Bacteriophages: a rich store of new antibiotics?

Kathryn Senior, Freelance writer

Identifying and harnessing the mechanism by which small bacteriophages lyse bacterial cells could open new avenues for antibiotic development. Thomas Bernhardt and colleagues (Texas A&M University, College Station, TX, USA) have shown that the phage-Qβ virion produces a protein that interferes with cell-wall biosynthesis[1], resulting in defects that cause the bacteria to explode like small balloons (Fig. 1). 'The most interesting thing about this phage, and a couple of others already identified, is that this lytic activity is due to the expression of a single gene, rather than several,' explains senior author Ryland Young. 'This makes the task of exploiting phages as a source of antimicrobials that much easier,' he adds.

Qβ reveals some interesting secrets

Qβ is a single-stranded RNA virus, a member of the *Alloleviridae* family that does not have a separate lysis gene. This function is performed by the maturation (A_2) protein, which is also responsible for adsorption of the phage to the sex pilus of its host and protection of the virion RNA against external ribonucleases.

In many phages, lysis occurs when a muralytic enzyme degrades murein, the cross-linked peptidoglycan that forms the tough structure of the protective bacterial cell wall. However, the lysis event initiated by Qβ does not involve muralytic activity and, until this study, the underlying mechanism of Qβ lysis had been something of a mystery.

Using a combined genetic and biochemical approach, Young's group found that the A_2 protein blocks cell-wall biosynthesis by inhibiting MurA, an enzyme that catalyzes one of the first steps that precedes the incorporation of a peptidoglycan precursor molecule into murein. Confirmation was obtained by showing direct inhibition of MurA *in vitro*, using purified Qβ virions. Although the exact mode of action of A_2 remains to be determined, it is thought that the protein binds to MurA, thus destabilizing its complexation with its substrate.

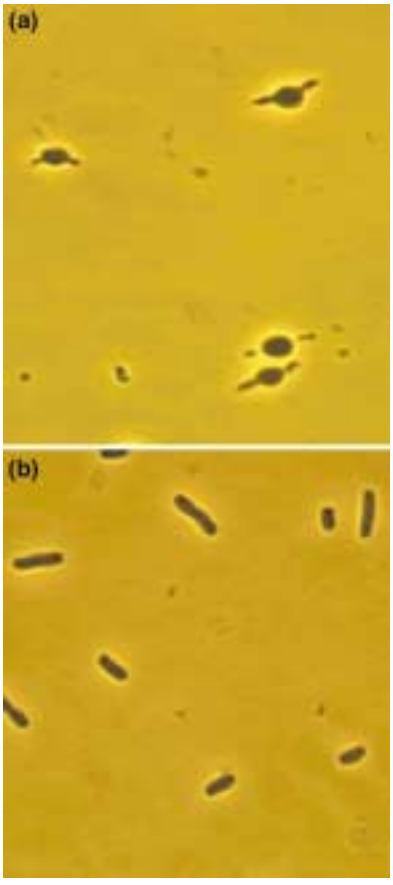

Figure 1. Photomicrograph of *Escherichia coli.* **(a)** *E. coli* are undergoing cell division when the Qβ virus attacks the cell wall, which, essentially, causes the *E. coli* to 'blow up'. **(b)** In the absence of the Qβ virus, *E. coli* continues to divide and reproduce.

'A_2 has been found to get into the cell with the phage-RNA genetic material during infection, but the amount that gets in is not enough to inhibit enough MurA to cause lysis,' comments Bernhardt. Substantial inhibition of cell-wall biosynthesis cannot occur until virion production is well established in the cell. 'It is, therefore, important to find the smallest portion of the lysis protein capable of inhibiting its target. That way, the lysis protein has the best chance of getting into the cell from outside in sufficient quantities to lyse the bacterial cell,' he explains.

