In searching for nuclear-encoded, apicoplast-localized proteins we have cloned ferredoxin-NADP⁺ reductase from Toxoplasma gondii and a [2Fe-2S] ferredoxin from Plasmodium falciparum. This chloroplast-localized redox system has been extensively studied in photosynthetic organisms and is responsible for the electron transfer from photosystem I to NADP⁺. Besides this light-dependent reaction in nonphotosynthetic plastids (e.g. from roots), electrons can also flow in the reverse direction, from NADPH to ferredoxin, which then serves as an important reductant for various plastid-localized enzymes. These plastids possess related, but distinct, ferredoxin-NADP⁺ reductase and ferredoxin isoforms for this purpose. We provide phylogenetic evidence that the T. gondii reductase is similar to such nonphotosynthetic isoforms. Both the P. falciparum [2Fe-2S] ferredoxin and the T. gondii ferredoxin-NADP⁺ reductase possess an N-terminal bipartite transit peptide domain typical for apicoplast-localized proteins. The recombinant proteins were obtained in active form, and antibodies raised against the reductase recognized two bands on Western blots of T. gondii tachyzoite lysates, indicative of the unprocessed and native form, respectively. We propose that the role of this redox system is to provide reduced ferredoxin, which might then be used for fatty acid desaturation or other biosynthetic processes yet to be defined. Thus, the interaction of these two proteins offers an attractive target for drug intervention.

Apicomplexan parasites are a large phylum of unicellular, obligate intracellular organisms, including such medically important members as Plasmodium spp., the causative agent of malaria (1), and Toxoplasma gondii, an opportunistic pathogen of immunosuppressed individuals and a common cause of congenital disease (2). The recent description and sequencing of a cellular 35-kilobase pair DNA element in several of these parasites, and its localization to a plastid-like organelle (3–6), called the apicoplast, has received great interest for at least two reasons. First, it provides evidence for a secondary endosymbiotic event in apicomplexa whereby their common ancestor engulfed an algal organism, giving rise to the organelle’s four membranes visible by electron microscopy (for review, see Ref. 3). Phylogenetic analysis of apicoplast-encoded genes supports this hypothesis (5). Second, the plastid-like organelle offers a new and promising target for rational drug design, because it is assumed to contain a number of unique metabolic pathways not found in the mammalian hosts (7). Recent reports have shown that at least the rationale of this approach is valid and that new drugs, which are urgently needed, especially for plasmodial infections, might be developed in the near future based on these targets (7, 8).

The proteins encoded by the fully sequenced Plasmodium falciparum and T. gondii plastid genomes are either ribosomal proteins or proteins involved in the replication of the episonal genome (3, 4), but are not sufficient to constitute the whole organelle and to provide insights into its experimentally proven essential function (9). It is, therefore, obvious that nuclear-encoded genes must exist that code for apicoplast localized proteins, similar to the situation of chloroplasts and plants (10).

In search for such proteins we describe in this report the cloning and functional expression of nuclear-encoded, but apicoplast-localized, ferredoxin-NADP⁺ reductase (FNR) from T. gondii and ferredoxin (Fd) from P. falciparum. This cellular redox system has been extensively studied for decades in photosynthetic organisms (11) and is responsible for the electron transfer from photosystem I to NADP⁺ for the provision of reducing equivalents needed in the Calvin cycle for CO2 fixation according to the following reaction: 2Fdred + NADP⁺ + H⁺ → 2Fdox + NADPH.

In this reaction the small [2Fe-2S] protein ferredoxin donates its electron to the FAD cofactor of FNR, which pairs two electrons for the subsequent reduction of FNR-bound NADP⁺ to NADPH (11). Thus, the presence of this redox pair in nonphotosynthetic apicomplexan parasites was unexpected at first. However, besides the light-dependent reaction, electron flow can also take place in the reverse direction in nonphotosynthetic plastids (e.g. from roots) from NADPH to Fd, which then serves as an important reductant for various plastid-localized enzymes like nitrite reductase, sulfite reductase, glutathione S-transferase, and photosynthetic PfFd, P. falciparum ferredoxin; TgFNR, T. gondii FNR; TPS, transit peptide sequence; RT-PCR, reverse transcription polymerase chain reaction; bp, base pair(s).

