

Pharmaceuticals (Duarte, USA), also describes the new data from Sirna as encouraging but notes that other recent studies also show *in vivo* effects of siRNAs. For example, he says, antibody-mediated *in vivo* delivery of siRNAs is looking very hopeful [4], and an siRNA designed to silence an oncogene expressed in Ewing sarcoma has inhibited human tumor growth in mice when packaged in a cyclodextrin polymer (designed by Calando) and targeted through the attachment of transferrin to the polymer (www.nci.nih.gov/NCICancerBulletin/NCI_Cancer_Bulletin_041905). 'Overall,' concludes Rossi, 'with all the

incremental improvements that are being made to siRNA delivery, I think we should have useful siRNA-based drugs in the very near future.'

References

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- 2 Ryther, R.C. *et al.* (2005) siRNA therapeutics: big potential from small RNAs. *Gene Ther.* 12, 5-11
- 3 Morrissey, D.V. *et al.* (2005) Activity of stabilized short interfering RNA in a mouse model of hepatitis B virus replication. *Hepatology* 41, 1349-1356
- 4 Song, E. *et al.* (2005) Antibody mediated *in vivo* delivery of small interfering RNAs via cell-surface receptors. *Nat. Biotechnol.* 23, 709-717

concluded that the LexA-controlled polymerases are necessary for the evolution of ciprofloxacin and rifampicin resistance. *In vitro* studies that deleted or mutated various genes involved in the recombination pathways, including *lexA*, confirmed the conclusions made from the animal studies.

Resistance to ciprofloxacin and rifampicin is, therefore, a consequence of proteins being induced to increase mutation rates significantly and not simply a chance occurrence of errors during genome replication. Interfering with the genes involved in these recombination pathways prevented the bacteria from becoming resistant.

Small-molecule inhibitors

'The real motivation was for drug design and looking for novel targets,' says Romesberg. 'Mutation plays a role and might be susceptible to this type of intervention. Inhibiting these pathways would have a significant impact on the evolution of resistance.'

He says they are currently trying to identify small molecules that could be administered in conjunction with antibiotics, preventing bacteria from acquiring resistance-conferring mutations in the first place.

'This is immensely important,' says George Drusano, physician scientist at Albany Medical College and Ordway Research Institute in Albany, NY, USA. 'We can roll back the past in certain circumstances and prevent the erosion of the susceptibility profile of clinically important organisms to this class of agents.'

Furthermore, the phenomenon might not be unique to bacteria. 'The evolution of resistance in chemotherapy is a real problem in breast cancer,' says Romesberg. 'In human cells, people just don't know what the pathways are.' He comments, 'one approach would be to do genome-wide screens in yeast to identify genes that, when deleted, would render the yeast susceptible to mutation'. For now, he and his colleagues are trying to determine whether this is a universal phenomenon in bacteria and other antibiotic classes, at the same time as devising a chemical approach to reversing resistance.

Reference

- 1 Cirz, R.T. *et al.* (2005) Inhibition of mutation and combating the evolution of antibiotic resistance. *PLoS Biology* 3, e176

Reversing the evolution of antibiotic resistance

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Bacteria develop resistance to antibiotics through gene mutations. Conventional wisdom has long held that these mutations are inevitable, caused by imperfect polymerases replicating a large genome under pressure. However, increasingly evidence suggests that bacteria are active players in the process, inducing proteins responsible for generating various types of mutations. Now, researchers at the Scripps Research Institute in La Jolla, CA, USA, led by Floyd Romesberg, are showing that interfering with these mutation-inducing pathways can reverse antibiotic resistance and render bacteria completely susceptible to the once ineffective antibiotic. 'Inhibiting these mutation pathways from the outset,' says Romesberg, 'could represent a novel approach to combating the evolution of antibiotic resistance' [1].

'Inhibiting these mutation pathways... could represent a novel approach to combating the evolution of antibiotic resistance'

Shutting down the SOS response

Resistance-conferring mutations arise from three recombination pathways involved in repairing DNA damage. The Scripps group examined the role of various genes encoding proteins involved in the bacterial SOS response to two antibiotics, ciprofloxacin and rifampicin

(members of the important quinolone and rifamycin classes, respectively).

To test whether the SOS response is necessary for ciprofloxacin and rifampicin resistance to occur, they infected mice with pathogenic *Escherichia coli* containing either wild-type or mutant LexA (a protein induced during SOS repair of DNA damage). Autoproteolysis of LexA causes derepression of three polymerases (Pol II, Pol IV and Pol V) involved in generating mutations.

They found that interfering with LexA prevented its autoproteolysis and the derepression of the LexA-controlled polymerases. As a result, *E. coli* was unable to turn on its SOS genes and could not evolve resistance to either ciprofloxacin or rifampicin. By contrast, *E. coli* with wild-type LexA resulted in the generation of *E. coli* subsequently becoming resistant to these antibiotics. They