A_2 could be a perfect antibiotic

The implications for antibiotic development could be immense. 'Single-gene lysis systems are extremely attractive for future antibiotic development because they represent polypeptide sequences that are known to inhibit bacterial cell-wall biosynthesis. This process is a favourable target because there are no human homologues and thus the side effects are predicted to be minimal,' explains Young. Although, to date, only two phages with the single-gene lytic system have been studied in detail, two more have been identified and the group expect that many more will be found. Furthermore, according to Young, there is a possibility that some single-gene lysis systems could work only against a particular bacterium, raising the possibility of developing specific antibacterials, as well as those with a broader spectrum of activity. Young is

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already discussing future strategies with several pharmaceutical companies.

Meanwhile, the fundamental research work continues. The next steps include resolving the known single-gene antibiotic polypeptides to the smallest possible sequence and elucidating the structure of each of these polypeptide antibiotics with their targets. 'These polypeptide antibiotics might be rational design tools for pharmaceutical companies to develop drugs that inhibit essential bacterial enzymes,' predicts Young.

Overcoming resistance

The possibility of developing several new classes of antibiotic is crucial as well as exciting. 'The need to define new targets for antimicrobial agents and to develop innovative strategies is clearly urgent,' says Charles Stratton, Director of Clinical Microbiology, Vanderbilt University School of Medicine (Nashville, TN, USA). In the past decade, the spread of multiple antibiotic resistance has become a major threat to the continued efficacy and use of current antibiotics. Stratton finds the approach of Bernhardt and colleagues of great interest. 'The main advantage of proteins produced by phages is that they target the cell wall, a structure that does not exist in human cells,' he says. He highlights the example of penicillin, the first antibiotic to be discovered. 'Penicillin is a β-lactam antibiotic and, although resistance to it is now a huge problem worldwide, it has proved to be one of the safest antibiotics because it targets peptidoglycan. Bacteriophage proteins that target different steps in cell-wall biosynthesis could be equally safe, but this premise must be proven,' he stresses. However, if it does prove correct, bacteriophages and the proteins they produce could become a major source of new antimicrobial agents.

Young agrees, and points out that phage proteins could have an added attraction: 'In principle, one could easily modify polypeptide antibiotics by changing the encoding DNA sequence. It is also possible to actually select random changes in a polypeptide that would overcome resistance, using the powerful tools of bacterial molecular genetics. But that really is looking into the future,' he concludes.

Reference

1 Bernhardt, T.G. *et al.* (2001) A protein antibiotic in the phage Qβ virion: Diversity in lysis targets. *Science* 292, 2326–2329

Milking nature for Alzheimer's treatment

Joanna Owens, Joanna.Owens@drugdiscoverytoday.com

A new drug derived from mammals' first-milk could provide an effective new treatment for Alzheimer's disease (AD). An interim report has recommended that ReGen Therapeutics (London, UK) be allowed to progress to the end-point of its 90-patient, multi-centre, double-blind, placebo-controlled clinical study of the efficacy of Colostrinin™ for treating AD.

The international steering committee of the trial, carried out in Poland, found that there were no adverse reactions related to Colostrinin and an encouraging trend of efficacy in patients treated with the drug compared with the placebo group. The committee also recommended that a further 18 patients should be enrolled to increase the statistical power of the trial.

The cost of AD

AD is an increasingly common, progressive neurodegenerative disease for which there is no cure. The disease slowly destroys the brain, impairing cognitive functions such as memory, abstract thinking and language function, and causes other symptoms such as attention deficit, depression, anxiety and agitation. The pharmaceutical industry is, therefore, under pressure to develop therapies for AD, which inflicts substantial emotional and financial costs to families, businesses and governments.

AD neurodegeneration is typified by the presence of amyloid plaques, tau tangles and loss of neurons[1]. The plaques consist of amyloid β (Aβ) deposits, which are formed as a result of the abnormal cleavage of amyloid precursor protein (APP)[2]. Tau tangles are caused by an overproduction of the tau protein[1], which, upon release from the cell, becomes heavily phosphorylated and glycated[3,4], and thus more insoluble. It is thought that both of these pathogenic processes could be a result of oxidative stress[1,3,4].

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