

Meeting report

The diversity of bacterial pathogenicity mechanisms

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A report on the international conference 'Molecular basis of bacterial pathogenesis', sponsored by the Federation of European Microbiological Societies (FEMS) and the Israel Center for the Study of Emerging Diseases, Ein Gedi, Israel, 23-27 January 2005.

One of the remarkable features of the recent FEMS meeting on the molecular basis of bacterial pathogenesis was the novel ways in which genome sequences are now being used to study bacterial pathogens. In the 10 years since the first complete sequence of the genome of a pathogenic bacterium - that of *Haemophilus influenzae* - was published, the genomes of almost all the major human pathogens have been sequenced. The first and most obvious use of these data was comparative genome analysis in order to understand what distinguishes pathogenic from nonpathogenic strains. While this approach continues to be useful for discovering new genes that cause disease (virulence genes, which are possible targets for new antibacterial drugs) and clusters of virulence genes (pathogenicity islands) in the genome, and for providing clues to how pathogens have evolved, several new approaches to using genome data were presented at the meeting. These include the development of new vaccines (reverse vaccinology), uncovering new biosynthetic pathways, studying how bacteria adapt rapidly to new environments and the beginning of a comprehensive comparison of genomics and proteomics.

The genetics of pathogenicity

Virulent strains of *Escherichia coli* can be divided into two classes: those that cause intestinal disease and those causing disease elsewhere in the body (extra-intestinal strains). Extra-intestinal *E. coli* (ExPEC strains) are the cause of a diverse spectrum of invasive human and animal infections, often leading to septicemia. Joerg Hacker (Institut für Molekulare Infektionsbiologie, Würzburg,

Germany) reported the analysis of the genomes of a number of pathogenic and commensal *E. coli* strains. Each genome could be divided into the 'core genome' and the 'flexible gene pool'; the latter comprises up to one third of the entire genome. For example, the uropathogenic *E. coli* strain 536 contains six pathogenicity islands, comprising more than 500 kb in total. These islands show a characteristic genetic architecture and determine properties such as adhesion to host cells, toxicity, invasiveness, resistance to serum and other virulence functions. The nonpathogenic commensal *E. coli* strain Nissle carries at least four 'symbiotic islands'. Hacker emphasized that the genomic islands of both pathogens and nonpathogens are unstable regions, as shown by their high deletion rates and the fact that they can be transferred from one strain to another following plasmid mobilization.

E. coli strains that cause the same disease and target the same host tissue also show large differences in the flexible gene pool, as Eliora Ron (Tel Aviv University, Israel) reported. Her results indicate that each step in the infection can be mediated by a number of alternative virulence factors, a conclusion that is consistent with previous studies indicating the existence of a 'mix and match' combinatorial system of virulence factors. The finding that the large pool of virulence genes in septicemic *E. coli* strains is independent of the host that is being infected implies a high risk of transmission of the pathogen from animals to humans (zoonosis).

The availability of genome sequences of pathogens enables the discovery and testing of vaccines without the need to grow the pathogen. Rino Rappuoli (Chiron Vaccines, Siena, Italy) reported the application of this procedure for the first time to serogroup B meningococcus (*Neisseria meningitidis*), a major cause of meningitis, which has resisted all conventional approaches to vaccine development. The genome sequence allowed the prediction of 600 potential antigens. Of the 350 that were expressed in *E. coli*, purified and used to immunize mice, 29 were found to induce bactericidal

antibodies. A subgroup of the genome-derived antigens is now being tested in clinical trials.

Avigdor Shafferman (Israel Institute for Biological Research, Ness-Ziona, Israel) presented genomic and proteomic analyses of the virulence-related genes of the anthrax bacillus, *Bacillus anthracis*, as potential vaccine candidates. Focusing on sequences that code for known virulence factors in *B. anthracis* or that contain motifs familiar from other pathogens, or that encode extracellular proteins, 200 candidate open-reading frames were selected for evaluation. Using high-throughput screening, several of these were selected as potential vaccine enhancers on the basis of the immunoreactivity of the encoded proteins with hyperimmune sera from *B. anthracis*-infected animals and by the ability of the DNA sequence itself to elicit a humoral immune response as a DNA vaccine.

