TECHNIQUES AND APPLICATIONS

All the king's horses and all the king's men…

Humpty Dumpty had a famous problem — he sat on a wall, had a great fall and…couldn't be put back together again. Genome biologists have had a similar problem until now — they could break genomes up and sequence them but not synthesize them *de novo*, or at least not in any efficient way. A group led by Craig Venter has now devised a method for assembling the complete genome of a bacteriophage from a pool of chemically synthesized oligonucleotides. Although still in need of a correcting mechanism, the method might in future be used to make synthetic chromosomes.

Driven by the desire to improve on previous attempts to assemble active viral genomes, Smith *et al.* chose the ϕX174 bacteriophage — the first living organism to have its 5,386 bp genome sequenced — to test their three-step approach to synthetic genome assembly. The first step involves oligonucleotide synthesis, based on both strands of the phage genome. In the second step, the oligonucleotides are phosphorylated, mixed and ligated to give, on average, 700-bp double-stranded fragments. Two rounds of polymerase cycle assembly are used in the third step to assemble these fragments into full-length genome molecules, which are PCR-amplified, linearized and tested for size by electrophoresis.

As the ultimate test of successful *in vitro* synthesis is that of molecular function, recircularized synthetic phages were electroporated into *Escherichia coli* to test for their infectivity. Careful comparison of infection efficiency between the wild-type and the synthetic phages indicated that some 9–10 inactivating mutations were present per synthetic genome, although at least one synthetic isolate was 100% identical to the original ϕX174 sequence. Smith *et al.* suggest that these point mutations probably result either from replication errors or are already present in the oligonucleotides.

Given the mutation rate, the authors point out that without an ability to select for the wild-type sequence, synthetic forms will carry, on

average, a point mutation per 2 kb. Undeterred by this, they propose that, in combination with DNA sequencing and site-directed repair, their method can be used to assemble larger genomes.

Synthetic genomics could solve the problems of dealing with degraded biological material or extinct species. Genome sequences could also be modified during synthesis to provide an alternative to genetic engineering. Although this application of synthetic genomics might be around the corner for microbial genomes, much larger, metazoan genomes might remain in the 'Humpty Dumpty' class for a while yet.

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References and links

ORIGINAL RESEARCH PAPER Smith, H. O. *et al.* Generating a synthetic genome by whole genome assembly: phiX174 bacteriophage from synthetic oligonucleotides. *Proc. Natl Acad. Sci. USA* (2003) doi: 10.1073/pnas.2237126100

FUNGAL VIRULENCE

Fungal defence? NO stress!

Phagocytes invaded by microorganisms fight back with the innate immune system and produce potent broad-spectrum antimicrobial molecules that can inhibit the growth of intracellular pathogens. These include reactive nitrogen species (RNS), such as nitric oxide (NO) and S-nitrosoglutathione (GSNO). Unsurprisingly, pathogenic microorganisms have evolved strategies to overcome innate host defences — but until now antimicrobial defence against reactive nitrogen species has not been linked to microbial virulence. Reporting in the latest issue of *Current Biology*, Joseph Heitman and colleagues at Duke University have established that fungal enzymes that counteract NO do have a role in virulence.

***Cryptococcus neoformans* is a fungal pathogen that can cause life-threatening infections of the central nervous system in immunocompromised patients. Following pulmonary infection, patrolling alveolar macrophages are a first line of defence against cryptococcosis. NO — generated by NO synthase, the product of the iNOS locus — exerts a fungistatic effect against *C. neoformans in vitro* and it is known that *C. neoformans* detoxifies NO activity in macrophages. Enzymes that are active against NO include flavohaemoglobin denitrosylase — found in yeasts and bacteria — which converts NO to nitrate, and GSNO reductase, which is conserved from bacteria to man and reduces GSNO to ammonia and glutathione disulphide. The *C. neoformans* flavohaemoglobin denitrosylase (*FHB1*) and GSNO reductase (*GNO1*) genes were identified by mining the TIGR and Stanford genomic sequences for *C. neoformans* with gene sequences from *Saccharomyces cerevisiae*.**

By analysing single and double gene disruption mutants, de Jesús-Berríos *et al.* showed that *C. neoformans* converts GSNO to NO (by the action of Gno1), which is subsequently metabolized by flavohaemoglobin denitrosylase (by the action of Fhb1). Using a mouse model for *C. neoformans*, which mimics human disease progression, they established that flavohaemoglobin denitrosylase activity contributes to virulence. By itself, GSNO reductase does not; however, mutants that lacked both enzymes were more attenuated for virulence than either single mutant, so GSNO reductase can contribute to counteract nitrosative challenge and promote disease progression.

The use of a mutant mouse that is unable to produce NO because it lacks the genes encoding the inducible form of NO synthase, restored the virulence of the fungal mutant lacking Fhb1. This validates the link between host defence (NO production) and microbial counterattack (flavohaemoglobin) with consequences for virulence. Combining mutations in flavohaemoglobin and superoxide dismutase (which defends against oxidative attack by phagocytes) attenuated virulence further. Plants and animals both produce NO to defend against pathogens, and it seems that the flavohaemoglobin family might have been conserved because these enzymes have a general role in microorganism retorts to the defences mounted by their hosts.

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References and links

ORIGINAL RESEARCH PAPER de Jesús-Berríos, M. *et al.* Enzymes that counteract nitrosative stress promote virulence. *Curr. Biol.* **13**, 1963–1968 (2003)

WEB SITE

Joseph Heitman's laboratory:
http://mgm.duke.edu/microbial/mycology/heitmn/