up to a few years ago, was a complete mystery. But there's still a great deal of work to do,' explains Thornton.

Alfred J. Spiro, Professor of Neurology and Pediatrics at the Albert Einstein College of Medicine (http://www.aecom.yu.edu) commented on these recent advances in our understanding of DM1, which also apply to DM2: this being caused by a CCTG expansion located in intron 1 of the zinc finger protein 9 gene [5], where research has revealed microsatellite expansions in RNA that could be pathogenic. He added, 'Comparative studies of these two disorders could shed some additional light on the basis of the multisystemic involvement in both'.

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Enlightening cholera bug reveals new virulence system

Graciela Flores, freelance writer

Researchers studying quorum sensing – the language of bacteria – have found a previously undiscovered set of instructions involved in controlling virulence in cholera infections.

Vibrio cholerae

Only in the past 10 years has it become obvious that bacteria can 'gang up' and act like an enormous multicellular organism in response to cell density [1]. Through subtle cross-talk, they synchronize behaviours such as bioluminescence, biofilm formation, sporulation and expression of virulence factors. Although they are continuously synthesizing signal molecules, called autoinducers, the bacteria do not sense these at low cell densities, because the signals never reach a critical concentration. Only at high cell densities do receptor proteins sense these autoinducer molecules, and turn the target genes either on or off [2].

'There are many other inputs, other than quorum sensing, into the regulation of the virulence cascade,' says Bonnie Bassler, Professor of Molecular Biology at Princeton University (http://www.molbio.princeton.edu), 'but what's very interesting is that, in *V. cholerae*, cell–cell communication controls the entire virulence regulon.'

Vibrio cholerae (Fig. 1) causes the disease cholera by producing an enterotoxin that provokes an acute intestinal illness. In most populations, it is of fairly mild impact and is indistinguishable from other infectious causes of diarrhoea. However, it causes 120,000 deaths worldwide, most of them in countries where cholera epidemics occur because of poor hygiene, the usual cause being contaminated water. Most cases can be treated successfully with oral rehydration fluids alone, but severe cases can require antibiotic treatment.

Then there were three

Altogether there are 50 genes responsible for virulence in *V. cholerae*. Among other products, they encode two main factors: the cholera toxin (TC) and the toxin-coregulated pilus (TCP), which helps the bacteria attach to the lining of the intestine. Until now, only two sensory systems were known to control the production of TCP and TC, as well as the other 48 genes [3]. The quorum-sensing system is an unexpected third.

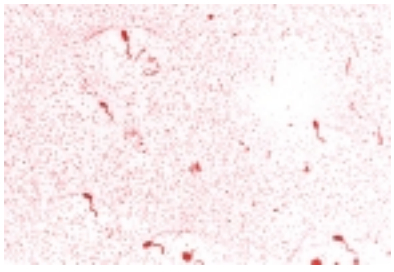

Figure 1. *Vibrio cholerae.* Leifson flagella stain (digitally colorized). Image courtesy of William A. Clark, US Centers for Disease Control and Prevention (http://www.cdc.gov/).

In Bassler's experiments, *V. cholerae* has revealed, one by one, its three parallel sensing systems that converge to

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regulate virulence [3]. System 1 is species-specific and is believed to participate in communication between bacteria belonging to the same species. It involves the CAI-1 autoinducer and its corresponding sensor CqsS. Through this system, one bacterium can tell another: 'I'm here.'

By contrast, system 2 is a universal language that bacteria belonging to different species can understand, enabling them to sense which other bacteria are around. It involves a signal molecule, the autoinducer AI-2, and its sensor, LuxPQ. 'This could give [the bacteria] a sense of 'yourself' versus 'everybody else',' said Bassler. By measuring the level of AI-2 signals, a bacterium can also know whether it is in the majority or in the minority, and behave accordingly.

Finally, there is system 3. 'We still don't know what it is,' said Bassler, 'it came as a surprise.'

Shedding new light on the cholera pathogen

This latest research is a collaboration between the Princeton group and a team from Dartmouth Medical School (http://www.dartmouth.edu) [4]. According to Melissa Miller, a graduate student in Bassler's lab and the first author of the paper, the researchers recognized from analyzing the *V. cholerae* genome that it possessed the more universal AI-2 system they had already described in *V. harveyi*, its close relative.

To determine whether the AI-2 system was functional in *V. cholerae*, Miller inserted the luciferase operon into its genome; this is responsible for light production in *V. harveyi.* Not only was the operon active but when they knocked out both the AI-2 system and the CAI-1 system, to their surprise, quorum sensing still controlled the luciferase operon. A triple mutant would be needed to finally eliminate quorum sensing in *V. cholerae*.

The therapeutic potential is obvious to Bassler. 'We could interfere with the system by adding autoinducer mimics that would turn off the virulence,' she explained. The laboratory is now conducting experiments in that direction. But first, they need to learn how the bacterium behaves *in vivo*. At present, they are working in a mouse model, looking at how *V. cholerae* might use independent signalling to control virulence, focusing on cross-talk within the gut.

Not alone in its complexity

'This work reaffirms the complexity of virulence gene regulation, something that is not unique to *V. cholerae*,' observed Barbara Iglewski, Chair of Microbiology and Immunology at the University of Rochester Medical Center (http://www.urmc.rochester.edu/). 'This is a story that's coming up in other organisms like *Pseudomonas aeruginosa*, which has three different extracellular signals regulated layer upon layer,' [5]. The method of introducing the luciferase operon could prove useful in other studies, she added.

The experiments also revealed another unexpected feature of *V. cholera*e. In contrast to what has been described for other quorum-sensing systems that regulate virulence, virulence factors in *V. cholera*e are turned off at high cell density. Miller suspects that the life cycle of the bacterium may explain this: *V. cholerae* spreads by returning to the water supply. 'At high cell density,' she speculated, 'the quorum-sensing system may shut off TCP production to stop bacteria from adhering to the intestinal wall, so that they are flushed into the environment.'

This work could point the way for the control of virulence in several organisms, offering great therapeutic potential.

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