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The evolution and diversity of kinetoplastid flagellates

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Five years ago, little was known about kinetoplastid evolution. Recent improvements in the taxon sampling for nuclear rRNA genes and several protein markers have transformed this understanding. Parasitism evolved at least four times in kinetoplastids. Obligate parasitic trypanosomatids are a relatively 'derived' group within kinetoplastids; their closest relative is likely to be the free-living *Bodo saltans*, and the ancestral trypanosomatids were probably parasites of insects. Although subject to recent controversy, trypanosomes (genus *Trypanosoma*) probably constitute a monophyletic group. Several unusual features of trypanosomatid genomes (e.g. *trans*-splicing, mitochondrial RNA editing and intron poverty) are common in kinetoplastids and pre-date the adoption of parasitism. The framework of relationships is becoming robust enough for real comparative approaches to be used to understand kinetoplastid biology.

The remarkable kinetoplastids

Kinetoplastids are a remarkable group of protists. They contain a range of ubiquitous free-living species – pathogens of invertebrates, vertebrates and even some plants. *Trypanosoma* species cause sleeping sickness and Chagas disease, whereas the leishmaniases kill and debilitate hundreds of thousands of people worldwide each year. Furthermore, these morphologically rather simple unicellular organisms are masters at finding unorthodox solutions to the problems of being a eukaryotic cell. Kinetoplastid peculiarities include: (i) complex and energy-consuming mitochondrial RNA editing; (ii) a unique mitochondrial DNA architecture; (iii) *trans*-splicing of all mRNA transcripts; (iv) the arrangement of genes into giant polycistronic clusters; (v) unprecedented modifications of nucleotides; (vi) the compartmentation of glycolysis; (vii) evasion of the host immune response using a variable surface coat; and (viii) the ability to escape destruction by migrating out of phagocytic vacuoles (for recent reviews, see Refs [1–7]). There must be other oddities that await discovery, a process that must be enhanced by the wealth of genomic data now available for the medically important trypanosomatids [8].

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Comparative methods are required for understanding the evolution of the bizarre aspects of kinetoplastid biology and for determining which elements could be directly associated with a parasitic life history. Until recently, the picture of the evolutionary history of kinetoplastids was not sufficiently robust to provide a framework for comparative approaches. However, recent diversity surveys and modern phylogenetic studies with new molecular datasets have transformed the knowledge about these organisms; many pieces of the missing evolutionary and systematic framework are now falling into place (Figure 1) and are beginning to impact on the understanding of cell evolution within kinetoplastids.

The history of kinetoplastid systematics

Since the end of the 19th century, the taxonomy of parasitic kinetoplastids has been based on the presence of life-cycle stages that are distinguished by morphology, whereas free-living kinetoplastids have not been studied in a particularly sophisticated manner in the past. Because of the dearth of morphological features, only 22 genera of kinetoplastids were established before 2005 and, despite extensive sampling and the introduction of electron microscopy, few of these have been described within the past 80 years. The higher-level systematics of the group are also conservative. The taxon Kinetoplastida was created 40 years ago [9] by uniting two groups – Trypanosomatidae and Bodonidae – that were previously considered to be unrelated groups of 'protozoa'. This distinction remained unchanged in classification systems because of the absence of strong hypotheses about the exact relationship between trypanosomatids and bodonids.

In contrast to some other taxa, only limited advances were yielded in the first decade of molecular studies of kinetoplastid phylogeny. This was primarily because of an uneven sampling of species across lineages (skewed towards taxa of medical importance, in particular *Trypanosoma* and *Leishmania*) and an unusual property of the most widely used information-rich molecular marker – the nuclear small subunit (SSU) rRNA. The gene encoding SSU rRNA underwent a massive evolutionary change in the early history of kinetoplastids. As a result, phylogenetic trees that were estimated from SSU rRNA sequences had an extremely long branch that

