Horizontal gene transfer in eukaryotic algal evolution

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Although seemingly innocuous, the power of something as simple as a drop of water, given a few millions of years over which to act continually, is immediately and awe-inspiringly obvious to someone peering over the edge of the Grand Canyon. Acting over a similarly immense time period but on vastly different scales, this massive effect from a weak, but constant, force embodies how natural selection has been able to direct the evolution of once primitive, maladapted biological structures to the remarkable and almost inconceivably diverse molecular machines found within extant organisms. This idea of natural selection as a slow-and-steady workhorse was central to Charles Darwin's evolutionary synthesis, as epitomized in his oft-repeated précis "Natura non facit saltum," Nature does not make leaps. Darwin would not live to see the discovery of genes as the vessel of inheritance and random mutation as the propagator of change, although these breakthroughs would serve to reinforce his prescient ideas.

To the contrary, the discovery of horizontal gene transfer (HGT) as a significant evolutionary driver may require an addendum to the Darwinian synthesis. A growing body of evidence indicates that many organisms, particularly prokaryotes, can and do make evolutionary leaps by sharing genes with one another, thereby opening a back door to an adaptation or ability that was already fine-tuned within another organism. Once thought to be an explanation of last resort when the data were not robust enough to give unambiguous results, with the recent availability of a wealth of whole-genome data, HGT has not only become respectable but has emerged as a central force in the evolution of many different prokaryotes (1–3). Of course, this idea came as no major surprise to many bacterial geneticists, who for decades have been selecting prokaryotes for their ability to take up and express exogenous genes (as Oswald Avery did some 60 years ago, demonstrating that DNA was the carrier of genetic information) (4).

The impact of HGT on eukaryote genomes has not been so clear-cut (3). The species concept of genetically segregated germ lines has been tied to eukaryote taxonomy since its inception, and the barriers against HGT in bacteria are magnified in eukaryotes by further complexities in transcription and translation, such as the need for correct splicing of RNA transcripts replete with introns. It can also be argued that sexual reproduction affords many eukaryotes the same advantage gained through HGT in bacteria. In this issue of PNAS, Archibald *et al.* (5) leap beyond the case-by-case examples that typify eukaryotic HGT and demonstrate that HGT has played a significant role in the evolution of a eukaryotic alga. In a collective analysis of 78 plastid-targeted proteins from this alga, they show that, even by conservative measures, ≈21% of these genes have likely been acquired by HGT. Their result stands to significantly expand the number of established cases of so-called transdomain HGT occurring between prokaryotes and eukaryotes and bolsters some novel ideas on evolutionary mechanisms in phagocytic eukaryotes (6).

The subject, *Bigelowiella natans*, is a member of a class of algae known as chlorarachniophytes that, in and of itself, is quite an evolutionary enigma. All plastid-containing eukaryotes acquired the ability to do photosynthesis when, perhaps ≈2 billion years ago, a primitive eukaryote engulfed a photosynthetic cyanobacterium. This so-called primary endosymbiotic event gave early eukaryotes an extremely powerful metabolic ability that previously was manifest only among photosynthetic bacteria, and also constituted, along with the enslavement of a proteobacterium that would become the mitochondrion, a massive horizontal transfer of genes into a primitive eukaryote. The modern progeny of this primitive photosynthetic eukaryote point to a single primary endosymbiotic event, although some evidence argues otherwise (e.g., refs. 7 and 8). It is also certainly feasible that endosymbiosis occurred multiple times, but many organisms were wiped out in the bottleneck of subsequent global catastrophes (e.g., global glaciations, refs. 9–11).

Not to be left behind, some eukaryotes acquired photosynthesis through the same mechanism, although not by engulfing a cyanobacterium but rather a eukaryotic alga (10, 12). Termed secondary endosymbiosis, this process is believed to have given rise to multiple independent groups of photosynthetic organisms, all of which bear the hallmark of plastids with three or more bounding membranes (13). That secondary endosymbiosis has occurred multiple times is also made clear in that it has occurred in various lineages after the radiation of the three flavors of primary algae (red, green, and glaucocystophyte), leading to secondary algae with quite varying plastid phenotypes. In primary and secondary endosymbiosis there has been a massive loss of genes from the endosymbiont genome, many of which have been transferred into the host genome, with each host-encoded plasmid-targeted gene now carrying a transit peptide sequence that directs it back to the plastid (secondary endosymbiont-directed proteins also carry an additional signal sequence to get them through the vestigial membrane system of the original plastid host) (14). Two groups of algae that evolved through secondary endosymbiosis, the cryptomondads and chlorarachniophytes (including *B. natans*) are particularly interesting because they still contain a relict nucleus called a nucleomorph, dramatically reduced in size, from the originally engulfed algae (10).

