# More plastids in human parasites?

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**Trypanosomatid parasites are disease agents with an extraordinarily broad host range including humans, livestock and plants. Recent work has revealed that trypanosomatids harbour numerous genes sharing apparent common ancestry with plants and/or bacteria. Although there is no evidence of a plastid (chloroplast-like organelle) in trypanosomatids, the presence of such genes suggests lateral gene transfer from some photosynthetic organism(s) during trypanosomatid evolution. Remarkably, many products of these horizontally acquired genes now function in the glycosome, a highly modified peroxisome unique to trypanosomatids and their near relatives.**

The holy grail for many parasitologists is to identify differences between parasite and host which can be exploited for therapy. In principle, any difference provides an opportunity to develop a treatment regime that selectively targets the parasite as long as the target is essential. Evolutionary distance is a reliable provider of biological divergence. Thus, the greater the time since two organisms shared a common ancestor, the greater the divergence as a result of incremental accumulation of small differences. Bacterial pathogens are a case in point. Many antibacterials in use today target unique prokaryotic biochemical processes such as cell wall formation. However, other highly successful antibacterials block ubiquitous and fundamental processes such as transcription and translation, which are sufficiently divergent from eukaryotic forms, so that pathogen-specific inhibition can occur. Alas, we do not enjoy such an advantage over our eukaryotic pathogens because they are our nearer cousins, and this narrows the window of targets considerably.

A remarkable twist in the evolution of eukaryotes is enabling us to reassess our arsenal for some parasites. Plastid organelles (known as chloroplasts in plants) are the product of an endosymbiotic merger of a cyanobacterial prokaryote with a eukaryote [1]. Typically, we associate plastids with photosynthetic organisms such as plants, seaweeds and unicellular phytoplankton. However, the recent discovery of a plastid (termed apicoplast) in apicomplexan parasites such as *Plasmodium* and *Toxoplasma* demonstrates that the rules are not so clear [2–4]. Although the apicoplast is no longer photosynthetic, the parasite has apparently become dependant upon this organelle for other metabolic functions, including fatty acid and isoprenoid synthesis [5]. When the plastid was first acquired, several anabolic pathways would have existed in two places in the new amalgamated cell: the cytosol and the plastid. Subsequent rationalization of this redundancy apparently led to loss of certain eukaryotic pathways with the prokaryotic ones introduced with the plastid being maintained. This resulted in the apicoplast being integrated beyond the point of no return for these parasites. Thus, these prokaryotic anabolic pathways, together with all the prokaryotic-type housekeeping functions on which they depend, are excellent targets for drug assault. Apicoplast DNA replication, transcription, translation, fatty acid and isoprenoid biosynthesis have all been validated as drug targets, and this could be just the tip of the iceberg [6]. Good news for the war on malaria and other apicomplexan diseases.

## A shady photosynthetic past for trypanosomatids?

We now learn that trypanosomatid parasites bear similar molecular hints of a possible photosynthetic past [7]. The Trypanosomatidae (Kinetoplastida) includes the agents of African sleeping sickness (*Trypanosoma brucei*), Chagas disease (*Trypanosoma cruzi*), and visceral and (muco)cutaneous leishmaniasis (*Leishmania* spp.). Together, these pathogens represent a threat to the health of >500 million people, infect >100 million people and cause >100 thousand deaths annually. Hannaert *et al.* report a collection of genes from *T. brucei* and *Leishmania mexicana* with intriguing plant and/or prokaryotic signatures [7]. Several of these represent genes involved in carbon metabolism, assigned to either glycolysis or the hexose-monophosphate pathway (HMP). Some of these genes are involved in the Calvin cycle in plastids, but are employed in either of the above processes in trypanosomatids, which do not possess a Calvin cycle. Other genes highlighted by Hannaert *et al.* are involved in fatty acid biosynthesis, aromatic amino acid and glycerol metabolism, reactive oxygen protection, respiration and proton pumping. Whereas many of these genes are apparently derived from prokaryotic sources (perhaps from plastids), others are reported to be more closely allied to plants [e.g. sedoheptulose-1,7-bisphosphatase (SPBase) and cofactor-independent phosphoglycerate mutase (PGAM)] [7].

