The tiny enslaved genome of a rhizarian alga

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At least twice in the history of life a predatory nonphotosynthetic protozoan cell ate a eukaryotic algal cell and enslaved it internally instead of digesting it, thereby becoming a chimeric photophagotrophic cell with two distinct nuclei and remarkably complex membrane topology—far surpassing that of animals or plants. In their descendants, the host nucleus became dominant, whereas that of the algal slave shrank by transfer of most of its genes into the main nucleus and retargeting the proteins they encode back into the enslaved chloroplast. Some descendants managed to transfer all essential genes and lose the enslaved nuclei altogether while retaining the algal chloroplast and plasma membrane (e.g., brown seaweeds), but in two groups of algae, cryptomonads (1) and chlorarachneans (2–4), the enslaved nucleus remains in the chimeric cell many millions of years afterward, raising fascinating questions for cell and evolutionary biology. How are functions shared between two evolutionarily unrelated nuclei, and how do their proteins integrate into one harmonious cell? The first inklings of answers came with the first nucleomorph genome sequence from a cryptomonad (1). In this issue of PNAS, Gilson et al. (5) report the first genome sequence of a chlorarachnean nucleomorph, the tiniest nucleus in nature.

Fig. 1 shows how these two unusual algal groups fit on the eukaryote evolutionary tree (6, 7). Cryptomonads belong to a vast branch, the chlorovolates, formed by a single enslavement of a red alga (8, 9) and containing eight phyla: two mainly photosynthetic [Ochrophyta (including brown algae, diatoms, and eight other algal classes) and Haptophyta]; two with one algal class each (dinoflagellates and cryptomonads) but many heterotrophs; and four entirely nonphotosynthetic (e.g., ciliate protozoa, and Pseudofungi) (10). By contrast, chlorarachneans belong to Rhizaria, a typically amoeboid group characterized by long, thin-branching pseudopods that often anastomose as a net. Rhizaria have two phyla: Cercozoa, wherein chlorarachneans are the only algae, and Retaria, including the largely marine Foraminifera and Radiolaria, which often cultivate algae temporarily in their cells, much as corals do, but never permanently enslave them as a true chloroplast with its own protein-import machinery like in chlorarachneans. Chlorarachnea is Greek for green spider, referring to the web-like body of the archetypal genus Chlorarachnion, in which green-chloroplast-containing cells form a multicellular network by temporary fusions of thread-like pseudopods that trap diverse prey for phagocytic engulfment and digestion.

Compared with other algae, chlorarachneans are little known partly because they live largely in subtropical waters. Japanese researchers found the biodiversity of chlorarachneans to be greater than previously recognized (11) and that they include classical amoeboid forms, others with cell walls, and flagellates like Bigelowiella (12) with potential as a laboratory model, being easier to grow; many display all three growth forms at different life-cycle stages.

Thus, chlorarachneans and cryptomonads represent entirely independent natural experiments in nuclear genome miniaturization that are most interesting to compare (13). What do such comparisons tell us? As expected, Bigelowiella nucleomorphs have far fewer genes than those of cryptomonads; only 331 protein-coding genes compared with 464 in the cryptomonomorph *Guillardia* (1). As in *Guillardia*, most genes are for housekeeping, merely to maintain the nucleomorph itself and the ribosomes that make its proteins. The only end-product functions of obvious direct value to the host are provided by 17 genes that encode proteins imported into the former green algal chloroplast. The 20-fold more numerous housekeeping genes are kept merely to allow expression of these 17 and are an evolutionary load, reflecting the sometimes bizarre imperfections of evolution by mutation and selection.

Organisms are not optimized, let alone designed, because selection is powerless without the requisite chance mutations. Thus, the nucleomorph of *Bigelowiella* was retained solely because 17 genes are essential for the chloroplast, just as were the cryptomonomorph nucleomorphs because of the 30 chloroplast proteins they encode (1). If, by chance, copies of all of these few genes had moved into the nucleus and their products successfully re-targeted into the chloroplasts through the four surrounding membranes, the nucleomorph genome would have disappeared. Such loss occurred in chromalveolates, both in the chromobiote sisters of cryptomonads and in alveolates (e.g., dinoflagellates and sporozoa like the malaria parasite) (10), but never in chlorarachneans.

However, euglenoid algae (members of Excavata) also have green plastids acquired from a green alga long ago. Their ancestor must once have had a nucleomorph and transferred all essential chloroplast protein genes into its own nucleus, re-targeted their proteins to the chloroplast, and then lost the nucleomorph entirely. It is possible that re-targeting was easier for euglenoids, in which proteins have to cross only three membranes and the targeting machinery via Golgi vesicles that fuse with the outermost membrane copes with proteins temporarily stuck in vesicle membranes by hydrophobic sequences (14). It is widely assumed that chlorarachneans

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enslaved a green alga independently of euglenoids (4), but the possibility that this occurred once only in a hypothetical common ancestor of Rhizaria and Excavata and offered evidence that both groups all lost chloroplasts (6, 8) is not yet decisively ruled out.

