The genome of *Rickettsia prowazekii* and some thoughts on the origin of mitochondria and hydrogenosomes

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Summary

The sequence of an α-proteobacterial genome, that of *Rickettsia prowazekii*,[1] is a substantial advance in microbial and evolutionary biology. The genome of this obligately aerobic intracellular parasite is small and is apparently still undergoing reduction, reflecting gene losses attributable to its intracellular parasitic lifestyle. Evolutionary analyses of proteins encoded in the genome contain the strongest phylogenetic evidence to date for the view that mitochondria descend from α-proteobacteria. Although both *Rickettsia* and mitochondrial genomes are highly reduced, it appears that genome reduction in these lineages has occurred independently. *Rickettsia*'s genome encodes an ATP-generating machinery that is strikingly similar to that of aerobic mitochondria. But it does not encode homologues for the ATP-producing pathways of anaerobic mitochondria or hydrogenosomes, leaving an important issue regarding the origin and nature of the ancestral mitochondrial symbiont unresolved. *BioEssays* 21:377–381, 1999.

> *L'idée s'imposa que les microorganismes avaient subi des pertes de fonction. Celles-ci apparurent comme la manifestation d'une évolution physiologique, définie comme une dégradation, une orthogenèse régressive.* (André Lwoff, 1944,[2] p. 11)
>
> ...leading to the notion that microorganisms underwent functional losses. These are the manifestations of physiological evolution, appearing as degradation, as regressive orthogenesis. (Authors' translation)

Introduction

The much awaited release of the complete genome of *Rickettsia prowazekii*[1] represents a signal advance for comparative microbiology. Though small, it is the first genome from the α-proteobacterial lineage of gram-negative eubacteria and is thus highly relevant to the understanding of mitchondrial origins. Indeed, data from its genome have hinted that the α-proteobacterial ancestor of mitochondria, most likely a sine qua non constituent of eukaryotic cells, may have been a close relative or even an ancestral member of the order Rickettsiales.[3,4] This paper briefly highlights some major issues emerging from this work, focusing on the downsizing of the genome as well as the nature of perspicous similarities and significant differences between *Rickettsia* and mitochondria.

Rickettsia prowazekii is the causative agent of typhus, one of the many deadly microbes affecting humans. As is true of almost all members of the order Rickettsiales, it is an obligate intracellular parasite.[5] The pathogenic process is simple but devastating. The bacterium is taken up by the host cell through induced phagocytosis. Immediately thereafter, it destroys the endocytic vacuole and lies free in the cytosol, where it multiplies and bursts the cell, releasing progeny ready to invade new target cells. During this process, the bacterium itself remains surrounded by its typical multilayered gram-negative cell wall. All interactions with the host cell

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are effected across these layers. These interactions ensure multiplication and dispersal of the bacterium, at the expense and death of the invaded cell.[5]

Reductive evolution of the genome

The 1.111-million base pair (Mb) genome of *R. prowazekii*,[1] as expected for an obligate intracellular parasite, is small compared with free-living eubacteria but is within the size range known for other eubacteria that can live as intracellular parasites.[6] The DNA of *R. prowazekii* carries 834 protein coding genes, 528 of which shared similarity with proteins of known function, translation constituting the largest class with 118 genes. Importantly, phylogenetic analyses encompassing 18 different *Rickettsia* proteins that also occur in mitochondrial genomes support an α-proteobacterial heritage for mitochondria,[2] the most extensive phylogenetic information yet marshaled in support of this view. Although small for a bacterium, *Rickettsia* contains an order of magnitude more protein-coding genes than the largest mitochondrial genome characterized, which encodes 67 proteins.[4] In line with mitochondrial genome reduction, more than 150 *R. prowazeckii* proteins were found to possess significant similarities to nuclear-encoded mitochondrial proteins from the yeast genome.[1]

Various intriguing aspects of the *Rickettsia* data have already been addressed in several recent papers.[3,7–9] Among these, reductive genome evolution (the loss of genes over time from an ancestral state with a larger genome) emerges as an important underlying theme. It has been emphasized[2] that the intracellular parasitic lifestyle of *Rickettsia* favors clonality, providing population genetic conditions under which Muller's ratchet—the rapid accumulation of deleterious alleles that cannot be eliminated by recombination in clonally (asexually) reproducing organisms—will take effect.[7,10] Theoretically, Muller's ratchet should rapidly increase genetic load and should therefore result in genome reduction, both for *Rickettsia* and for mitochondria.[1,7] Congruent with this view, some operons and gene clusters are clearly shared between mitochondrial genomes and *Rickettsia*, but there are some significant differences as well,[1,3] indicating that mitochondrial and *Rickettsia* genomes underwent reduction independently of one another[1,3] and that this process started from an ancestral genome that was probably larger than 1.1 Mb.

Uniquely among all prokaryotic genomes sequenced to date, a surprisingly large fraction (24%) of the *Rickettsia* genome consists of noncoding DNA.[1] Moreover, analysis of these noncoding DNA regions demonstates the presence of many pseudogenes, i.e., genes that have only recently been inactivated through mutation during the process of genome reduction.[1,7] This finding is consistent with earlier conclusions from the same laboratory[10–12] that genome reduction in *Rickettsia* has not yet reached its endpoint[7] and that Muller's ratchet may indeed still be at work.