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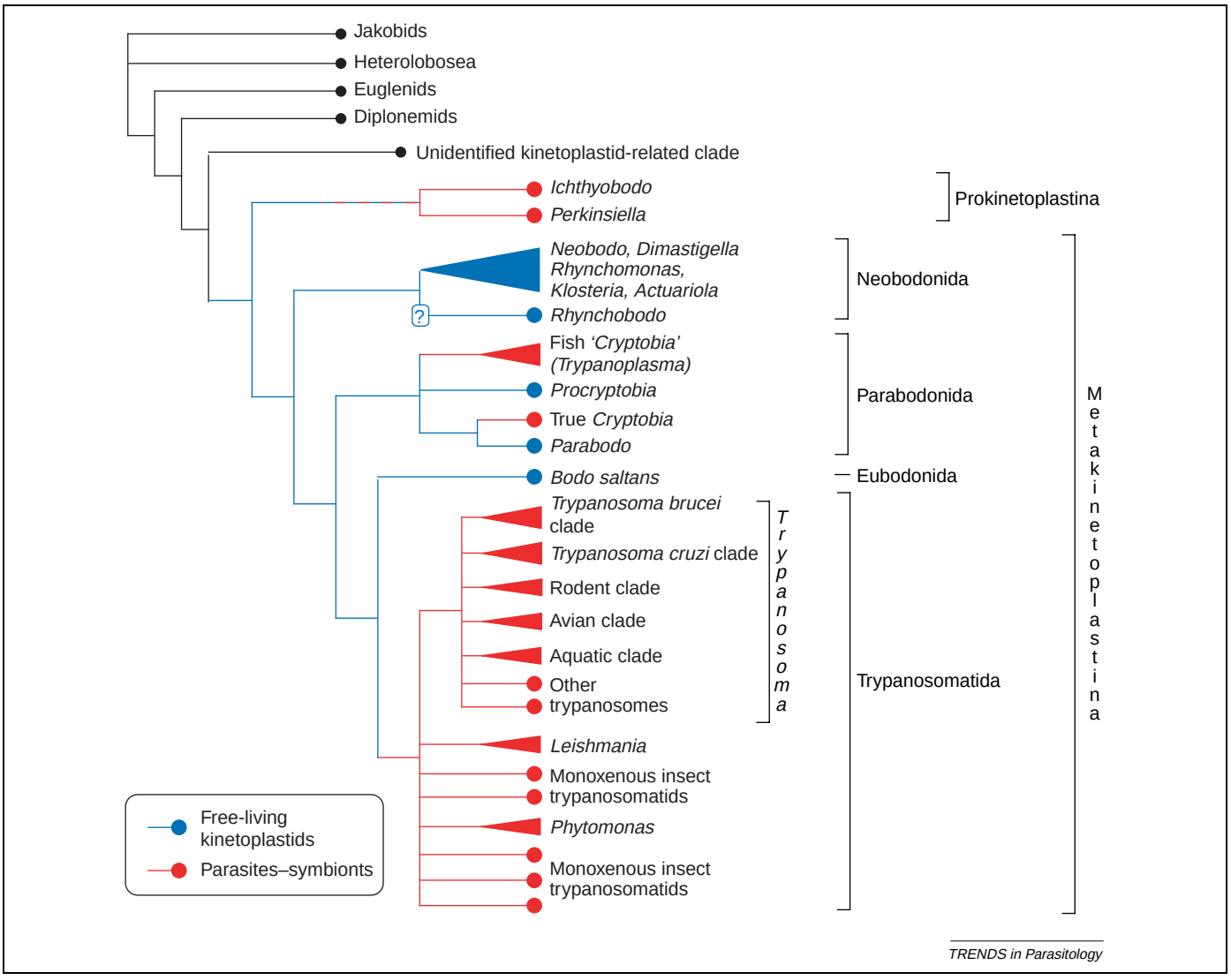

Figure 1. Evolutionary relationships among kinetoplastids as estimated by recent taxon-rich SSU rRNA gene trees and protein phylogenies. The new higher-level classification of kinetoplastids that was introduced in Ref. [26] is used. The placement of trypanosomatids as a sister group to Eubodonida follows HSP phylogenies [13,22]. The 'unidentified kinetoplastid-related clade' is known only from environmental SSU rRNA gene sequences [25]; the organismal identity is not yet known. SSU rRNA and HSP analyses differ as to whether *Rhynchobodo* forms a specific clade with other neobodonids [21]. Black lines represent branches outside the kinetoplastid group; red–blue line indicates unknown status; circles denote single or a few known representatives of a particular clade; triangles denote several known representatives of a particular clade; question mark represents unstable clade position.

connected kinetoplastids to other eukaryotes, in contrast to the relatively short deeper internal branches within kinetoplastids. This property made the correct estimation of relationships within kinetoplastids extremely difficult and has been an ongoing problem at multiple taxonomic levels.