Given that apicomplexan parasites contain a plastid, we must ask the question, do trypanosomatids also contain a plastid? The answer is almost certainly, no. Unlike apicomplexans, where a mysterious, but unidentified, organelle was well described at an ultrastructural level before

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molecular evidence for a plastid [8], there is no such evidence of a plastid in trypanosomatids. Two explanations for plant-like genes remain. The simplest explanation, favoured by Hannaert *et al.*, is that the trypanosomatid parasites once contained a plastid endosymbiont, but have since lost it. Endosymbiont genes were transferred to the nucleus during the tenancy of the plastid, but only a trace of such an organelle now remains. Hannaert *et al.* note that trypanosomatids fall within the phylum Euglenozoa, which includes euglenid algae (Figure 1). These algae have green plastids surrounded by three membranes, which indicates a secondary plastid [i.e. an engulfed eukaryotic (algal) endosymbiont similar to the apicoplast] [9]. Therefore, it is possible that the common ancestor of the Euglenozoa contained this plastid, but that it has since been lost in several lineages during Euglenozoa diversification (Figure 1).

This hypothesis for an ancient plastid acquisition in Euglenozoa followed by plastid loss in the kinetoplastid lineage has some attractive features. Lateral gene transfer is probably accelerated by having an in-house source of foreign DNA such as an endosymbiont [10]. In addition, trypanosomatids harbour not only genes found in plastids and bacteria, but also genes (such as SBPase) that are only known from eukaryotes. While this early eukaryotic endosymbiont hypothesis requires organelle loss within the Euglenozoa (from multiple lineages to explain the current occurrence of plastids in this phylum) (Figure 1), plastid loss is not without precedent. For instance, secondary plastids have apparently been lost from oomycetes [11] and possibly ciliates [12].

Although attractive, the early acquisition hypothesis (Figure 1ai) should be embraced cautiously at this point. For example, recent ultrastructural analysis of Euglenozoa suggests that only certain euglenids, with specialized cytoskeletal adaptations that allowed for the ingestion of other eukaryotic cells, contain a plastid [13]. Euglenids lacking these adaptations can only phagocytose bacteria and lack a plastid [13]. According to this hypothesis, euglenid plastids were acquired relatively recently and only by some members of this group. Trypanosomatids therefore could not have inherited such a plastid if it were acquired so recently (Figure 1). However, some kinetoplastids contain their own bacterial endosymbionts (e.g. *Crithidia oncopelti* and *Cryptobia vaginalis*). Thus, it is conceivable that independent endosymbionts could have been an alternative source for some of the plastid and/or prokaryotic-type genes (Figure 1).

An alternative possible source of evolutionarily unrelated genes is food items. Ford Doolittle's 'You are what you eat' hypothesis posits rampant gene transfer from prey to consumer in the microscopic world [14]. Just as a lysing organelle can provide a surge of genetic material with an opportunity for integration into the nucleus, a partially digested bacterial food particle also provides opportunity for gene transfer (Figure 1aii). Many free-living kinetoplastids, such as bodonids, are bacteriovores, and a long history of bacterial gene integration during kinetoplastid evolution is possible. In a similar vein, some modern trypanosomatids parasitize plant tissues. If ancestral trypanosomatids also parasitized plants, they could have directly acquired genes from their hosts. The question of how trypanosomatids gained their plant and prokaryote-type genes will only become clear with further molecular data (notably from other protists) that will enable more thorough analyses of their evolutionary affinities. For many of the genes presented by Hannaert *et al.*, limited taxon representation allows for only preliminary phylogenetic conclusions at this point (http://www.icp.ucl.ac.be/~opperd/supplementary/table.html). Indeed, broader taxon sampling undermines the plant connection for fructose bisphosphate aldolase [15], as it also does for SBPase that was previously only known from plants and algae, but has recently been reported from fungi [15]. If further phylogenetic analyses of laterally transferred genes consistently point to a single genetic source, then the endosymbiont hypothesis will be supported. Alternatively, if the genes derive from multiple independent lineages, then multiple independent gene transfers, most likely from ingested food items, will be more probable. The inclusion of euglenid sequences will be crucial to these analyses.