Deciding which history is true is important for estimating the age of chlorarachnean nucleomorphs. Judging from sequence trees (15), the last common ancestor of chlorarachneans might be marginally older than Cercozoa, which probably go back 540 million years (My) (16). If, instead, chlorarachneans acquired plastids independently of euglenoids, their nucleomorphs are probably 135–380 My old. Cryptomonad nucleomorphs are as old as chromalveolate, probably ~570 My (16). Thus, chlorarachnean nucleomorphs are no older than those of cryptomonads and are possibly much younger, yet they did shrink more. Thus, degree of compaction is not simply related to elapsed evolutionary time, as also shown by chromalveolate that lost nucleomorphs and were no older than cryptomonads that did not.

Nucleomorph genome reduction involves three processes: transfer of gene copies to the nucleus, their acquisition of bipartite targeting signals for protein import across four membranes (17), and deletion of the original nucleomorph copy. This gene-transfer process has both chance and deterministic aspects. The role of historical accident is shown by only 2 of the 17 chloroplast protein genes in the enslaved 

\[ \text{Guillardia}. \]

Given originally ~1,000 chloroplast protein genes in the enslaved algal nuclei, shared retention of these two is probably just a coincidence.

There appears no rhyme or reason why certain genes were successfully transferred and others were not (5). It is probably through historical accidents that chlorarachnean and cryptomonad failed to transfer all of their genes to the nucleus, whereas chromubiotics, algae, and euglenoids independently succeeded. Retention of Toc75/Tic20 but not Tic22 genes for chloroplast protein import, unlike 

\[ \text{Guillardia}, \]

also is likely accidental.

Determinism is evident in the way intergenic spacers have been pared down to as little as 1 nt or less: some genes overlap by up to 110 nt. These very short spacers and overlapping genes must reflect systematically greater reproductive success of deletions compared with insertions. Elimination of intergenic noncoding DNA, pseudogenes, and gene duplicates attest to strong selection for small genomes (18), but chlorarachnean nucleomorph genes riddled with introns [852 in all, 160 in intron size was dramatically reduced to much as a designer could have. Yet intron size was dramatically reduced to 18–21 nt (mostly 19 nt), suggesting a novel, possibly length-dependent mechanism of splicing.

Unlike in cryptomonads, the nucleomorph lost genes for tubulins and proteasomes, raising the question whether these proteins are imported into the periplastid space or are dispensable with. Previously, divergent nuclear-encoded tubulins were found with leaders, suggestive of targeting signals (19); thus, it is likely that they are imported and that the nucleomorph divides by a relict mitotic spindle as in 

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What remains for the future? With unpublished sequences of 

\[ \text{Bigelowiella}. \]

cryptomonad chloroplast and mitochondrial genomes completed in Canada and Japan, we greatly need nuclear genome sequences for 

\[ \text{Bigelowiella} \text{ and } \text{Guillardia}. \]

nucleomorphs to discover the conundrum whether chlorarachnean and euglenoid plastids were enslaved in a common ancestor or not may depend on elucidating the chlorarachnean protein-targeting systems.

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11. Cavalier-Smith, T. & Maier, U. G. (2001) *Chromosoma* **119**, 570 My (16). Thus, chlorarachnean nucleomorphs must be marginally older than Cercozoa, which probably go back 540 million years (My) (16). If, instead, chlorarachneans acquired plastids independently of euglenoids, their nucleomorphs are probably 135–380 My old. Cryptomonad nucleomorphs are as old as chromalveolate, probably ~570 My (16). Thus, chlorarachnean nucleomorphs are no older than those of cryptomonads and are possibly much younger, yet they did shrink more. Thus, degree of compaction is not simply related to elapsed evolutionary time, as also shown by chromalveolate that lost nucleomorphs and were no older than cryptomonads that did not.

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What remains for the future? With unpublished sequences of *Bigelowiella* chloroplast and mitochondrial genomes completed in Canada and Japan, we greatly need nuclear genome sequences for *Bigelowiella* and *Guillardia* to understand how four genomes cooperate to make such complex chimeric cells. Rhizaria are the only major group in the living world with no complete nuclear genome sequences. For fuller understanding of the cell biology and lifestyles of these unusual organisms, which may be the most abundant predators on Earth as Cercozoa dominate the soil and Retaria dominate marine sediments and the oligotrophic ocean vastnesses, we need genome sequences from divergent heterotrophic Rhizaria, especially Cercozoa (far easier to cultivate). A final answer to the conundrum whether chlorarachnean and euglenoid plastids were enslaved in a common ancestor or not may depend on elucidating the chlorarachnean protein-targeting systems.