Central metabolism

Rickettsia prowazekii inhabits the cytosol of mitochondrion-bearing cells. Its reduced gene content tends to reflect specialization toward this nutrient- and precursor-rich ecological niche. Enzymes for the biosynthesis of amino acids, nucleic acid precursors, and lipids have become largely unnecessary for the parasite. Accordingly, many of the genes for these pathways have been eliminated during evolution.[1]

The machinery associated with *Rickettsia*'s ATP generation is of particular interest, in view of the suspectedly close relationship between this organism and mitochondria.[1,3,4] No enzymes for glycolysis are encoded. A complete system, however, is present for the utilization of pyruvate, generated in the cytosol of the host cell. This system comprises the multienzyme pyruvate dehydrogenase complex, tricarboxylic acid (TCA) cycle, a cytochrome-based electron transport chain, cytochrome oxidase, and an F_1F_0 ATPase, which harnesses the transmembrane proton gradient for ATP formation.[9] This corresponds protein-for-protein to the ATP generating system found in aerobic mitochondria and its enzymes are close orthologues of their mitochondrial counterparts.[2,8] Interestingly, *Rickettsia*'s ATP/ADP exchange transporter shares no sequence similarity with mitochondrial ATP/ADP exchange transporters[1]; rather, it is related to ATP/ADP transporters of chloroplasts.[13]

Rickettsia's identified gene complement agrees well with its biochemistry as currently understood,[5,9] but there were also some surprises. Pyruvate dikinase and acetate kinase, for example, have no obvious function on the metabolic map of this organism. Such ''unnecessary'' proteins might be used by *Rickettsia* in an unusual manner, or they might just be unneeded leftovers from the larger genome of *Rickettsia*'s free-living ancesors that have not (yet) fallen prey to reduction. With the genes known, it is now the task of biochemical studies to uncover the metabolic function of the products of such genes.

Rickettsia and the ancestral mitochondrion

What does the exquisite similarity and clear homology of the ATP-producing machinery of modern aerobic mitochondria and *Rickettsia* tell us? In order to have a clearer understanding of this point, we need to contrast the biology of the mitochondrion, in whatever form we encounter it, to the biology of the intracellular parasite. Although most of this is general knowledge, a brief summary should aid the discussion.

Aerobic mitochondria and *Rickettsia* are functionally integrated into their contemporary eukaryotic ''host'' cells in ways that appear very similar ways at first sight. They both import one or more metabolites that arise from glycolysis, among which pyruvate plays a major role. The mechanisms of

pyruvate oxidation and of the concomitant ATP generation are practically identical. Their respective membranes both contain an ADP/ATP translocase.[1] *Rickettsia* and aerobic mitochondria are virtually interchangeble with regard to their interaction with the cytosol on a generalized metabolic map[9] in that both can be depicted as DNA-bearing. double membrane-bounded compartments of oxidative phosphorylation. They differ, however, in the manner in which the ATP is used. Most of the ATP synthesized by mitochondria is exported to the cytosol. By contrast, *Rickettsia* uses itself the ATP it produces and, in the early stages of development, even imports ATP from the host cell.[9]

Key to the unerstanding of ATP synthesis in *Rickettsia* is the fact that it is strictly oxygen dependent[1,5] and the overall similarity of ATP synthesis between *Rickettsia* and mitochondria applies to aerobic mitochondria only. Many anaerobic mitochondria—and their relatives, the hydrogenosomes—are known that produce ATP without using oxygen as a terminal electron acceptor. These anaerobic organelles use proteins and/or pathways that are known to exist among (facultatively) anaerobic eubacteria,[14–20] but for which the obligate aerobe *Rickettsia* has no homologues. Examples of such anaerobic mitochondria include the nitrate-respiring ones of some fungi [14] and some ciliates,[15] the fumarate-reducing ones of parasitic worms[16] and some trypanosomes,[17] the wax-fermenting ones of *Euglena*,[18] the hydrogen-producing ones of the ciliate *Nyctotherus*,[19] not to mention the hydrogenosomes, fermentative ATP-producing organelles of many amitochondriate protists.[20] The list of mitochondrial and hydrogenosomal enzymes needed for such anaerobic ATP-producing pathways that are found among (facultatively) anaerobic eubacteria, but have no recognizable homologues in the *Rickettsia* genome, includes nitrate and nitrite reductase,[14] acetate : succinate CoA transferase,[16,17,20] pyruvate : ferredoxin oxidoreductase,[20] and iron-only hydrogenase,[19,20] to mention just a few. Because *Rickettsia*'s genome has retained primarily such genes as are needed to survive as an obligately aerobic, mitochondrion-like parasite, its sequence cannot address the origins of such ATP-producing pathways in anaerobic mitochondria and hydrogenosomes.

