effector; Raf, PI3-kinase or RalGEFs. They then tested the ability of the cells to form colonies in soft agar (a phenotype characteristic of cancer cells). Although there had been hints that Ras-induced tumourigenesis in humans and mice might not be identical [5], the results came as a surprise: activation of the Raf and PI3-kinase effector pathways, either alone or together, was not sufficient to cause transformation of human cells. Instead, activation of the RalGEF effector pathway did the trick.

'Here is a pathway that has been pretty well ignored, because it did not play a big role in making murine cells tumourigenic,' says Christopher Counter, senior author of the study. 'We find that it is far more important in human cells than it appears to be in mouse cells, at least in the approach we used to study Ras-induced transformation.'

Open questions

Anton Berns, Scientific Director of The Netherlands Cancer Institute (http://www.nki.nl/) in Amsterdam, believes that the paper is interesting, but warns not to overinterpret the results. 'I do not think it is justified to conclude that mouse models, at least in respect to the Ras pathway, are very different,' he said. Berns points towards recent reports that B-Raf is mutated in several human tumours [6] and suggests that both the Raf and the RalGEF effector pathways might be important. 'RalGEF might certainly be a little bit more significant in humans than in mice. But then, this conclusion is based on *in vitro* assays.'

Cannon strikes a similar note: 'I would have liked to have seen whether RalGEF is really malfunctioning in primary human tumours – whether it is overexpressed or mutated.'

'This finding does not diminish the importance of mouse models,' agrees Channing Der, co-author of the study. 'What it tells us is that we need to use both systems [mouse models and cultured human cells], because each system has its strengths and its limitations. We need to be cautious of extrapolating from one to the other.'

This applies to their own studies, Der says. His team is studying human cells, which is an advantage, but in cell culture, which is a disadvantage. In mouse studies, the species difference is a concern, but the fact that they are *in vivo* gives them 'a great advantage'. The message, he says, is to use both these systems rigorously and concurrently, to learn the most likely scenario for humans.

Implications for treatment

Counter and colleagues are now aiming to antagonize the RalGEF pathway as a means of treating *ras* mutation-positive cancers, such as lung, colon and pancreatic cancer. Counter concludes, 'There is no question that the best candidates have come from experiments in mice, and although they are clearly important in human cancers, it looks like there might be at least one other pathway that is open for targeting, and that is the RalGEF pathway.'

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Muscular dystrophy: toxic RNA to blame

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Researchers probing the genetic cause of adult-onset muscular dystrophy have found a totally new way for mutations to cause hereditary disease. Their research shows that the loss of muscle control in the most common form of this disease, myotonic dystrophy type 1 (DM1), is a result of a mutation that creates a toxic form of RNA [1–3]. The researchers call this 'a new way that a genetic mistake can harm the body.'

Myotonic dystrophy

Characteristics of myotonic dystrophy are hyperexcitability (myotonia; the ability to contract but not relax the muscles), progressive muscle wasting, insulin resistance, cardiac defects, cataracts and neuropsychiatric disorders. Most muscular dystrophies only affect the muscles.

The gene for DM1, found on chromosome 19, encodes a protein kinase (DMPK) that is found in skeletal muscle, where its pathophysiological role remains to be defined. Symptoms of the disorder become progressively worse as it is passed down through generations: the disorder has an incidence of about one in 8000

DM1 differs from many other hereditary diseases by having a variable genetic factor. (In most genetic disorders, you either have the gene or you do not.) In DM1, the severity of the disease varies

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with the number of mutations and the age of onset of the disease. At present, the disorder is incurable but not unmanageable.

CTG triplet

In DM1, mistakes in gene copying result in the amplification of a CTG triplet. Severely affected individuals have an expansion of up to several thousand copies of this triplet, whereas unaffected people have fewer than a dozen copies of the repeat. However, the portion of the gene that is repeated does not itself encode a protein, making this a particularly puzzling mutation.

Research teams led by Thomas A. Cooper from the Baylor College of Medicine (http://public.bcm.tmc.edu) [1] and Charles Thornton at the University of Rochester Medical Center (http://www.urmc.rochester.edu) [2,3], examined skeletal muscle cells from human DM patients and also from mice that were bred to express the mutation. Both research groups found that the skeletal muscle of classical DM lacks a membrane protein, the cell chloride channel (CIC-1), which is a key component in muscle relaxation.

Muscling in

The genetic defect that underlies DM was discovered 10 years ago, but up until now it was not known how this defect causes the disease. Thornton and his team found that the faulty gene produces mRNA that prevents the chloride channel being synthesized in muscle. This channel is crucial to maintaining the proper electrical flow in muscles; without it, the signals in the muscles stay switched 'on' for too long and the control of muscle function becomes unstable.

