EVOLUTION

A Green Algal Apicoplast Ancestor

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Apicomplexan parasites, including the human pathogens *Toxoplasma* and *Plasmodium*, contain a vestigial plastid, the apicoplast. This chloroplast-derived organelle is the remnant of a secondary endosymbiosis between an ancestral apicomplexan and a photosynthetic organism whose origin is moot (*1*–*4*). We have identified two distinctive apicomplexan nuclear genes that suggest a green algal ancestry for the apicoplast.

COXII, a subunit of the mitochondrial cytochrome oxidase, is usually encoded by a mitochondrial *cox2* gene. However, members of at least three genetic lineages lack *cox2* in the mitochondrial DNA (mtDNA). Some leguminous plants express full-length COXII from the nucleus (*5*). Members of the algal class Chlorophyceae also lack *cox2* in the mtDNA (*6*). Two such algae, *Chlamydomonas reinhardtii* and *Polytomella* sp., have two distinct nuclear *cox2* genes: *cox2a* encodes COXIIA, corresponding to the NH_2-terminal membrane domain of orthodox COXII, and *cox2b* encodes COXIIB, corresponding to the COOH-terminal domain (*7*). COXIIA and COXIIB are imported into the mitochondrion (*7*). A truncated *cox2* in the mtDNA of the chlorophyte alga *Scenedesmus obliquus* (*8*) has been recognized as a *cox2a* homolog. We predicted that this alga would also have a nuclear *cox2b* (*7*) and have confirmed this (fig. S1) (*9*). The frequency of *cox2a* and *cox2b* in Chlorophyceae suggests an ancestral chlorophyte *cox2* split in the mtDNA, before independent transfers of *cox2a* and *cox2b* to the nucleus. *S. obliquus* appears as an intermediate, retaining *cox2a* in the mtDNA. The incompatibility between the *S. obliquus* mitochondrial and nuclear genetic codes (*8*) may have developed after the transfer of *cox2b* to the nucleus, preventing the subsequent migration of *cox2a*.

The third group lacking mtDNA-encoded *cox2* is the Apicomplexa (*10*). We identified *cox2a* and *cox2b* cDNAs from *Toxoplasma gondii* and cloned the corresponding nuclear genes (fig. S1). We also identified nuclear *cox2a* and *cox2b* in the apicomplexans *Plasmodium falciparum*, *P. yoelii*, *Theileria annulata*, *T. parva*, and *Eimeria tenella*.

Several lines of evidence suggest a common ancestry for apicomplexan and green algal *cox2* genes. Both algal and apicomplexan *cox2* genes appear to have been split in identical locations (fig. S1). All predicted COXIIA proteins have a 125– to 170–amino acid NH_2-terminal extension that is a cleavable mitochondrial targeting sequence in algal COXIIA (*7*). COXIIB proteins have shorter NH_2-terminal extensions (43 to 60 residues), not cleaved in algae, with no equivalent in conventional COXII (*7*). The conserved PxxxPxxY motif in this region of COXIIB also implies a common origin for apicomplexan and chlorophyte *cox2b* genes (fig. S1). In phylogenetic analyses of the available COXIIA and COXIIB sequences and representative orthodox COXII sequences, apicomplexans group with chlorophytes (Fig. 1). In contrast, apicomplexan sequences of mtDNA-encoded cytochrome b group independently from green algal sequences. The data strongly argue that apicomplexan *cox2a* and *cox2b* derive from green algae, not from an independently split apicomplexan *cox2*. *T. gondii cox2a* has an intron that is conserved in location and phase with introns in *cox2a* of *C. reinhardtii* and *Polytomella* sp. (figs. S1 and S2). This suggests a lateral transfer of *cox2a* and *cox2b* from the green algal nucleus to the apicomplexan nuclear genome.

Previous analyses have favored either a green algal (*2*) or a red algal (*1*, *4*) apicoplast precursor. Because the mtDNAs of a wide variety of red algae contain a single conventional *cox2* (*6*), the nuclear *cox2a* and *cox2b* genes of the Apicomplexa indicate a green algal endosymbiont (*2*) from the class Chlorophyceae. This is a remarkable example of lateral gene transfer, in that a single apicomplexan cytochrome oxidase subunit (COXII) has been replaced by two proteins (COXIIA and COXIIB) of green algal origin. The use of functional split genes as atypical molecular markers has helped to clarify the complex evolution of the causative agents of malaria and toxoplasmosis.

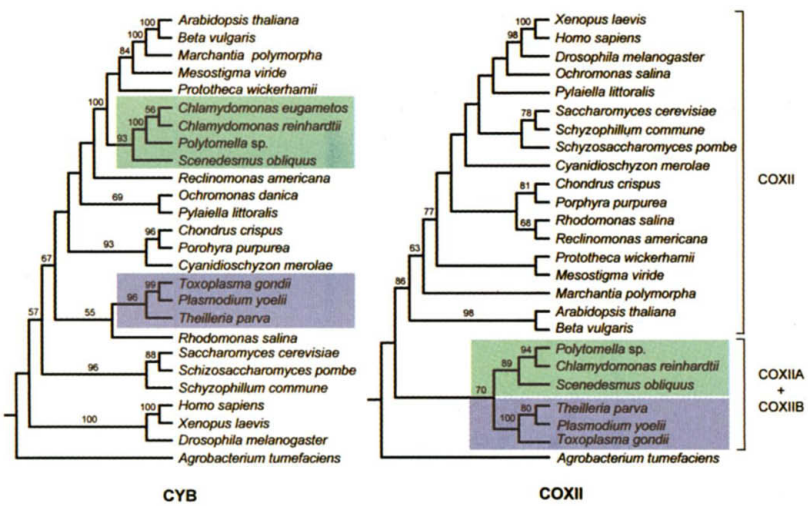

Fig. 1. Maximum likelihood (ML) analyses showing the position of apicomplexan parasites based on cytochrome b (CYB) and COXIIA + COXIIB sequences. COXIIA and COXIIB (excluding MTS and extensions) were fused in silico as a single polypeptide and aligned with orthodox mitochondrial COXII sequences. Bootstrap support >50% is indicated above branches. See materials and methods in supporting online material (*9*).

References and Notes

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11. Sequencing of *T. parva* and *P. yoelii* is carried on by the Institute for Genomic Research. We thank J. M. Quinn and S. Merchant for the *S. obliquus* library and A. Gómez-Puyou, D. W. Krogmann, and D. A. Maslov for critical comments. Work supported by TW01176 (NIH), HL59646 (NIH), and IN207201 (UNAM).

Supporting Online Material
www.sciencemag.org/cgi/content/full/298/5601/2155/DC1
Materials and Methods
Figs. S1 and S2

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