Where did kinetoplastids come from?

Since the 1980s, kinetoplastids have been considered to be related to the euglenids, with these two protist groups together representing a distinct deep branch within the eukaryotic tree, as estimated in early SSU rRNA analyses. Rather than a deep branch, molecular phylogenies based on multiple proteins indicate that kinetoplastids and euglenids are specifically related to two different groups of protozoa, the heterolobosеid amoeboflagellates and small free-living bacterivorous flagellates called jakobids [10,11]. The distant ancestors of kinetoplastids were probably morphologically and ecologically similar to jakobids.

Within this assemblage, protein phylogenies and a shared non-canonical mitochondrial genetic code provide support for the notion, based on ultrastructural features, that the closest relatives of kinetoplastids are not euglenids but are, instead, an obscure group called diplonemids [12,13]. Diplonemids are free-living surface-associated protozoa that are occasionally reported as being facultative parasites of invertebrates [14,15]. The close relationship between kinetoplastids and diplonemids is leading to greater interest in this overlooked group.

Basal relationships

Early molecular phylogenetic studies of kinetoplastids focused on the medically important trypanosomatids, virtually ignoring the various bodonids [16]. Indeed, the first SSU rRNA studies to cover a wide diversity of bodonids emerged only in 2000. However, all bodonid sequences determined before 2002 clustered with trypanosomatids at the end of the long basal kinetoplastid

branch, and analyses gave different accounts of basal relationships within kinetoplastids, including the possibility that trypanosomatids are ancestral [17–20].

Recent studies have benefited from two important developments. First, additional informative molecular markers, namely heat-shock proteins (HSPs), have been introduced that include a broad sampling of kinetoplastid diversity but do not display a long basal branch [13,21,22]. Second, two 'new' lineages have been sampled that 'break' the long basal branch in SSU rRNA trees. The first lineage includes two kinetoplastid groups – *Ichthyobodo*, which is an ectoparasite of fish [23], and *Perkinsiella*, which is a morphologically highly reduced endosymbiont of certain amoebae [24]. The second lineage was discovered in environmental PCR surveys of the benthos surrounding deep hydrothermal vents [25]. It is unknown whether members of this lineage are kinetoplastids or another type of eukaryote (Figure 1). Both HSP phylogenies [21,22] and SSU rRNA analyses that include the new branch-breaking lineages [14,26] indicate that the main radiation of kinetoplastids consists of four primary subgroups: trypanosomatids and three clades of bodonids. Except for the placement of one bodonid (*Rhynchobodo*), the composition of these four groups is the same in the two datasets.

It is clear that trypanosomatids are descended from within bodonids and that the old systematic division of kinetoplastids into these two groups is artificial. In its stead, a new system has been proposed that divides kinetoplastids into Prokinetoplastina (*Ichthyobodo* and *Perkinsiella*) and Metakinetoplastina (other bodonids and trypanosomatids) [26]. The three clades of bodonid organisms within the Metakinetoplastina are named Neobodonida, Parabodonida and Eubodonida. The parasites and commensals assigned to the genera *Cryptobia* and *Trypanoplasma* form two separate groups within the Parabodonida, whereas *Bodo saltans* – which is the most extensively studied free-living kinetoplastid – belongs to the Eubodonida.

One key question remains controversial – which bodonid groups are the closest relatives of trypanosomatids? Many recent SSU rRNA phylogenies indicate that trypanosomatids branched-off early from other metakinetoplastids [14,26,27]. By contrast, recent HSP90 and HSP70 trees indicate, with reasonable statistical support, that trypanosomatids are nested within metakinetoplastids [22]. The well-sampled HSP90 dataset indicates that trypanosomatids are related most closely to Eubodonida, which is consistent with an earlier analysis of a partial mitochondrial gene sequence [28]. At the time of writing, the nested position of trypanosomatids is the better-supported scenario and, thus, seems more likely. This hypothesis highlights *B. saltans* (the only confirmed eubodonid) as being a key organism for understanding the evolution of trypanosomatids.