Novel metabolic mechanisms

Glutathione biosynthesis in many bacteria proceeds through the consecutive action of two separate enzymes, encoded by *gshA* and *gshB*. Yair Aharonowitz (Tel Aviv University) presented evidence that in the intracellular pathogen *Listeria monocytogenes* the synthesis of glutathione is carried out by a multidomain protein, called GshF, which integrates the two primary catalytic activities. Analysis of the genome sequences of several other mammalian pathogens indicated the presence of the *gshF* gene, and molecular phylogenetic analysis suggests that *gshF* genes probably originated when a *gshA* ancestor recruited a gene encoding a member of the ATP-grasp superfamily by gene fusion, and was subsequently spread by horizontal gene transfer.

Werner Goebel (University of Würzburg, Germany) reported the application of high-throughput comparative transcriptome and proteome analysis and metabolic flux measurements using ¹³C-glucose in an effort to understand the molecular basis of the intracellular growth of *L. monocytogenes*. His results showed that storage and waste products of the host cells were the major carbon and nitrogen sources for intracellular listeriae. Complementing this study, Juergen Krept (University of Würzburg) has used the complete genome sequence of *L. monocytogenes* to identify previously unknown virulence factors. A large gene cluster determines the uptake and metabolism of maltose, maltotriose, and maltodextrins. Growth experiments indicated that the maltose pathway was important for the growth and survival of the bacterium in cell culture.

From genome to proteome

One of the highlights of the meeting was the review by Michael Hecker (University of Greifswald, Germany) of five years of research going from the genome to the proteome of *Bacillus subtilis*. The elegant technique developed by Hecker

for analyzing the proteome involves separating the proteins by two-dimensional chromatography and then analyzing the trypsin digest of each protein by nuclear magnetic resonance (NMR). As the sizes and amino-acid sequences of all the peptides generated from the trypsin digest of each protein can be predicted from the genome sequence, the proteins can be identified in a few minutes. Using this powerful technique, protein synthesis, stability, secretion, post-translational modification and protein damage were determined under a variety of environmental conditions. In essence, this approach brings the blueprint of life (the genome) together with the real life of proteins. Hecker also presented new information on the proteomics of *Staphylococcus aureus*, including its extracellular proteins and protein-expression networks.

Carmen Buchrieser (Institut Pasteur, Paris, France) has determined and analyzed the complete genome sequence of two strains of *Legionella pneumophila*, the cause of legionnaire's disease: Paris (3,239 genes) and Lens (3,129 genes). Three different plasmids were identified, and nearly 13% of each genome is distinct from the other. A large number of genes encode eukaryotic-like proteins or motifs that are predicted to modulate host-cell functions to the pathogen's advantage, including tetratricopeptide repeats, ankyrin repeats, F-box domains, coiled-coil domains, serine-threonine protein kinase domains, apyrase domains and sphingosine-1-phosphate lyase domains. Thus, the genome reflects the history and lifestyle of *L. pneumophila*, a pathogen of human macrophages that co-evolved with amoebae, which is probably the reason that it has eukaryotic-like proteins.

Bacterial virulence is due to a remarkable variety of properties. Pascale Cossart (Institut Pasteur) described how the intracellular pathogen *L. monocytogenes* exploits the endocytic machinery of mammalian cells. This bacterium invades normally non-phagocytic cells by inducing phagocytosis. After lysing the phagosome membrane surrounding it, the bacterium multiplies in the cytosol and moves within the cell by polymerizing actin of the host cell's cytoskeleton. Philippe Sansonetti (Institut Pasteur) reported how the interaction of *Shigella flexneri* with epithelial cells encompasses contact with membrane rafts on the host cell through engagement of the cell-surface protein CD44, activation of a type III secretory apparatus and release of bacterial Ipa (invasion plasmid antigen) proteins into the cell. A cascade of signals elicited by GTPases causes rearrangement of the cytoskeleton, allowing the bacteria to be taken up by macropinocytosis, a general endocytic mechanism by which large amounts of the extracellular fluid and its contents are nonspecifically engulfed. A paracrine pathway involving calcium fluxes and secretion of ATP via cell junctions that Sansonetti calls hemiconnexions facilitates cytoskeletal rearrangements, thereby allowing the bacteria to enter the cell and spread from cell to cell. High concentrations of *Shigella* in epithelial cells result in production of the chemokine interleukin-8,

inducing massive recruitment of leukocytes that are responsible for the destructive inflammatory process characteristic of shigellosis, a disease characterized by dysentery.

A recurring theme in biology is unity despite diversity. One of the main take-home messages of the meeting was that not only is there a wide diversity of pathogenic bacterial species, but there is even an enormous diversity of virulence genes in strains of the same species. Hopefully, further analysis of the genomes of bacterial pathogens will reveal some unifying principles underlying this complexity.

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