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NEWS AND VIEWS

A systems biology *tour de force* for a near-minimal bacterium

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Ever since the 1930s, when a handful of physicists, chemists, and biologists banded together into the phage school led by Max Delbruck, there have been efforts to understand life at its simplest and most fundamental level. In the ensuing decades up to the present, we have achieved a complete understanding of the genetic and chemical structure of a number of viruses, and know in many cases the role of all of their genes. The same cannot be said for cells. The simplest cells are bacteria, but they generally contain thousands of genes. The desire to understand how cells work has long attracted biologists to work with simple, near-minimal cells because of the assumption that smaller organisms with fewer molecular parts will be less complex and easier to understand. Whole genome sequencing and decades of biochemical studies have shown that the simplest known cells that are capable of growth in laboratory media are the atypical bacteria called mycoplasmas. The interest in these tiny bacteria as model systems to study cellular function led to two of them, *Mycoplasma genitalium* and *Mycoplasma pneumoniae*, being among the first few bacteria of which the genomes were sequenced (Fraser *et al*, 1995; Himmelreich *et al*, 1996). As those genome sequences were published in the mid-1990s, molecular and systems biology techniques have advanced to the point that the DNA sequences can be leveraged to gain a much deeper understanding of the biology of these minimal cells. A trio of papers published in *Science* by seven research groups coordinated by Peer Bork, Anne-Claude Gavin, and Luis Serrano offer an unprecedentedly detailed analysis of the transcriptional regulation, proteome organization, and metabolic regulation of the minimal bacterium *M. pneumoniae* (Güell *et al*, 2009; Kühner *et al*, 2009; Yus *et al*, 2009).

The analysis of transcriptional regulation by Güell *et al* (2009) is based on a combination of spotted arrays, strand-specific tilling arrays, and transcript sequencing. Their in-depth analysis of *M. pneumoniae* transcription under various growth conditions precisely defines transcriptional units, promoters, and termination signals. Previously, a general lack of obvious standard −35 and −10 transcriptional promoter regions as well as hairpin termination signals led to the conjecture that mycoplasmas had evolved some cryptic set of promoters and terminators; however, this analysis showed that most of the operons had canonical or slightly altered standard sigma 70 promoter regions and RNA hairpin termination sites. As is the case in more conventional bacteria, many of the operons are partially transcribed under different conditions. They report more than 100 previously un-annotated transcripts, most of which were antisense to known genes. Analysis of bacterial small RNAs over the last few years shows a much larger role for these previously unrecognized transcripts in regulation of gene expression at many levels. In sum, the *M. pneumoniae* transcriptional regulation analysis shows an unexpected complexity that is similar to what is present in conventional bacteria such as *Escherichia coli* and *Bacillus subtilis*.

The proteome organization study by Kühner *et al* (2009) goes far beyond the 2-D gel/mass spectrometry approaches used in previous large-scale studies of mycoplasma proteomes (Wasinger *et al*, 2000; Ueberle *et al*, 2002; Jaffe *et al*, 2004). *M. pneumoniae* protein complexes were identified using tandem affinity purification and mass spectrometry (TAP-MS). This approach was used previously only for analysis of *Saccharomyces cerevisiae*, which revealed homooligomeric and heteroligomeric protein assemblies and many 'moonlighting' or multifunctional proteins associated with multiple cellular machines (Gavin *et al*, 2006; Krogan *et al*, 2006; Tarassov *et al*, 2008). In the current analysis, the TAP-MS data are selectively complemented using sophisticated modeling and cryo-electron tomography analysis to provide insights into the structural anatomy of *M. pneumoniae*. More than 100 protein complexes were identified from 1058 high-confidence interactions between soluble proteins. This included almost 90% of the soluble proteins, which is a value similar to that reported for yeast. Many of the complexes were unexpected, such as a complex of five aminoacyl tRNA synthetases. The associations also led to

new annotations for a number of *M. pneumoniae* genes. The simple observation from this study is that even in a minimal cell the proteome organization is similar to that in more complex organisms. In addition, the wealth of data generated radically improves our perspective on the number and content of molecular machines in a mycoplasma cytoplasm.

The metabolism modeling paper by Yus *et al* (2009) is not unlike a growing number of microbial metabolic reconstruction studies appearing in the literature. Still, it is a vast improvement over previous analyses of mycoplasma metabolism because of its integration of the new understanding of *M. pneumoniae* metabolism borne from the accompanying transcription and proteome papers, and a rigorous experimental investigation of monitoring biomass indicators, metabolites, and ^{13}C glucose utilization that gave insight into metabolic directionality, fluxes, and energetics. Importantly, the authors present the first defined medium for these bacteria. The formulation of that media was predicted by the *M. pneumoniae* metabolic model.

These three papers should be read as chapters in a larger story rather than as stand-alone scientific studies. Although each report offers a set of vignettes about *M. pneumoniae* biology that will give readers a sense of one 'ome' of this near-minimal organism, the power of these massive studies is realized only by integrating the views of the *M. pneumoniae* transcriptome, proteome, and metabolomes. For those seeking more detailed information, there is a massive set of supplementary online material that will catalyze the expansion of our understanding of the molecular machines that comprise this perhaps not so simple organism.

In reading these papers, one must be careful to avoid not seeing the forest because of the trees. For instance, in the proteome paper the authors only characterized a few well-known protein complexes, such as the RNA polymerase and pyruvate dehydrogenase, rather than more deeply investigate some of the many novel complexes they reported. Many of the protein associations reported have not been observed in any other bacterial systems. This could compel some readers to dismiss the result in the absence of solid proof that these unexpected biological entities exist. We find it simply begs further analysis. Our team at the J. Craig Venter Institute uses mycoplasmas as platforms to learn the first principles in the design of cellular life (Lartigue *et al*, 2007, 2009; Gibson *et al*, 2008). Towards that aim, we can ascribe no function to almost 100 of approximately 370 protein coding genes in *M. genitalium* that are apparently essential for life (Hutchison *et al*, 1999; Glass *et al*, 2006). These systems biology analyses of the closely related *M. pneumoniae* give us our first clues about most of those genes. Although we realize that some of the data offered in these three papers may be artifactual, we suspect the vast majority is not. For any given unknown protein or molecular machine, the knowledge of its transcriptional regulation and protein–protein associations can be the catalyst for further study of the role that this protein has in the cell, and for advancing one step closer to the long-sought understanding of cellular life at its simplest and most fundamental level.

Conflict of interest

The authors declare that they have no conflict of interest.

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