Trypanosomatid phylogeny

Early rRNA phylogenies using few taxa and simple phylogenetic methods of analysis unexpectedly recovered the genus *Trypanosoma* as being a paraphyletic grade at the base of trypanosomatids rather than being a natural group. By contrast, subsequent improved analyses of SSU rRNA data and preliminary examinations of protein markers usually indicated that *Trypanosoma* is monophyletic [29–32]. This debate has recently been reopened, with analyses of taxon-rich sets of SSU rRNA gene sequences providing support for both paraphyly [18,33] and monophyly [34] of trypanosomes. Nonetheless, in light of recent re-analyses of SSU rRNA datasets and the emergence of well-sampled protein datasets [22,34,35], the evidence for monophyly of *Trypanosoma* species now seems to be stronger and the debate seems to be closed again (Box 1).

Importantly, the reconfirmation of trypanosome monophyly identifies key phylogenetic groupings within the genus *Trypanosoma* [32,34] that are supported by information from several independent sources. Most *Trypanosoma* species that are studied using molecular means fall into a small number of clades correlated with factors such as host taxon, ecology and, especially, vector taxon. Familiar groupings include the African salivarian trypanosomes (*Trypanosoma brucei* and relatives, which are transmitted by tsetse flies) and the predominantly New World grouping of *Trypanosoma cruzi* and related species (which are transmitted by triatomine bugs). The *Trypanosoma* genus also includes a 'rodent clade' (presumably transmitted primarily by fleas), an 'avian clade' (transmitted primarily by black flies and hippoboscid flies) and an 'aquatic clade' (transmitted by leeches), although the robustness of such groupings require further testing as more potential hosts are examined. The aquatic clade highlights the interplay among hosts, ecology and vectors. Whereas other clades of *Trypanosoma* are transmitted mostly between amniotes by insect vectors, members of the aquatic clade are transmitted between fish or amphibians by aquatic leeches. However, this clade also includes trypanosomes of platypuses and aquatic tortoises [32,34,36] that are also presumed to be transmitted by leeches [35,37], thus providing evidence of host switching. Interestingly, a different trypanosome subclade seems to have switched to terrestrial leech vectors [35].

What of trypanosomatids other than the *Trypanosoma* genus? Trypanosomatids are traditionally classified into nine genera and include both monoxenous insect parasites and dixenous taxa that alternate (or are presumed to alternate) between insects and vertebrates (or plants). The investigation of these organisms in molecular studies was limited until recently, when dozens of strains were isolated from insects in two extremely different regions – Costa Rica [38] and northern Russia [39]. The analysis of several molecular markers demonstrates that none of the accepted monoxenous genera (*Crithidia*, *Blastocrithidia*, *Herpetomonas*, *Leptomonas* and *Wallaceina*) is monophyletic; only the dixenous *Leishmania* and (presumed) dixenous *Phytomonas* seem to be natural groups (the vertebrate-infecting *Endotrypanum* is represented by a single sequence that is closely related to *Leishmania*). This pattern is striking because *Leishmania* and *Phytomonas* have been sampled more extensively than the other genera and it is unlikely that either is an undetected polyphyletic group. *Phytomonas* and *Leishmania–Endotrypanum* emerge separately from within the monoxenous taxa.

Box 1. Are trypanosomes monophyletic? A controversy resolved (again)

Because of their medical importance, trypanosomatids – especially members of the genus *Trypanosoma* – are the most-extensively studied kinetoplastids. An understanding of their evolution is of both applied and intrinsic scientific interest. However, it will be important to determine whether trypanosomes constitute a single monophyletic group.

Since 1997, several studies of SSU rRNA genes have supported the idea that trypanosomatids are monophyletic [21,29,31,32,43,64], as have nearly all phylogenetic analyses of protein-coding genes [30,65–68] that have been performed to address this issue. Recently, however, Hughes and Piontkivska [18,33] contended that previous SSU rRNA analyses had examined an inadequate or biased taxon selection within kinetoplastids and/or that the analyses did not include appropriate outgroups. They presented re-analyses of SSU rRNA data in which trypanosomes (and trypanosomatids) always seemed to be paraphyletic; in their first analysis [18], in particular, *Trypanosoma vivax* appeared outside the main group of trypanosomes when some methods were used. The authors also suggested that existing protein gene phylogenies that support trypanosome monophyly are too taxon-poor to be reliable [19,33].

