Experimental Search for Minimal Organisms and the Last Universal Common Ancestor

Reconstructing the Ur-Organism

Two questions that should be closely related have historically been studied with very different approaches. One is what constitutes a minimal living system, whether minimal cell or minimal self-contained ecosystem. The other is what actual system was the last universal common ancestor (LUCA) of all modern cells. As the LUCA is supposed to have been a bottleneck through which all life passed before diversifying into modern forms, it is treated as a self-sufficient organism and would be a candidate for a minimal cell.

Attempted reconstructions of the LUCA have largely been inferences in molecular phylogeny. Modern genes are grouped by common function and where possible by sequence homology, and primordial forms are traced back through the tree of life. In contrast, the search for minimal organisms has been mostly experimental, based on survey of short natural genomes and further random removal of genes. Current understanding of metabolism and control is still too primitive for theoretical approaches to have significantly affected this program.

The experimental search for a minimal microbial genome began in the early 1960s, culminating with *Mycoplasma genitalium*, which has only 482 protein-coding genes and 580,076 base pairs in the wild type. Unfortunately this starting point cannot lead to either a minimal free-living organism or a model for the LUCA, because *Mycoplasma* and other small-genome organisms like *Rickettsia* and *Chlamydia* are obligate pathogens known as “cell wall defectives.” Descended from free-living Gram-positive bacteria with ~4000 genes by loss of genes and functions, the Mycoplasmataceae depend on their hosts for most primary biosynthesis, and even for osmotic regulation. They are consummate heterotrophs, and their minimality reflects a high degree of ecological specialization rather than primitiveness.

It has since become known that single cells can be autotrophic on environmental CO₂, reductant (H₂), water, H₂S, NH₃, phosphate, and trace minerals, and thermophilic organisms of this type with 1500–2000 genes from both the Archeal (such as *Methanococcus jannaschii*) and Bacterial (such as *Aquifex aeolicus*) domains have been studied. Their genomes are smaller than those of the smallest phototrophs, and the search for minimal organisms continues with the goal of understanding the limits of small life.
autotrophs (cyanobacteria or green sulfur bacteria at 2000–4000 genes) and presumably smaller than the unions of genes from heterotrophic communities. It is therefore plausible that in the emergence of life autotomy preceded heterotrophy and chemotrophy preceded phototrophy and that the LUCA was itself a thermophilic chemoautotroph.

We can estimate a minimal genome size to support life by adding the gene number for cell synthesis from *M. genitalium* to the number of genes for autotrophic intermediary metabolism from the sequenced genome of *A. aeolicus*. Gene deletion experiments on *Mycoplasma* have yielded viable organisms with ~380 genes [1]. The anabolism of *Aquifex* appears to require fewer than 400 genes. Because the functions in the two sets partly overlap, together they yield a lower bound on the genome for an autotroph of ~750 genes. The lower bound omits genes for feedback control of cell processes, because it is not known whether these are required by a minimal cell. Most autotrophs thus have roughly twice the number of genes believed to be essential for metabolic and macromolecular synthesizing functions.

These observations suggest a major experimental project in biogenesis: using gene deletion from autotrophic anaerobes to recover minimal truly free-living cells, in effect extracting candidates for the LUCA from existing organisms as a complementary approach to inferring them phylogenetically. The genes in modern autotrophs identified as “nonessential” in such deletion experiments could then be systematically studied as adaptations either to variable environments or to survival in a competitive microbial world.

Ideally the project would be systematic and comprehensive. Its first stage would entail assembly of data on all known bacterial and archaeal autotrophs, including minimal growth media, growth conditions, pathway information, and complete sequencing and annotation. The information that currently exists is scattered through the primary literature and needs to be gathered and curated. Data on most properties of known autotrophs—which are neither pathogens nor candidates for drug production or bioremediation—need to be acquired.

The second step in the project would be to culture as many autotrophs as feasible and carry out random parallel gene deletions as has been done for cell-wall defectives [1,2], leading to a minimal genome for each species consistent with viability and growth in a minimal medium. Considerable ingenuity may be required to approach a true minimal genome, perhaps combining in vitro evolution with gene deletion. The pathways of autotrophs are likely ancient, but their proteins are modern, and may have come to depend on cellular complexity not available in earlier organisms.

The third step would be to compare the various genomes in search of common features and significant differences. Similarities might be interpreted as features of the LUCA or restrictions on the environment in which it could have lived. Differences might indicate parallel paths for evolution of viable cells, illuminating the split between bacterial and archaeal domains in the selection of membrane lipids or the innovation of DNA polymerases [3]. Further variation could be elicited by changing the growth conditions for individual “minimized” species. A key question about the nature of core metabolism is whether thermodynamic forces and flux balance alone can stabilize metabolic pathways under variable conditions or whether allosteric and feedback controls are essential.

Fifty years ago the proposed project would not have been feasible, but today advances in instrumentation, computing, and our understanding of microbiology place it within reach. With the replacement of the prokaryote/eukaryote dichotomy by Carl Woese’s three domains (Archaea, Bacteria, Eukarya) and growing knowledge of the distinctions among them, we have come to appreciate that the oxidizing heterotrophs, including humans, provide a very narrow window on the organization and principles of biology. A systematic study of the chemo-autotrophs and their minimal forms would not only bring us closer to reconstructing the ur-organism, it would help us understand the stability of metabolism and cell reproduction and the necessary conditions for persistence of life.

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References


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