SYSTEMS BIOLOGY

Big surprises in a little package

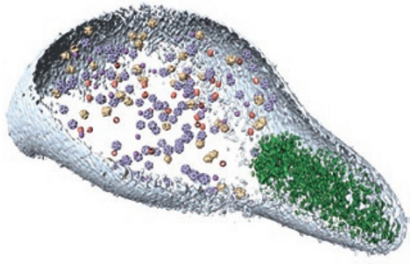
An in-depth, systems biology approach to analyzing a 'reduced genome' bacterium reveals startling complexity.

Even by bacterial standards, *Mycoplasma pneumoniae* can be considered a genomic lightweight: the evolutionary process has left it with a 'reduced' set of merely 689 protein-coding genes.

As such, some were skeptical when a consortium of researchers from the European Molecular Biology Laboratory in Heidelberg and Center for Genomic Regulation in Barcelona, led by Peer Bork, Luis Serrano and Anne-Claude Gavin, declared their intention to subject this tiny bug to a comprehensive 'omics' analysis. "They thought that to look at protein-protein interactions or even transcription in prokaryotes might be 'overdoing it'," says Gavin.

However, even those in the group were surprised by the level of sophistication they encountered. "We were very optimistic: we thought that after three years and a little bit of work, we'd even have a full computational model of this bacterium that would grow and divide in the computer," says Serrano. "Unfortunately, what we found was an unexpected degree of complexity, and we are far from understanding how it works."

To characterize the *M. pneumoniae* metabolome, the researchers compiled a rough map of biochemical reactions by pairing information from the literature with their own analysis of the organism's genome and enzymatic content (Yus *et al.*, 2009). Experimental confirmation of the accuracy of this map required the determination of this organism's minimal 'diet' of essential compounds. "That was a major experimental hurdle," recalls Bork. "If you don't know what the bug eats, you can't make conclusions about its responses." Their effort paid off, however, offering new insights into the activity of 57 metabolic genes. They also uncovered an unexpected level of gene multifunctionality, with 32 enzymes executing a total of 91 reactions—nearly half of the processes on their metabolic map.



Whole-cell tomogram of *M. pneumoniae* incorporating multiprotein complex localization data for ribosome (yellow), pyruvate dehydrogenase (light purple), RNA polymerase (dark purple) and GroEL chaperone complex (red). Reprinted from Kühner *et al.*, 2009 with permission from *Science*.

In spite of a gene set that includes only eight known transcription factors, *M. pneumoniae* can respond to diverse environmental changes, including pH shifts, amino-acid starvation or growth on alternate carbon sources, suggesting the existence of alternative regulatory mechanisms. "We have seen complex and specific responses that are very similar to what you see with bacteria like *Lactococcus lactis*, which has over 100 transcription factors," says Serrano, "and we don't know where this regulation is coming from."

Close analysis of the *M. pneumoniae* transcriptome via tiling arrays and RNA deep sequencing only revealed additional surprises (Güell *et al.*, 2009). Prokaryotic genes are generally organized into units known as operons, which are subject to coordinated regulation and expression, and the researchers authoritatively identified hundreds of mono- and polycistronic operons. However, they also observed strong evidence for an additional level of subtler transcriptional control, with many 'alternative' sets of genes from different operons that were subject to coexpression under specific environmental conditions, such as heat shock. "A quarter of them seem to vary under different conditions, which was surprising to me," says Bork. "The transcription landscape is much more eukaryote-like than expected."

The team also tackled the *M. pneumoniae* proteome, combining tandem affinity purification mass spectrometry with sophisticated new tools for bioinformatic analysis to characterize protein-protein interactions for 411 proteins—60% of this bacterium's annotated proteome (Kühner *et al.*, 2009). Their data revealed hundreds of protein complexes, more than half of which were previously uncharacterized, including an unexpectedly large proportion of homomultimers, which comprised more than a third of all complexes identified. The authors noted a surprising amount of previously unrecognized cross-talk between complexes, including evidence that certain ribosomal proteins also participate in transcription, thereby directly coupling gene activity with protein synthesis. In addition, by pairing structural models derived from their data with electron tomographic imaging, they directly visualized the localization of large complexes such as ribosomes in individual bacteria.

The authors suggest that this newly revealed complexity reveals how informative a 'simple' bacterium can be. "Despite being a very small and reduced prokaryotic cell, the basic principles are still there," says Gavin. "It's a perfectly relevant organism for systems biology." Accordingly, even as the team continues to hack away at additional aspects of *M. pneumoniae*, such as post-translational modification, they also plan to share their data and invite the international systems biology community to help fill in the gaps. "We hope that other people will jump into the fight," says Serrano, "and that in [a few] years we can say that we truly understand an autonomous living system."

Michael Eisenstein

RESEARCH PAPERS

Güell, M. *et al.* Transcriptome complexity in a genome-reduced bacterium. *Science* **326**, 1268–1271 (2009).

Kühner, S. *et al.* Proteome organization in a genome-reduced bacterium. *Science* **326**, 1235–1240 (2009).

Yus, E. *et al.* Impact of genome reduction on bacterial metabolism and its regulation. *Science* **326**, 1263–1268 (2009).