After the analysis by Hughes and Piontkivska in 2003 [19], Hamilton *et al.* [34] undertook the first extensive protein-coding gene-based phylogenetic study of trypanosomatids (including 37 trypanosomes), using sequences of the glycosomal form of GAPDH (gGAPDH). Meanwhile, Simpson *et al.* [22] assembled an HSP90 dataset with a broad sampling of kinetoplastids. Using outgroups that included euglenids, both analyses supported trypanosome monophyly with high statistical support. The gGAPDH analyses provided strong support for the placement of *T. vivax* with other African salivarian trypanosomes (e.g. *Trypanosoma brucei*), which is consistent with other characteristics – including antigenic variation [69,70] – and several previous rRNA studies [29,31,32,43]. Wherever tested, the results were robust, regardless of the analysis method and whether nucleotides or amino acids were analysed.

Hamilton *et al.* [34] also evaluated the effects of different outgroups, methodologies and alignment on SSU rRNA analyses. Alignment and taxon selection had a major effect on the phylogenetic trees recovered. The first study by Hughes and Piontkivska [18] is notable because, by using only two closely related euglenids as outgroups (excluding, for example, diplonemids), the long branch between kinetoplastids and the outgroup is exaggerated, presumably favouring long-branch attraction between the outgroup and rapidly evolving ingroup taxa. A more recent analysis that includes many outgroups to metakinetoplastids [33] can be reassessed after structural issues with the alignment are resolved. Indeed, *T. vivax* (previously identified as rapidly evolving) branches outside the salivarian trypanosomes only when euglenid outgroups are used [34]. Although the criticisms by Hughes and Piontkivska of previous rRNA studies were reasonable, their analyses and alignments showing trypanosome paraphyly are questionable* and weigh only lightly against the now considerable evidence from a range of other data that supports trypanosome monophyly, including both ribosomal and protein-coding sequences.

* The validity of several of the 'multiple' bodonid outgroup taxa crucial to the most recent analysis by Piontkivska and Hughes [33] is also open to debate; other studies indicate that these taxa are variants of the same morphospecies [14].

Some trypanosomatids that are traditionally assigned to the genera *Crithidia*, *Blastocrithidia* and *Herpetomonas* contain a single endosymbiotic bacterium in their cytoplasm that divides synchronously with the host cell and seems to be closely related to the γ-proteobacterium *Bordetella* [40]. The flagellates can be cleared of the endosymbiont by treatment with antibiotics but such aposymbiotic strains have a different profile of glycoconjugates and their interaction with insect cells and guts is impaired [41]. All endosymbiont-bearing trypanosomatids that have been studied belong to a monophyletic assemblage, which indicates that this mutualistic symbiosis happened only once and has been retained because of a selective advantage for the protist [40].

Interestingly, although SSU rRNA trees recover *Trypanosoma* as being the deepest branch within the trypanosomatids in most cases, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) phylogenies indicate that *Trypanosoma* could emerge from within the monoxenous forms [34]. The exact placement of *Trypanosoma* within trypanosomatids deserves further investigation.

Evolution of parasitism

Parasitism evolved many times within kinetoplastids. Assuming that there were no reversions to a free-living state, there were at least four independent adoptions of obligate parasitism or commensalism that involved: (i) the *Ichthyobodo–Perkinsiella* clade; (ii) fish-infecting *Cryptobia* (i.e. *Trypanoplasma*) species; (iii) 'true' *Cryptobia*, which is commensal in snails; and (iv) trypanosomatids (Figure 1). Each parasitic group has free-living relatives that are at least as closely affiliated with it as the nearest parasitic lineage; in the case of trypanosomatids, the adjacent free-living lineage is probably Eubodonida.

