Biofilm studies yield targets against cystic fibrosis

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The bacterium *Pseudomonas aeruginosa*, principal culprit in the persistent lung infections of cystic fibrosis (CF) patients, is not only capable of growing in the oxygen-depleted mucus of CF-affected airways but actually thrives there, and develops remarkably robust biofilms in complete anaerobiosis.

This finding sent a team led by Daniel J. Hassett, Associate Professor of Molecular Genetics at the University of Cincinnati College of Medicine (http://www.med.uc.edu), on a quest for bacterial proteins that are specific to such biofilms, and they found a few unique ones that they believe are potential targets for therapeutic agents. These findings were published in the October 2002 issue of *Developmental Cell* [1].

A great environment for bacteria

CF is one of the most common fatal genetic disorders in the USA, is most prevalent in the Caucasian population and occurs on average in 1 in 3300 live births. A mutation in a gene that encodes a chloride channel – the cystic fibrosis transmembrane conductance regulator – produces partially functional or completely dysfunctional channels. Depending on where the gene is mutated and on whether the person carries one or two copies of the mutated allele, the prognosis varies widely: heterozygous individuals are fine for life; those who are homozygous for the mutation acquire CF; and if patients have the most common CF allele – DF508 – they typically die at around the age of 31.

CF patients develop thick mucus secretions resulting from disruption of the salt–water balance. These disruptions clog bronchial tubes in the lungs and plug exit passages of the pancreas and intestines, leading to loss of function of these organs. And it is in this thick mucus – depleted of oxygen by the metabolic activity of aerobic bacteria, neutrophils and even epithelial cells – where *P. aeruginosa* thrives [2].

After finding that *P. aeruginosa* forms biofilms that have three times more bacteria and contain 1.8-times more live cells than dead cells when grown in the absence of oxygen, Hassett's team used isogenic mutants to see if proteins known to be important in aerobic biofilms were also central in anaerobic biofilm – and indeed they were.

The researchers targeted gene products involved in quorum sensing and in the formation of flagella and pili. Predictably, the pilus-deficient (pilA) and flagellum-deficient (fliC) mutants formed poor aerobic or anaerobic biofilms. The quorum-sensing mutants, lasR, rhlR and lasRrhlR all grew robust aerobic biofilms, but in the absence of oxygen no mutant could match the biofilms made by wildtype organisms [2].

No action without a quorum

However, the most interesting result was still to come. 'When we impaired the quorum sensing, we found that even though they did form a nice biofilm, they were dead,' explained Hassett.

The precise basis for the death of the rhlR mutants was the accumulation of nitric oxide (NO), which the authors demonstrated by various means, such as sequestering NO and knocking out the enzyme that synthesizes NO. In both cases, the survival of the bacteria was dramatically increased.

'This is proof to any skeptical reader that NO is the culprit in killing,' said Hassett, who is already envisioning a few chemotherapeutic agents. The idea is to use homoserine lactone analogues to interfere with bacterial communication, which, they believe, is causing the NO buildup. 'If bacteria don't know what the message means,' he said, 'they will undergo this process of poisoning themselves.'

Postdoctoral researcher Mark Shirtliff of the Center for Biofilm Engineering at Montana State University (http://www.erc.montana.edu/) agrees that targeting this quorum-sensing system is a promising approach [3]. 'By interfering with this system specifically,' he said, 'you might promote the bacteria to commit suicide'.

But Clay Fuqua, Assistant Professor of Biology at Indiana University in Bloomington (http://www.indiana.edu), believes that the effect of rhlR quorum-sensing mutants – decreased bacterial survival in biofilms – is in some way an artifact of cultivation. 'The toxicity is due to increased accumulation of NO that results from the mutation,' he said. 'This observation may be extremely useful in developing antimicrobial chemotherapies, but that does not necessarily indicate a central role of rhlR [and hence quorum-sensing] specifically in anaerobic biofilms.'

More than one potential target

Fuqua is actually more interested in yet another finding reported in the paper. When the authors looked for bacterial products specific to anaerobic biofilms via proteomic analysis, they found an outer membrane protein – a channel-forming porin called OprF – that was

1359-6446/02/$ – see front matter PII: S1359-6446(02)02524-2

upregulated about 40-fold in anaerobic biofilms. The protein was also in the sera of chronically infected patients. The authors showed that with increasing chronicity of the infection, progressively more antibodies against OprF were formed. Thus, Fuqua thinks OprF is a promising target.

