Not so simple after all

A renaissance of research into prokaryotic evolution and cell structure

Biologists have long thought that the internal workings of prokaryotes—the smallest and simplest organisms, including bacteria—are well understood, and have accordingly considered eukaryotic organisms and their cells to be more fascinating objects for study. During the past decade, however, the focus of some research has begun to turn back to prokaryotes—particularly because genomic analyses have shown the enormous flexibility and adaptability of these seemingly simple organisms. Moreover, recent structural research has shown that the cell components of prokaryotes might also be more complex than previously thought. It has long been held that prokaryotic cells lack the internal structure and organization of eukaryotic cells, which have various membrane-bound organelles, such as mitochondria, chloroplasts and the nucleus. Indeed, prokaryotes are assumed to be the predecessors of some eukaryotic organelles—permanently captured after a period of endosymbiosis.

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The view that bacterial cells are structurally simpler than those of 'higher' organisms is reflected in many text books and web sites with comments such as: "the ribosome is the only prokaryotic organelle"—if, indeed, this highly complex and sophisticated protein-synthesizing machinery is an organelle in its own right. These types of statement are correct in the sense that prokaryotes contain no membrane-bound organelles; but there is no rule that says an organelle must be enclosed by a membrane. Many eukaryotic ribosomes are freely suspended in the cytoplasm where they manufacture cytosolic proteins. Furthermore, the word 'organelle' itself is being replaced by the terms 'functional unit' or 'compartment', which are increasingly used to describe coherent structures within a cell

that perform clearly defined sets of tasks—such as mitochondria, which create energy by aerobic respiration, or chloroplasts, which do so by photosynthesis.

By using this definition, a series of recent discoveries show that prokaryotes also contain distinct functional units, or micro-compartments, which could reasonably be called organelles. One of the main breakthroughs was made at the University of California Los Angeles, USA, by a team led by Todd Yeates. They revealed structural details of micro-compartments in bacteria and found that these highly organized protein assemblies resemble viruses; they consist of thousands of protein subunits assembled in a viral-like structure or scaffold (Kerfeld *et al*, 2005).

For Yeates, the resemblance of micro-compartments to viruses is not coincidental, even if the exact evolutionary history remains uncertain. "The question remains open as to whether viruses and bacterial micro-compartments represent a case of convergent or divergent evolution," he said. "At this point, there isn't really any substantial evidence to support either case [...] If it turns out to be a case of convergent evolution, this will reinforce the idea that highly ordered protein assemblies occur relatively often by chance during evolution, and so have arisen multiple times independently, and in different functional contexts. If it turns out to be a case of divergent evolution—meaning bacterial micro-compartments share a common ancestry with some virus—the situation will be reminiscent of the endosymbiotic hypothesis, which holds that organelles in eukaryotes derived from prokaryotic organisms." The endosymbiotic hypothesis is now widely accepted in the case of mitochondria and photosynthesizing organelles, which include chloroplasts in algae and plants, because comparative studies have revealed clear similarities with the genomes of relevant bacteria (van der Giezen, 2005), as well as structural similarities between some organelles and bacteria (Alcock *et al*, 2008).

It is much harder to perform such structural analysis of prokaryotic cells because of the smaller scale, but there is plenty of evidence that micro-compartments could have evolved by divergent evolution with bacteria 'capturing' a virus and using both its genes and structural features for its own ends. For quite a long time, biologists thought that bacteriophages—the viruses that infect bacteria—operated largely as pathogens that replicate inside the host before destroying the cell through lysis and spreading to neighbouring bacteria. But now it seems that stable endosymbiotic relationships might be the norm, according to Nancy Moran, Principal Investigator in the Department of Ecology and Evolutionary Biology at the University of Arizona (Tucson, AZ, USA). "Since a phage is vertically transmitted when the bacterial host cell divides, it is fairly easy for selection to favour the phage benefiting its host, rather than killing it through lysis," she said.

In support of this idea, the number of bacteria that are found to depend on phages for crucial functions increases almost by the day. "It has been shown that phage genes encode proteins useful in every part of the bacterial life cycle," said Martha Clokie from the Department of Infection, Immunity and Inflammation at the University of Leicester, UK. Many of these functions are concerned either with infection or evading host defences, and include the production of some potent toxins, such as the shiga toxins made by the *Escherichia coli* phages. Phage genes in the bacterium *Streptococcus mitis* encode proteins that enable the cell to bind to blood platelets and coat heart valves, thus causing inflammation of the inner heart (Bensing *et al*, 2001). *Salmonella enterica* phages help their host bacterium avoid eukaryotic host defences. Phages are also responsible, at least in some cases, for the development of antibiotic resistance (Chiu *et al*, 2006).

Phages also assist bacteria with the physical task of invading host cells by donating structural features—notably, the phage heads that the virus originally evolved to improve its own pathogenicity. But, as Clokie pointed out, there is also speculation that phages can help to protect bacteria from oxidative stress, which again helps them to invade cells and avoid host defences. "This is more speculative, but genes encoding a periplasmic copper and zinc-cofactored superoxide dismutase, which protect the bacterial cells from oxidative stress, have been found on

phages [that] occur in the most pathogenic strains of *E. coli* and *Salmonella*," she said.

These findings are highly significant, not only for understanding virulence, disease and resistance, but also for prokaryotic and viral co-evolution. They suggest that bacteria and viruses can 'hunt together' and that there is not always a clear distinction between the two in terms of disease. Indeed some of the most dangerous bacteria seem to rely on phages for their most potent virulence factors and toxins. The implications for structure are interesting as well. At the very least, it seems that some phages have become almost permanent components of bacteria, as the work by Yeates and others suggests.