In addition to these important biochemical differences between *Rickettsia* and anaerobic ATP-producing organelles, there are key differences in the mode of genome reduction realized by *Rickettsia* and organelles. Obviously, genome reduction in aerobic mitochondria has proceeded to an even far greater extent,[4] and in some (but not all) hydrogenosomes no genome at all has been detected.[19,20] But the major difference is that much of mitochondrial and hydrogenosomal genome reduction was achieved by transfer of genes to the host's chromosomes. Genetic integration of these exported genes and reimportation of the products of some into the organelle was a key process in the evolutionary emergence of the mitochondrion.[4,21–23] In the case of *Rickettsia*, the genes were apparently simply lost.

Thus, the analysis of the *Rickettsia prowazekii* genome[2] lends further, virtually incontrovertible, support to the notion of the common ancestry of mitochondria and contemporary α-proteobacteria.[3,4,22,24] A number of considerations argue, however, that this common ancestor had a much richer genetic endowment than today's Rickettsiales. *Rickettsia*'s similarity to aerobic mitochondria could, in principle, reflect convergent evolution[3]; in other words, it might stem from ecological specialization to the oxygen-, pyruvate-, and nutrient-rich intracellular environment of a mitochondrion-bearing cytosol. Metabolic pathways found in mitochondria and hydrogenosomes, but not in *Rickettsia*, suggest that the free-living common ancestor of mitochondria and hydrogenosomes might have harbored genes for these diverse pathways of ATP synthesis.[25] The mitochondria studied to date do not contain glycolytic enzymes, and *Rickettsia*'s genome does not encode proteins of the glycolytic pathway.[1,4] However, a number of nuclear-encoded enzymes of the glycolytic pathway in the eukaryotic cytosol nonetheless appear to derive from the α-proteobacterial ancestor of mitochondria.[25–28] So, when larger proteobacterial genomes, particularly from free-living species such as *Rhodobacter*, *Paracoccus*, or others become available, we may find many more nuclear genes in yeast and other eukaryotes with proteobacterial ancestries than previous studies have revealed.[26,29,30] Anaerobic ATP-producing pathways in organelles are sufficiently well documented and sufficiently widespread among eukaryotes that their evolutionary histories need to be specifically accounted for in models for the origins of mitochondria and hydrogenosomes.[25]

Other intracellular parasites

Symbiotic or parasitic existence of eubacteria within the confines of an eukaryotic cell is a widespread phenomenon. The sequencing of complete genomes of such organisms can give us a deeper insight into the nature of the dependence of the intracellular inhabitant on the cell harboring it. Although this dependence is predominantly nutritional, other types of dependence occur as well, a topic beyond the scope of this brief commentary. Living inside another cell clearly can relieve the parasite from the necessity of keeping a complete metabolic machinery and can result in evolution by functional losses, accompanied by reduction of the genome.

Of microorganisms with completely sequenced genomes the smallest genomes are indeed found in certain intracellular parasites. The genome of *Mycoplasma* species comprises 580–820 kbp[31–33] and that of *Chlamydia trachomatis* 1.04 Mb.[34] A number of genes of central energy metabolism are missing from these genomes as well, but the pathways retained are not the same as in *Rickettsia*. The presence of a

complete system of information transmittal and processing shows that all these organisms retained their genetic autonomy.

Such extensive genome reduction is not unknown among eukaryotic parasites, either. We mention only the group Microsporidia,[35] as the most extreme example known. All members of this group live within eukaryotic cells, usually, but not always, free in the cytosol. For some time, Microsporidia were regarded as one of most ancestral eukaryotic groups,[36] but cytological and molecular evidence increasingly supports the notion that they are highly modified fungi.[37,38] The record for small genome size is held by a parasite of rabbits, *Encephalitozoon cuniculi* with a genome of 2.9 Mb, which consists of bona fide eukaryotic chromosomes.[39] This genome is smaller than that of either *Escherichia* or *Synechocystis*.[6] Microsporidia have a relatively simplified morphological organization; most remarkably, they contain no morphologically recognizable mitochondria.[35] Although biochemically almost nothing is known of the metabolism of Microsporidia, they seem to represent an interesting counterpoint to *Rickettsia*: the aerobic, prokaryotic parasite has evolved to become similar to a mitochondrion, whereas the anaerobic eukaryotic parasite has relinquished its mitochondria, or reduced them to unrecognizable structures.

Conclusions

The striking similarities between mitochondria and *Rickettsia prowazekii* clearly indicate an α-proteobacterial ancestry of the organelle. The prominent biological and metabolic differences between them suggest, however, that their independent histories from a common, free-living ancestor followed separate paths and that the metabolic similarities reflect convergent reductive evolution. Such findings underscore the interdependence of biochemical and genomic evolution. Finally, it is very important to note that considerable evidence is accumulating to suggest that all known eukaryotes either (1) possess mitochondria or (2) possessed a mitochondrial symbiont in their evolutionary past.[25,38] More than ever, the origin of mitochondria bears upon our understanding of the origin and eraly evolution of eukaryotes. The elucidation of genomes of many more α-proteobacteria, particularly of free-living forms, holds much promise for further insights into these questions.

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