It has long been debated whether trypanosomatids were parasites of insects or of vertebrates ancestrally. Vertebrate-first models were supported by the idea that trypanosomatids descended from haemoparasitic cryptobiids that infected fish and by the proposed basal paraphyly of *Trypanosoma* within trypanosomatids. As discussed, recent molecular data refute both of these ideas. The insect-first model proposes that *Trypanosoma* and *Leishmania* are descended from parasites of blood-sucking insects that survived accidental transmission into a vertebrate host during feeding [42]. The origin of *Leishmania* and, possibly, *Trypanosoma* from within living insect trypanosomatids supports this hypothesis. In the insect-first scenario, trypanosomes that infect aquatic hosts and that are now transmitted by leeches are presumed to be derived, not ancestral; the existence of a distinct aquatic clade within the genus *Trypanosoma* is consistent with such a hypothesis [32,34–36,43]. If *Trypanosoma* and *Leishmania* are descended from parasites of biting insects, it is unlikely that their origins were earlier than the invasion of land by vertebrates (~370 million years ago) because aquatic vertebrates are unlikely targets of biting insects [34]. *Leishmania* species have less rRNA sequence divergence than do *Trypanosoma* species [44] but, judging by recent reports of fossils [45], they originated at least 100 million years ago.

The transmission of an insect trypanosomatid into a warm-blooded host must have occurred many times but it was successful only rarely. However, any success would have opened a large niche to the parasite because transfer to other vertebrate hosts would presumably be an easy

step; certainly, some of the more 'successful' extant parasites have relatively cosmopolitan host ranges. So far, only *Trypanosoma* and *Leishmania* (and *Endotrypanum*) have passed through this bottleneck and left surviving descendents. However, rare (or overlooked) accounts indicate that other insect trypanosomatids could be 'on the way into' vertebrate hosts [46,47], possibly as a result of changes in the 'immunological landscape'. One possible transfer was recently caught in the act: a trypanosomatid that was isolated from rats and dogs in Egypt seems to be closely related to parasites of hemipterid bugs [39]. Similarly, some *Crithidia* and *Herpetomonas* species can, at least experimentally, infect mouse dermal fibroblasts [48].

The latest molecular studies of trypanosomatids and bodonids mandate a reassessment of the diversity of these groups. Observations of several trypanosomatid species within one insect specimen and, conversely, finding the same parasite in a wide range of insect hosts over a large geographic area disprove a strict 'one host, one parasite' concept [39]. Therefore, although the idea that there is a trypanosomatid species for every insect species [49] might not be fully supported, the diversity mapped so far mostly in hemipteran and dipteran insects must represent only the tip of the iceberg [50]. Attempts to understand kinetoplastid evolution are complicated further by studies of morphology that, in the character-poor kinetoplastids, seem to be deceptive. Morphologically distinct leptomonad promastigotes that would previously have been assigned to different species are genetically identical and, thus, belong to a single species [51]. Conversely, the ubiquitous free-living morphospecies *Neobodo designis* includes organisms that are several percent dissimilar in their SSU rRNA sequences and that might have extremely different autecologies (e.g. salinity preferences) [52,53] (Box 2).

Understanding kinetoplastid genomes

Trypanosomatids have a unique mitochondrial genome architecture [5,6,20]. Their mitochondrial DNA is called kinetoplast and, as one of the largest organellar genomes, contains two classes of molecules: dozens of maxicircles and thousands of minicircles. Minicircles are circular but non-supercoiled molecules that are typically ~1 kb in size and linked together (catenated) into a network that resembles chainmail armour. Maxicircles encode most of the mitochondrial genetic information but many transcripts are extensively edited by the insertions and/or deletions of uridines: a process controlled by numerous minicircle-encoded guide RNAs [4,5].

Box 2. Outstanding questions

- What is the extent of the genetic diversity of insect trypanosomatids?
- Are trypanosomes really the basal group within trypanosomatids?
- What sort of organisms are the 'unidentified kinetoplastid-related clade'?
- Are the nuclear genomes of free-living kinetoplastids organized and transcribed like those of trypanosomatids?
- What do the giant mitochondrial genomes of bodonids encode?