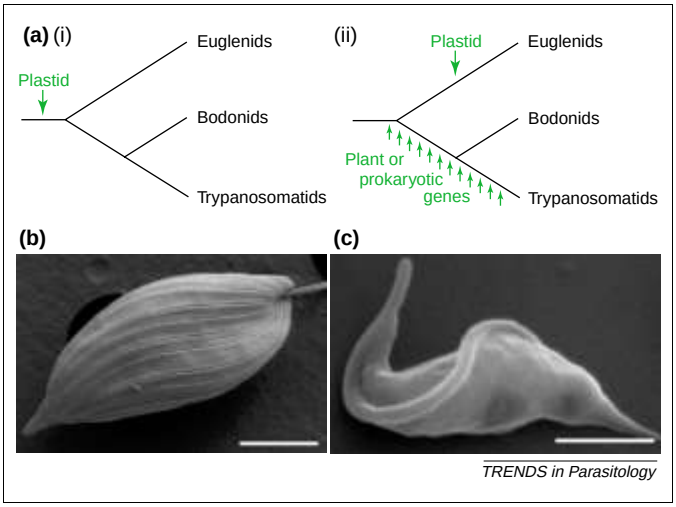


**Figure 1**. Plastid origins in Euglenozoa evolution. **(a)** Two hypotheses accounting for plant and/or prokaryote-like genes in trypanosomatid parasites. **(i)** A plastid was acquired by an ancient common ancestor, and plastid genes were transferred to the nucleus before plastid loss in the non-euglenid lineages (note, independent plastid loss must also have occurred in several non-photosynthetic euglenid lineages). **(ii)** Alternatively, the euglenid plastid was acquired after the euglenid group diverged. In this case, a source(s) of plant and/or prokaryote-like genes might have been accumulated later gene transfers from food items or other external sources, or perhaps even independent endosymbionts that were subsequently lost. Scanning electron micrographs of the euglenid, *Phacus caudata* (courtesy of Brian Leander) **(b)** and the trypanosomatid, *Trypanosoma brucei* (courtesy of Louis De Vos) **(c)**. Scale bars = 5 μm (b,c).

## Glycosome: a possible drug target

Other plant or prokaryote-like genes will probably be unearthed as *Trypanosoma* and *Leishmania* genome sequencing programs near completion (see Table 1 for sequencing project websites). It is now important to understand how these genes have been integrated into trypanosomatid metabolism to explore any opportunities for selectively targeting them with drugs. Remarkably, a large portion of these genes is implicated in the functions of a unique kinetoplastid organelle, the glycosome [7]. Glycosomes are remodelled peroxisomes that have adopted pathways including glycolysis and purine salvage, in addition to maintaining more typical peroxisome

**Table 1. Websites for trypanosomatid sequencing projects**

| Parasite sequencing project | Website address |
|---|---|
| *Trypanosoma brucei* | http://www.sanger.ac.uk/Projects/T_brucei/<br>http://www.tigr.org/tdb/mdb/tbdb/index.shtml |
| *Trypanosoma cruzi* | http://www.tigr.org/tdb/e2k1/tca1/ |
| *Leishmania major* | http://www.sanger.ac.uk/Projects/L_major/ |

functions such as β-oxidation of fatty acids [16]. The integrity of the glycosome is essential (for *T. brucei* at least) as perturbation of glycosome protein uptake is lethal [17,18], and this implies that glycosomes are a reasonable target for drugs.

Relocation of pathways to a compartment such as a peroxisome seems a relatively unlikely event, given that it would typically require relocating several proteins (presumably independently) before any functional pathway could be established. Hannaert *et al.* suggest that lateral transfer of plant-like and bacterial genes could have been important in the remodelling of the trypanosomatid peroxisome into a glycosome. For example, up to four out of the seven to nine glycolytic enzymes in the glycosome could be derived from lateral gene transfer. Pathway relocation would probably have required duplication of genetic material to tinker with the new, while the old continues to fulfill the essential function. Lateral gene transfer might have provided the necessary duplication and therefore helped drive peroxisome remodelling.

The next step is to begin re-targeting proteins to their new compartment. In the case of peroxisomes, this involves the addition of a relatively simple peroxisomal-targeting sequences (PTS) either at the C-terminus (a simple tripeptide extension known as PTS1) or near the N-terminus (PTS2) [19]. Several enzymes of carbon metabolism (e.g. glucosephosphate isomerase, aldolase and triose-phosophate isomerase) contain a functional PTS, but are located in both the glycosome and the cytosol [20]. This is believed to allow dual functions of these enzymes; gluconeogenesis or the HMP in the cytoplasm and glycolysis in the glycosome. The relative distribution of enzymes varies among trypanosomatid parasites (typically *Leishmania* spp. favour the cytosolic location, whereas *T. brucei* favour the glycosome) and this could reflect the relative requirement of these pathways for each parasite [20]. So, it could have been that re-targeting each protein to the peroxisome was a gradual process that allowed this compartment to slowly explore new capabilities without immediately affecting the enzyme's cytosolic responsibilities.