Although he is personally more excited by the quorum-sensing target, Shirtliff mentioned some successful attempts by other authors to develop a vaccine against the OprF protein [4]. The vaccine protected mice fromchronic pulmonary infection with *P. aeruginosa* and could also be applied in this case.

Meanwhile, Hassett is happy with both potential targets and is prepared to talk with companies that he expects will be interested in these results. 'We have a surface-exposed drug target and we have the talking-machinery drug target,' he said. 'It's very exciting.'

References

1 Yoon, S.S. *et al.* (2002) *Pseudomonas aeruginosa* anaerobic respiration in biofilms: relationships to cystic fibrosis pathogenesis. *Dev. Cell* 3, 593–603

2 Worlitzsch, D. *et al.* (2002) Effects of reduced mucus oxygen concentration in airway *Pseudomonas* infections of cystic fibrosis patients. *J. Clin. Invest.* 109, 317–325

3 Erickson, D.L. *et al.* (2002) *Pseudomonas aeruginosa* quorum-sensing systems may control virulence factor expression in the lungs of patients with cystic fibrosis. *Infect. Immun.* 70,1783-1790

4 Price, B.M. *et al.* (2002) Enhancement of the protective efficacy of an oprF DNA vaccine against *Pseudomonas aeruginosa*. *FEMS Immunol. Med. Microbiol.* 33, 89–99

Chemical geneticists unify their data

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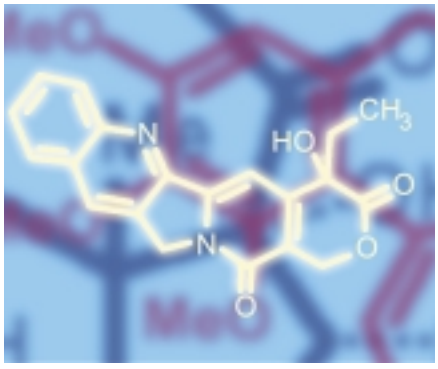
The first large-scale database of small molecules, to be released shortly, will be a boon to the field of chemical genetics, says Brent Stockwell, a researcher at the Whitehead Institute (http://www.wi.mit.edu/home.html). The collection promises to help researchers rapidly test thousands of mechanisms in mammalian cells, which have been beyond the reach of traditional genetics.

The annotated compound library (ACL) will be available to the public once the details have been finalized and published, Stockwell said at a recent conference at the New York Academy of Sciences (http://www.nyas.org).

A special challenge

Chemical geneticists – who study the effects created when specific small-molecule chemicals are added to cells – have to overcome several disadvantages that classical geneticists do not encounter, as they study the effects of random (or even intentionally induced) mutations [1]. Where other geneticists can trace a phenotype back to the mutated gene with relative ease, identifying the specific target of a small molecule can be challenging.



Unlike site-directed mutagenesis, it can also take 'years and years, if [it is] at all possible, to make one small molecule that's specific for a single target,' said Kevin Shokat, Associate Professor of Cellular and Molecular Pharmacology at the University of California in San Francisco (http://www.ucsf.edu).

When researchers do have success with a specific molecule, they have to plough through reams of literature to match it with the relevant molecular mechanisms [2]. Creating a GenBank-like database of small molecules can overcome some of those handicaps, says Stockwell. For example, he says, if scientists screen 2000 compounds, and find that 50 can actively inhibit angiogenesis, the ACL website can calculate statistically relevant themes among those compounds. Based on the mechanisms that are enriched among the molecules, the researchers can form a 'mechanistic hypothesis,' he said.

A range of chemical space

There are currently two main types of small molecules: drug-like synthetic compounds that are simple and of low molecular weight, and novel natural products of unknown mechanism that are more complex and have higher molecular weights [3].

The ACL lists both simple and complex compounds, which have known mechanisms and targets and span a greater range of chemical activity, explained Stockwell. 'We're sampling a great range of chemical space,' he said.

The database currently lists nearly 2000 compounds, but Stockwell and his colleagues hope to expand that number to 5000. The compounds are annotated in a systematic fashion with 138 molecular descriptors and 12,700 different mechanistic terms. Over time, Stockwell says, the number of terms could be expanded to 60,000.

In one demonstration of the database's power, the researchers tested

  PII: S1359-6446(02)02523-0