How did this system evolve? Bona fide minicircles have been examined from all of the major metakinetoplastid groups [20]. RNA editing is thought to occur in all minicircle-bearing taxa because guide RNA production is the only known role of minicircles but, at present, there is no direct evidence of editing in deeper-branching kinetoplastids (i.e. Prokinetoplastina and Neobodonida). Some bodonids have large supercoiled minicircles, which indicates a more primitive condition [20]; however, these taxa all belong to one derived subgroup – the Parabodonida [21]. Thus, small open-circle minicircles are probably ancestral within metakinetoplastids. By contrast, none of the non-trypanosomatids that has been studied concatenates its minicircles into one continuous network. Thus, the network seems to be a late evolutionary development that ensures faithful replication and that could have led to the diminution of the presumably redundant mitochondrial DNA of bodonids. In trypanosomatids, the minicircle network replicates in two strikingly different ways: the kinetoplasts of *T. cruzi*, *Leishmania tarentolae* and *Crithidia fasciculata* seem to rotate during replication, whereas *T. brucei* has a stationary kinetoplast [54]. Analyses that have included additional species show that a continuum exists between both forms, represented by kinetoplasts that seem to have rotated to several different degrees (J. Lukeš, unpublished). Nothing is known about the kinetoplast of prokinetoplastids other than that it is huge and occupies a significant portion of the cell [24].

The search for the origins of the kinetoplastid mitochondrial genome structure requires analysis of the genomes of the closest relatives of this group – diplonemids and euglenids. Recent studies of diplonemids demonstrate a different, yet equally unprecedented, organization of their mitochondrial genome. It consists of dozens of circular molecules, each with only several hundred base pairs of coding sequence. Mature mRNAs are generated by *trans*-splicing several of these gene fragments together using an unknown mechanism [15]. Although the plastid (chloroplast) genome of *Euglena gracilis* was one of the first plastid genomes to be sequenced, the mitochondrial genome of this so-called model protist is recalcitrant to study [55] and only one gene fragment is known [56]. It might be that euglenid mitochondrial genomes are also aberrant and that the diversity of mitochondrial genomes in kinetoplastids, diplonemids and euglenids approaches that of all other eukaryotes combined.

The nuclear genomes of trypanosomatids are as unusual as their mitochondrial counterparts, yet are streamlined rather than complex. Because of their medical importance, several *Trypanosoma* and *Leishmania* spp. are the subjects of genome-sequencing projects [8]. The three completed 'tritryp' genomes are ~48–59% coding sequence and are almost devoid of introns [8]. Most protein-coding genes are arranged in massive polycistronic clusters such that dozens of adjacent functionally unrelated genes are co-transcribed [57]. The mRNA is cleaved into single-gene transcripts that are *trans*-spliced to small 'spliced leader' RNAs [1]. The completed genomes have a strong conservation of gene order, with many

breakpoints between (or near the ends of) co-transcribed gene clusters [8]. *Trans*-splicing of a capped spliced-leader RNA to transcripts synthesized by different RNA polymerases is important for an ambitious plan to create an expression system in *L. tarentolae* – a model trypanosomatid that grows quickly in a relatively cheap medium – that might, in several respects, be a superior system to the established bacterial- and yeast-based systems in terms of the overexpression of human proteins [58].

There are no genomic data available from non-trypanosomatid kinetoplastids [16], so the extent of polycistronic transcription in these taxa is currently unknown, although spliced-leader RNA genes are present in euglenids, diplonemids and kinetoplastids [59]. Interestingly, no spliceosomal introns have been found in the protein-coding gene sequences that have been reported for various bodonids [22], suggesting that intron poverty might be a general feature of kinetoplastids (by contrast, euglenid and diplonemid genes often contain introns [13,60]). Therefore, we propose that there was a marked streamlining of the nuclear genome early in the history of kinetoplastids. If gene order is highly conserved across kinetoplastids, these data might become a powerful tool for evolutionary inference when there is a broader sample of kinetoplastid genomes.

A case of plastid envy?

One of the most exciting discoveries in evolutionary parasitology was that of the relict plastid of Apicomplexa. It has recently been proposed that kinetoplastids also descended from an ancestor with a plastid because of the presence in trypanosomatids of genes that are similar to the plastid-associated genes of plants or cyanobacterial genes [61]. This idea is appealing given the close relationship between kinetoplastids and euglenids, with some of the latter being photosynthetic. However, the hypothesis has been criticized because of phylogenetic evidence that the euglenid plastid was acquired within euglenids [61,62] and because some of the 'plastid-like' genes are more widely distributed among eukaryotes than was first thought [63]. Recent examinations of the completed genomes of trypanosomatids, however, did not find a signature of past plastid symbiosis [8]. Thus, it seems that individual events of lateral gene transfer from multiple sources could be a better explanation of the presence of such genes in the kinetoplastid genome, the phylogeny of which deviates significantly from the rest of the genome.

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