Finally, the glycosomal membrane is known to be at least slightly permeable to glycolytic intermediates [21] and this might have provided a further key to incremental relocation of pathways such as glycolysis. Alternatively, if a plastid endosymbiont is implicated in trypanosomatid evolution, it is possible that genes for plastid transporters, which often have remarkably broad substrate specificities [22,23], could have accelerated glycosome evolution by allowing the import or export of glycolytic intermediates. To date, no carbon transporters have been identified for the glycosome at a molecular level to test this hypothesis. The genes do exist in apicomplexan parasites where they are probably crucial to apicoplast metabolism [5]. Completion of trypanosomatid sequencing projects should tell us if any such transporters have similar roles in trypanosomatid glycosomes.

## Conclusions

The revelation that trypanosomatids contain several evolutionarily divergent genes is a reminder that many of the parasites that inflict so much suffering on people and their livestock have had a long and complicated evolutionary history. In the case of trypanosomatid parasites, this ancestry probably stretches back several hundred million years, and could have involved associations with photosynthetic organisms and perhaps even an autotrophic lifestyle. The challenge now is to unravel this lengthy and convoluted chain of evolution, so that it can be exploited to control the scourge of modern trypanosomatids.

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### References

1 Cavalier-Smith, T. (1982) The origins of plastids. *Biol. J. Linn. Soc.* 17, 289–306
2 Wilson, R.J.M. *et al.* (1996) Complete gene map of the plastid-like DNA of the malaria parasite. *J. Mol. Biol.* 261, 155–172
3 McFadden, G.I. *et al.* (1996) Plastid in human parasites. *Nature* 381, 482
4 Köhler, S. *et al.* (1997) A plastid of probable green algal origin in apicomplexan parasites. *Science* 275, 1485–1488
5 Gardner, M.J. *et al.* (2002) The genome sequence of the human malaria parasite. *Nature* 419, 498–511
6 Ralph, S.A. *et al.* (2001) The apicoplast as an antimalarial drug target. *Drug Resist. Updat.* 4, 145–151
7 Hannaert, V. *et al.* (2003) Plant-like traits associated with metabolism of *Trypanosoma* parasites. *Proc. Natl. Acad. Sci. U. S. A.* 100, 1067–1071
8 McFadden, G.I. *et al.* (1997) Plastids in apicomplexan parasites. *Pl. Syst. Evol.* 11, 261–287
9 Delwiche, C.F. and Palmer, J.D. (1997) The origin of plastids and their spread via secondary endosymbiosis. *Pl. Syst. Evol.* 11, 51–86
10 Huang, C.Y. *et al.* (2003) Direct measurement of the transfer rate of chloroplast DNA into the nucleus. *Nature* 422, 72–76
11 Andersson, J.O. and Roger, A.J. (2002) A cyanobacterial gene in nonphotosynthetic protists – an early chloroplast acquisition in eukaryotes. *Curr. Biol.* 12, 115–119
12 Fast, N.M. *et al.* (2001) Nuclear-encoded, plastid-targeted genes suggest a single common origin for apicomplexan and dinoflagellate plastids. *Mol. Biol. Evol.* 18, 418–426
13 Leander, B.S. *et al.* (2001) Trends in the evolution of the euglenid pellicle. *Evolution Int. J. Org. Evolution* 55, 2215–2235
14 Doolittle, W.F. (1998) You are what you eat: a gene transfer ratchet could account for bacterial genes in eukaryotic nuclear genomes. *Trends Genet.* 14, 307–311
15 Rogers, M.B. and Keeling, P.J. Lateral transfer and re-compartmentalisation of Calvin cycle enzymens of plants and algae. *J. Mol. Evol.* (in press).
16 de Souza, W. (2002) Special organelles of some pathogenic protozoa. *Parasitol. Res.* 88, 1013–1025
17 Furuya, T. *et al.* (2002) Glucose is toxic to glycosome-deficient trypanosomes. *Proc. Natl. Acad. Sci. U. S. A.* 99, 14177–14182
18 Guerra-Giraldez, C. *et al.* (2002) Compartmentation of enzymes in a