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timate synthase, and lipid desaturases (12–14). These plastids possess related, but distinct, FNR and Fd isoforms for this purpose (15). We provide phylogenetic data that imply that T. gondii FNR and P. falciparum Fd are similar to nonphotosynthetic isoforms and that reduced Fd is the likely biosynthetic product of this interaction.

**EXPERIMENTAL PROCEDURES**

**Parasites**—T. gondii tachyzoites of strain RH were grown in human foreskin fibroblasts and purified by centrifugation through 3-μm Isopore membrane filters (Millipore) as described previously (16). P. falciparum (strain 3D7) schizonts and schizont stages were cultured and enriched by Gelafluinid floatation as described previously (17).

**Molecular Cloning Techniques**—DNA extraction from T. gondii was performed by acid phenol extraction in the presence of LiCl as described previously (18). DNA and RNA from P. falciparum were obtained from mixed stages after saponin lysis (19). Genomic P. falciparum DNA was isolated as described previously (19). Total RNA from 10^9 trophozoites and schizonts each was isolated with TRI reagent (Sigma) according to the manufacturer’s protocol. mRNA was isolated from 100 μg of total RNA using the Poly(A)/Tract mRNA isolation kit from Promega. The final mRNA was dissolved in 250 μl of water.

PCR amplification of the partial FNR sequence from genomic DNA was performed using Taq polymerase (Amersham Pharmacia Biotech) and 5'-GGGTTACCTGACTACCTCAACGTGCACA-3' (5484) and 5'-GGGTTACCTGACTACCTCAACGTGCACA-3' (5484) as primers. The reaction was heat-inactivated at 72 °C for 7 min and then extended for 1.5 h at 42 °C in reaction buffer containing 100 μM dNTP. The reaction was heat-inactivated at 72 °C for 7 min and then extended for 1.5 h at 42 °C in reaction buffer containing 100 μM dNTP. The reaction was heat-inactivated at 72 °C for 7 min and then extended for 1.5 h at 42 °C in reaction buffer containing 100 μM dNTP.

The GST C-terminal FNR fusion protein (GST-TgFNR) was purified from transformed E. coli BL21(DE3) (Novagen) on immobilized glutathione (Amersham Pharmacia Biotech) using the protocol of (27). For antibody production, 200 μg of GST-TgFNR emulsified in “Antibody Multiplier” adjuvant (Linaris GmbH, Bettingen, Germany) were injected three times subcutaneously into a New Zealand rabbit at an interval of 4 weeks.

NDPH-dependent diaphorase activity of FNR preparations was determined by DCPIP reduction assay according to Ref. 28. It was set up in a microtiter plate in a final volume of 100 μl as follows: 125 μM NDPH and 120 μM DCPIP were mixed in 50 μl Tris-HCl, pH 8.0, and the reaction started by the addition of 20 pmol of purified 6His-Ubi-TgFNR. The change in absorbance at 630 nm was followed over time in a microplate reader.

**Computerized Sequence Analysis**—Protein sequence alignments were performed using CLUSTAL X (29) and optimized by visual inspection. Phylogenetic tree construction was then performed using this optimized alignment and either the maximum parsimony method (PROTPARS) implemented in the PHYLIP 3.5 package (30), the neighbor-joining/distance matrix method from CLUSTAL X (with correction for multiple substitutions), or the quartet puzzling method for maximum likelihood tree building (31). The final trees were drawn using TreeView (32). Signal sequence prediction was performed using the SignalP server.