In 2000, Thornton and co-workers developed a mouse mimic of DM and found that the mRNA itself caused the symptoms by accumulating in muscles. The group, led by Cooper, had previously found that the DM mutation was also responsible for interruption of the process of alternative splicing (Fig. 1), which regulates gene expression and protein production [4].

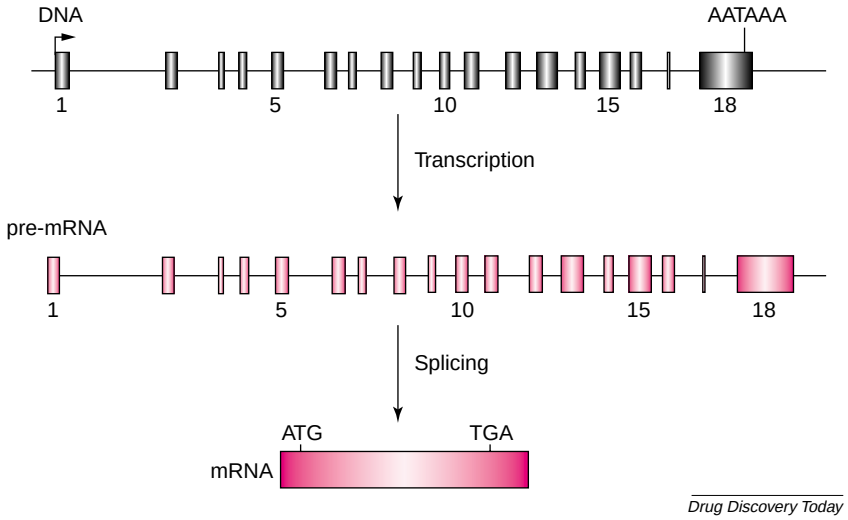

Figure 1. The process of alternative splicing regulates gene activation and the production of protein. The abnormal mRNA from the myotonic dystrophy protein kinase gene, *DMPK*, builds up in the nucleus and prevents other RNAs from being spliced correctly, leading to the electrical problems that result in the characteristics of myotonic dystrophy type 1. Figure courtesy of Tom Cooper (Baylor College of Medicine; http://public.bcm.tmc.edu).

'Somehow the abnormal mRNA coming from the myotonic dystrophy gene [*DMPK*] builds up in the nucleus and prevents other RNAs from being spliced correctly. This is what causes this electrical misbehaviour in patients with myotonic dystrophy,' explains Thornton.

Results of the recent study showed that DM1 is caused by a CTG amplification in the 3′ untranslated region (UTR) of the *DMPK* gene. The CUG-binding protein, which is present in increased levels in striated muscle from individuals with DM1, regulates abnormal splicing of the CIC pre-mRNA. Expression of these repeated regions reduces transmembrane chloride conductance to levels so low that the muscles cannot function properly, causing myotonic dystrophy.

'It is timely to find a disease that affects regulation of alternative splicing, because the human genome project is revealing that most human genes undergo this process,' explains Cooper. 'This is a new mechanism for genetic disease in humans that could play a role in other disorders.'

Thornton adds, 'We believe this is the first example of an abnormal gene that has a harmful effect in the human body because the RNA that the gene produces is toxic. Bad RNA is the culprit, and good RNA is the victim.'

Future perspectives

'This is something of a breakthrough,' Cooper says, 'because this disease has been such an enigma since its discovery over 10 years ago. We now know the mechanism of pathogenesis; still, how to address the cause of the disease will require some fine-tuning of the mechanistic insights.'

The teams are now looking into understanding the mRNA flaw and how it might cause the characteristic muscle stiffness and weakness. However, although this is an important step in our understanding of DM1, the scientists stress that any possible new treatments for the disorder are still years away. 'In just a few years, we have gained a much better understanding of a process that,

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.

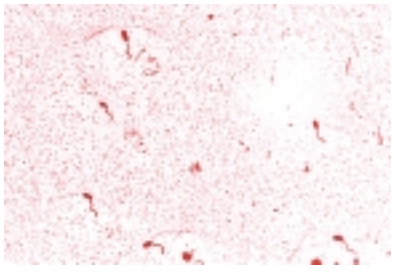

Figure 1. *Vibrio cholerae.* Leifson flagella stain (digitally colorized). Image courtesy of William A. Clark, US Centers for Disease Control and Prevention (http://www.cdc.gov/).

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

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