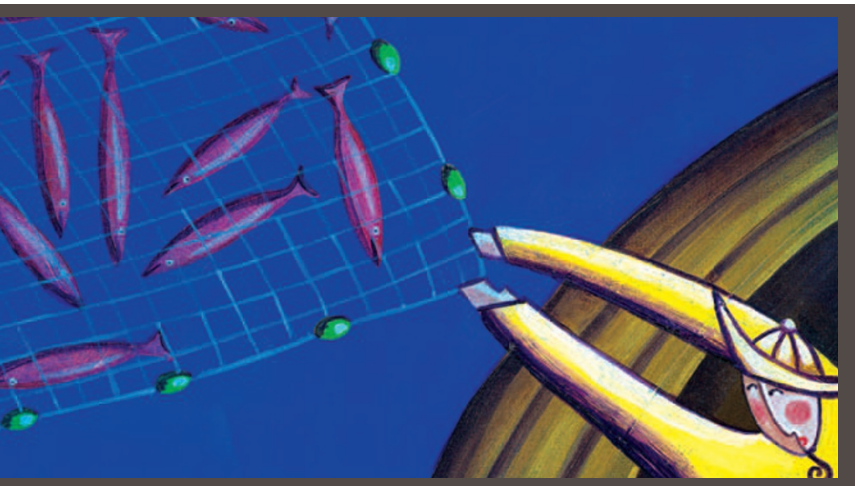
> "The authors therefore suggest that the clumping phenomenon is a form of fratricide"

cytometry and a specially adapted TUNEL (terminal deoxyribonucleotide transferase-mediated dUTP nick end-labelling) assay. The peptide-dependent phenotypes observed — filamentation, defective chromosome segregation, anucleation and increased DNA damage — combined with other *in vitro* data were consistent with a scenario in which the peptides mediate their bactericidal effect by trapping HJs (or other branched intermediates) generated during recombination-dependent repair of collapsed replication forks. Although it was not conclusively demonstrated that the peptides bind branched intermediates *in vivo*, further confirmation of this hypothesis was provided by comparison of the effects of the peptides in a series of mutants of different backgrounds and the fact that peptide-induced damage was synergistic with some DNA-damaging agents, such as UV light.

The HJ-trapping hexapeptides used in this study were initially identified in the Segall lab in a screen of synthetic peptide combinatorial libraries to identify inhibitors of lambda integrase site-specific recombination, and indeed these peptides have been extremely useful in dissecting recombination pathways and will continue to be useful analytical tools. Given their broad-spectrum bactericidal activity and the fact that the development of spontaneous resistance is unlikely, this work shows that they might also have potential as antibacterial agents, and provides proof of principle that DNA repair might be a novel target for the development of new antibacterials.

Sheilagh Molloy

ORIGINAL RESEARCH PAPER Gunderson, C. W. & Segall, A. M. DNA repair, a novel antibacterial target: Holliday junction-trapping peptides induce DNA damage and chromosome segregation defects. *Mol. Microbiol.* (doi: 10.1111/j.1365-2958.2005.05009.x)
FURTHER READING Bold, J. L., Pinilla, C. & Segall, A. M. Reversible inhibitors of lambda integrase-mediated recombination efficiently trap Holliday junction intermediates and form the basis of a novel assay for junction resolution. *J. Biol. Chem.* **279**, 3472–3483 (2004)
WEBSITE
Segall lab website: http://segall-lab.com



No experimental data were available to prove whether a DNA receptor is involved, but active binding by the DNA-uptake machinery was ruled out. One interesting hypothesis raised by the authors is that the long strands of DNA released from the lysed non-competent cells form a 'fishing net' that causes the cells to adhere together in the acidic conditions. Fortunately for Håvarstein *et al.*, the clumping phenomenon is no longer 'the one that got away.'

Sheilagh Molloy

ORIGINAL RESEARCH PAPER Håvarstein, L. S. *et al.* New insights into the pneumococcal fratricide: relationship to clumping and identification of a novel immunity factor. *Mol. Microbiol.* (doi: 10.1111/j.1365-2958.2005.05021.x)
FURTHER READING Guiral, S. *et al.* Competence-programmed predation of noncompetent cells in the human pathogen *Streptococcus pneumoniae*: genetic requirements. *Proc. Natl Acad. Sci. USA* **102**, 8710–8715 (2005) | Tomasz, A. & Zanati, E. Appearance of a protein 'agglutinin' on the spheroplast membrane of pneumococci during induction of competence. *J. Bacteriol.* **105**, 1213–1215 (1971)

VIROLOGY

Targeted integration

HIV preferentially integrates into active genes in the human genome but, until now, how the virus targets these sites has been unknown. Frederic Bushman and colleagues show for the first time that a cellular protein called lens epithelium-derived growth factor (LEDGF/p75) controls the site of HIV DNA integration in human cells. Reporting in *Nature Medicine*, they outline a mechanism by which this protein directs HIV to active genes.

Because integration-target preferences differ among different retroviruses, researchers have suggested that DNA- or chromatin-bound nuclear proteins might interact with retroviral integration complexes, promoting viral integration at nearby DNA. In particular, the transcriptional co-activator LEDGF/p75 was proposed as a potential tethering protein because it binds tightly to HIV integrase and is linked to the chromosomal localization of integrase. Bushman and co-workers knocked down LEDGF/p75 in three human cell lines and showed that the integration of HIV into active genes was reduced compared with controls. Then they focused on genes that were regulated by LEDGF/p75, identifying these loci by transcriptional profiling. In several human cell lines, LEDGF/p75-regulated genes were preferred HIV-integration sites, whereas in cells depleted of LEDGF/p75, this differential integration pattern was not observed. Furthermore, knockdown of LEDGF/p75 resulted in increased HIV integration into chromosomal regions with a higher GC content.

The authors conclude by proposing a model for HIV DNA-integration targeting. LEDGF/p75 binds to RNA polymerase subunits and also to transcription factors, which results in its enrichment at active genes. Simultaneous binding to HIV integrase could tether HIV to these active loci. LEDGF/p75 comprises an AT hook, a motif that binds preferentially to AT-rich DNA. This could explain why LEDGF/p75 depletion shifts HIV integration to GC-rich DNA.

Shannon Amoils

ORIGINAL RESEARCH PAPER Ciuffi, A. *et al.* A role for LEDGF/p75 in targeting HIV DNA integration. *Nature Med.* **11**, 1287–1289 (2005)
FURTHER READING Bushman, F. *et al.* Genome-wide analysis of retroviral DNA integration. *Nature Rev. Microbiol.* **3**, 848–859 (2005)