Additional transfer of genes has undoubtedly occurred from the relict nucleus into the host genome (15), although despite these complex transfer events these genes in the host genome should have a phylogenetic signal consistent with the engulfed algae and thus should be grouped more broadly with cyanobacteria. Genes present in the host before endosymbiosis should cluster with other eukaryotic genes and thereby can be used to classify the original host. For most nuclear-encoded algal genes these stratifications are indeed observed, and in *B. natans* a variety of evidence clearly indicates a green algal endosymbiont origin. However, as Archibald *et al*. (5) show, many of the plastid-targeted genes from *B. natans* clearly diverge from this expectation. These horizontally transferred genes span a varied swath of functions, including chlorophyll biosynthesis, carbon fixation, and ribosome structure, and cluster with a similarly broad range of taxa other than green algae. Several of their trees, which, importantly, encompass much of the available taxonomic sampling for each sequence, are particularly robust based on bootstrap values and conserved sequence motifs, providing strong

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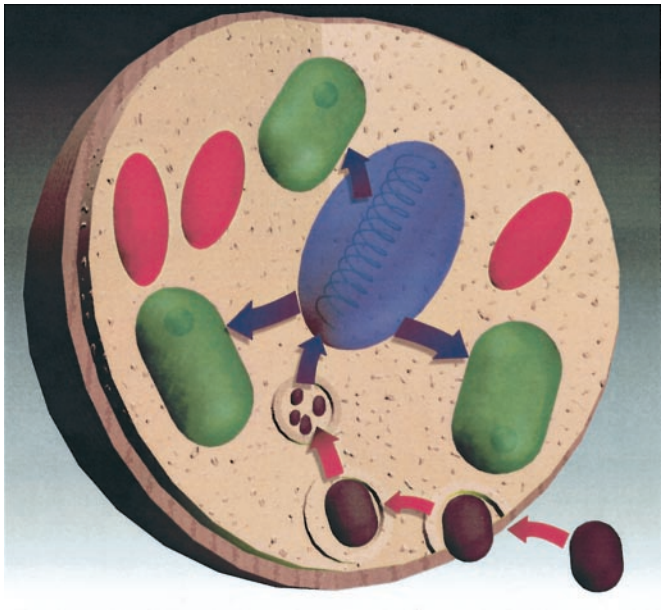

Fig. 1. Stepwise conceptual image of one mechanism of HGT that, as proposed by Archibald *et al.* (5), might operate in the chlorarachniophyte alga *B. natans*. Red arrows show phagocytosis and subsequent digestion of a bacterium or protist, from which foreign DNA has survived digestion and become incorporated into the algal nucleus (flow of HGT-acquired genetic information indicated with blue arrows). Although the genes studied herein by Archibald *et al*. are directed for function to the plastid, the significant number of horizontally transferred genes they found may only be the tip of the iceberg in phagocytic protists such as *B. natans*.

support for HGT having played a significant role in the evolution of this organism.

Because all of the genes studied by Archibald *et al.* are encoded within the nucleus of *B. natans* but operate within the plastid, the signal and transit peptides are absolutely necessary. One can surmise that this necessity would strongly favor HGT in and among algae, where nuclear-encoded genes targeted to plastids must navigate a similar maze of endomembranes through the direction of transit peptides, and additional signal peptides in the case of other secondary algae. Indeed, a majority of genes studied by the authors support this expectation, favoring phylogenies consistent with HGT from streptophytes or red algae into the *B. natans* genome.

Most intriguingly, two of the genes from their analysis indicate HGT from different bacteria, significant not only as an example of prokaryote-to-eukaryote gene transfer but also because these acquired genes initially would have not had the proper leader sequence for import into the plastid. Whether the appropriate targeting sequence was incorporated *de novo* through gene conversion or some other mechanism of homologous or orthologous replacement is not clear, but this remarkable finding certainly invokes new ideas on how genes are assimilated into a genome.

Microbiologists have long known about phenotypes that favor promiscuous plasmid sharing among bacteria, responsible for the epidemic spread of antibiotic resistance. Although no plasmid analog exists in eukaryotes, Archibald *et al*. suggest that HGT in *B. natans* may occur in the same way it has for the many endosymbiotic events that have happened over the past 2 billion years, by engulfing other organisms (Fig. 1). Compared with the green alga *Chlamydomonas reinhardtii*, which is photoautotrophic and in which no parallel evidence of HGT is found, *B. natans* is mixotrophic, meaning that it can live phagocytotically and photosynthetically. Models have been proposed whereby small snippets of DNA from an engulfed microbe are able to escape digestion, e.g., from protist lysozomes, and migrate to and subsequently be incorporated into the host genome (6, 16). One can imagine the series of fleetingly small probability events proceeding from engulfment to incorporation of a strand of foreign DNA into the genome to a new gene overcoming genetic drift to become fixed in the population. In a certain sense, this is the same cumulative effect as random mutations in single genes or dripping water, but it now operates on a different level, an entire gene. However, the essential point is that those probabilities are nonzero and over time have made a significant contribution to the genome of this organism (6). The real boon of Archibald *et al.*'s hypothesis, perhaps best synopsized by Ford Doolittle's epigram "you are what you eat" (16), is that it is eminently testable as more eukaryotic genome data become available. It is already apparent that the magnitude of HGT varies dramatically in different lineages of algae, with the proposed explanation of the phagocytotic lifestyle as a likely but not proven explanation for the observed mosaic pattern. Whether this is a more general mechanism for HGT in a wider range of eukaryotes, including nonalgal taxa, is not yet apparent.

So although Nature herself may not make leaps, it now seems clear that many organisms, eukaryotes and prokaryotes, are certainly able to mimic evolutionary jumps through HGT.

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