Yeates used electron microscopy to construct a three-dimensional model of the carboxysome, which enhances CO₂ fixation in cyanobacteria (Kerfeld *et al*, 2005). Ribulose biphosphate carboxylase (RuBisCO)—the most important enzyme involved in CO₂ fixation in bacteria—is sequestered into the carboxysome where it concentrates CO₂. The carboxysome was first discovered in the 1960s, but its structure is only now becoming clear. It is surrounded by a protein shell 100–200 nm in diameter, which resembles that of a virus—about 10 times

larger than the ribosome in prokaryotes and 1,000 times the volume.

Further work by Thomas Bobik, Associate Professor in the Department of Biochemistry, Biophysics and Molecular Biology at Iowa State University (Ames, IA, USA), has shown that carboxysomes are just one of several types of bacterial micro-compartment with similar viral-like structures but different functions (Bobik, 2007). Some are found in non-photosynthetic bacteria where they are involved in various catalytic reactions for releasing carbon, nitrogen and energy for metabolism. *Escherichia*, *Klebsiella*, *Clostridium*, *Fusobacterium*, *Shigella*, *Listeria*, and *Yersinia* all use such micro-compartments to produce carbon through the degradation of 1,2-propanediol, ethanolamine, or both (Bobik, 2007).

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In fact, all these micro-compartments resemble organelles in various ways. Although they are not membrane-bound, they help

to confine and retain various compounds to facilitate reactions that could not take place as effectively—if at all—elsewhere in the cell. The function of the micro-compartment depends on its structure as a whole and not just its constituent genetic components.

However, according to Yeates, further insights will only come from detailed structural analysis. This is not straightforward for such small structures, but he notes that: “[o]ur view is that structural studies are going to be key to unravelling how micro-compartments are built, how they are organized, and how they confer an advantage on the cells in which they have evolved [...] Some recent EM studies have helped confirm the basic icosahedral architecture of the carboxysome, but the deviations from perfect regularity [...] are presenting difficult challenges for high-resolution reconstructions.”

In practice, scientists are therefore forced to search indirectly for micro-compartments by analysing bacterial genomes. Bobik and colleagues, for example, searched GenBank for known shell genes of micro-compartments and analysed the surrounding clusters, which revealed that at least 25% of bacterial genomes carry micro-compartment gene homologues. Furthermore, the surrounding clusters contained genes for enzymes that are already known to be associated with micro-compartments. Analysis of these enzymes led Bobik to conclude that there are at least seven types of bacterial micro-compartment—all similar in structure but with different functions (Bobik, 2007)—some of which have yet to be identified physically. “A long-term goal is complete understanding of the molecular mechanisms of micro-compartments. I think molecular and structural studies will advance our understanding of protein–protein interactions and mechanisms substantially in the next few years,” Bobik commented.

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Another avenue of research is to establish what role, if any, viruses have had in the origin of micro-compartments, given the structural and functional similarities.

The idea that they might have had a role is supported by the fact that some viruses carry genes on behalf of their hosts. For example, the genes that encode the photosystem II core reaction centre protein, known as D1 and the HLIP protein, and which protect the system from intense light, are present in the genomes of three phages that infect the marine cyanobacterium *Prochlorococcus* (Lindell *et al.*, 2004). This enables the bacterium to lighten its genetic load, while the viruses act as sources of portable components that can be recruited almost on demand to cope with or exploit new environments.

Another interesting question arising from the discovery of micro-compartments is whether these structural units also exist in those eukaryotic organelles that evolved from bacteria. So far, structural studies have failed to yield evidence of viral-like components in eukaryotic organelles, although as Yeates noted, it is early days. Some of the most promising candidates for this kind of research are the chloroplasts of some algae, within which the RuBisCO enzyme is highly concentrated in protein bodies known as pyrenoids. “Whether there is any relationship between prokaryotic carboxysomes and the pyrenoid bodies of algae is unknown, but it seems like a plausible hypothesis for testing,” Yeates said.

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The first eukaryotic organelle to be linked to a viral ancestry was the nucleus itself. The hypothesis suggests that a large DNA virus could have invaded small bacteria and, rather than causing apoptosis, it established a permanent presence in the cytoplasm (Bell, 2001). The virus then retained some of its own genes while acquiring others from the host genome, eventually usurping the latter's role completely. In fact, although the nucleus does share some features of DNA viruses—linear rather than circular chromosomes, for example—there are also some problems with the theory, according to Tom Misteli in the Cell Biology of Gene Expression Group at the US National Institutes of Health (Bethesda, MD, USA). A significant problem is that, although the heads of phages are made of pure protein, this structure has been

lost in lower eukaryotes, such as yeast, whereas higher eukaryotes have regained a proteinaceous network beneath the nuclear envelope. “It is difficult to imagine that lower eukaryotes lost the protein component of the nuclear envelope just for higher eukaryotes to regain it,” Misteli said. An alternative theory argues that at least some of the viral features of both the nucleus and perhaps even prokaryotic micro-compartments were the result of convergent or parallel evolution.

These details notwithstanding, there is now little doubt that viruses have had an important role in the development of both prokaryotes and eukaryotes through co-evolution and lateral gene transfer. Some prokaryotes cannot even exist in the absence of phages, which might thus blur the line between a micro-compartment and an endosymbiotic virus, just as some eukaryotes cannot survive without bacterial endosymbionts. However, more detailed studies are required and genomic analyses needed before we can say whether or how endosymbiotic phages evolved into micro-compartments in prokaryotes. Regardless, this area of enquiry is one that makes the field of prokaryotic research exciting once again.

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