microbody, the glycosome, is essential in *Trypanosoma brucei*. *J. Cell Sci.* 115, 2651–2658
19 Parsons, M. *et al.* (2001) Biogenesis and function of peroxisomes and glycosomes. *Mol. Biochem. Parasitol.* 115, 19–28
20 Nyame, K. *et al.* (1994) Subcellular distribution and characterization of glucosephosphate isomerase in *Leishmania mexicana*. *Mol. Biochem. Parasitol.* 67, 269–279
21 Visser, N. *et al.* (1981) Subcellular compartmentation of glycolytic intermediates in *Trypanosoma brucei*. *Eur. J. Biochem.* 118, 521–526
22 Pohlmeyer, K. *et al.* (1998) A high-conductance solute channel in the chloroplastic outer envelope from pea. *Plant Cell* 10, 1207–1216
23 Flugge, U.I. (1999) Phosphate translocators in plastids. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50, 27–45

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Letters

# Molecular epidemiology pitfalls: some important clarifications

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Constantine presents the interest and danger in using molecular tools for epidemiological inferences, and provides useful advice on how to avoid pitfalls [1]. However, there are some important inaccuracies, which need to be addressed here.

The definition and use of the term homoplasy is not totally accurate in Constantine's *Opinion* [1]. Homoplasy does not refer to samples. The definition of homoplasy is the identity between two alleles that are not identical by descent, but by state. This occurs when an allele mutates into the same state as a previously existing allele, or when two different alleles cannot be distinguished by the technique used. According to Rousset [2], the effect of homoplasy on measures of population subdivision (i.e. $F$ statistics) is weak and simply corresponds to the infinite allele model (where homoplasy never occurs because mutation always creates new alleles) with a higher mutation rate [i.e. $u' = ku/(k-1)$, where $k$ is the number of possible allelic states and $u$ is the mutation rate]. The effect of a limited number of possible alleles (homoplasy) is not null, but is weakly detectable on $F_{st}$ only (measure of population differentiation) and only for very low $k$ (number of possible allelic states). According to equations 3 and 6 of Ref. [2], the difference will never exceed 0.01 in most situations if $k > 2$ and the mutation rate $u \leq 10^{-4}$. Thus, two samples will rarely appear to be the same (if ever) just because of homoplasy. It seems that there is confusion, on one hand, between species and between populations and, on the other hand, between population genetics and phylogenetic analysis. Indeed, they do not deal with the same problems. Phylogenetic analyses are correct for studying species divergences and can also be applied to fully clonal species. Population genetics tools are more appropriate to study populations where recombination occurs.

The breeding system definition also appears to be inaccurate. Heterogamy (mating preferentially occurs between phenotypically divergent partners) is disregarded. Moreover, the Hardy–Weinberg equilibrium is not a breeding system, as suggested in the Glossary of Ref. [1], but is an expected genotypic distribution under a specific set of different assumptions and one of these assumptions involve the breeding system (i.e. random association of gametes). This particular genotypic distribution, known as $[p + (1-p)]^2$ in the di-allelic case, can be mimicked by partial clones [3] or with other special parameters sets that are in disagreement with the Hardy–Weinberg assumptions. Similarly, the definition of linkage equilibrium given confuses the Hardy–Weinberg assumptions with the genetic consequences expected in populations fitting such assumptions. A population that follows Hardy–Weinberg assumptions can maintain linkage disequilibrium between different loci for a very long time. Indeed, under Hardy–Weinberg assumptions, the rate of decrease in linkage between two genes with recombination rate $r$ is proportional to $(1-r)^t$, where t equals the number of generations [4]. Because Hardy–Weinberg equilibrium is reached in a single generation [4], it is easy to imagine populations in Hardy–Weinberg equilibrium at each locus with a significant linkage between loci.

The sentence on the maximum gene flow that is sufficient to prevent differentiation is arbitrary and thus meaningless. An effective number of migrants of one is very low and would lead to $F_{st} = 0.2$, an amount of divergence that could be found between different species [5] and thus between significantly divergent samples. Any migration rate is sufficient to prevent divergence by drift alone and comments on the amount of gene flow, considering the significant expected variance of $F_{st}$ estimates [6], is non-informative. In addition, Constantine's comment on the ideal populations on which population genetics analysis are said to be based appear to be unfair

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