**RESULTS**

**Cloning and Genomic Organization of the T. gondii Gene Coding for Ferredoxin-NADP^+/+reductase**—Searching the T. gondii EST data base for putative apicoplast localized proteins we found a single entry among the approximately 10,000 sequences (EST zy19d02.r1) showing significant homology to the C terminus of exclusively chloroplast-localized plant-type FNR (33). To rule out the possibility that this short sequence was a cloning artifact or contamination its existence in the genome of the parasite was verified by PCR with genomic DNA isolated from purified RH strain. A single product of 400 bp was obtained, which was, 150 bp longer than the reported EST sequence. To prove that the amplicon was indeed related to EST zy19d02.r1 that and the additional bp reflect an intron, the PCR product was sequenced. The resulting sequence proved it to be the presumed EST containing exactly 150 bp of noncoding DNA, the boundaries of it conforming to the usual exon-intron junctions of eukaryotes (data not shown). This result confirmed that the single EST zy19d02.r1 was of T. gondii origin and prompted us to isolate the full-length cDNA. Using digoxigenin-labeled EST zy19d02.r1 to screen a λ ZAP

2 F. Seebauer and N. Thomsen, manuscript in preparation.

3 Please contact the corresponding author for further information.

**Apicomplexan Plant-type Fd and FNR**

4 The Lycopersicon esculentum cDNA cDNA.fasta sequence was assembled from EST sequences published in the dbest data base using the Lasergene package (DNASTAR), yielding an overlapping contig with no ambiguities in the consensus sequence (data not shown). All other sequences were taken as published in the dbest data base.
Fig. 1. Multiple alignment of 26 plant-type FNR sequences. Identical residues with TgFNR shared by more than 30% of the sequences are shaded. FAD and NAD(P(H) binding motifs are indicated by solid and broken boxes, respectively. Signature sequences are marked by dark shading. The position of the predicted signal peptide cleavage is indicated with "V" and the position of the C-terminal intron by an arrow. The following sequences (with their annotations) were used: T. gondii, GenBank™ accession number AJ242627; Volvox carteri, GenBank™ accession number U23928; Chlamydomonas reinhardtii, GenBank™ accession number U10545; Arabidopsis thaliana nps-1, GenBank™ accession number AAF19773; A. thaliana nps-2, SwissProt number CAB81081; Pisum sativum root, SwissProt number Q41014; L. esculentum, ESTs contig; Nicotiana tabacum root, SwissProt number U10545; Oryza sativa root, GenBank™ accession number D17410; O. sativa embryo, GenBank™ accession number D87547; Z. mays root, GenBank™ accession number AB035644; Z. mays leaf-2, SwissProt number AB035644; A. thaliana ps, GenBank™ accession number AL161503; Mesembryanthemum crystallinum leaf, GenBank™ accession number M25528; Spinacia oleracea leaf, GenBank™ accession number X07981; N. tabacum leaf, GenBank™ accession number Y14032; Capsicum annum ps, GenBank™ accession number AJ250375; F. sativum embryo, GenBank™ accession number M21449; Vicia faba leaf GenBank™ accession number U14095.
The N-terminal 150-aa sequence as well as the whole FNR sequence are able to target a genetically fused fluorescent reporter protein into the plastid organelle of the parasite has now been provided (58), demonstrating that the putative TgFNR sequence is an apicoplast-located protein containing a bipartite apicoplast targeting signal.

The TgFNR Protein Contains Unique Sequence Features Compared with Its Plant Counterparts—The alignment in Fig. 1 revealed a significant homology of the T. gondii sequence with its plant and algal counterparts over the entire sequence (not encompassing the putative TPS) but, not surprisingly, the most conserved residues being the binding sites for cofactors FAD and NADP(H). However, there are several differences with possibly functional consequences. Most obviously, TgFNR contains a large insertion of 31 aa (with regard to Anabaena variabilis FNR) at position 233, located immediately N-terminal to one FAD binding motif and flanked by two adjacent prolines (Fig. 1). This extension might influence the dynamics and/or accessibility of cofactor binding as well as electron transfer rates. Interestingly, the root isofoms and the green algal FNRs contain 3 to 4 aa insertions at the very same position. There are 4 additional smaller insertions in TgFNR (between 3 and 8 aa in length), one of them being again conserved in the root and green algal FNRs, which would, however, not be expected to significantly influence the overall structure of TgFNR or its function.

Another significant difference related to the 31-aa insertion point in TgFNR is the exchange of a positively charged lysine (Lys75 in the A. variabilis mature sequence, equivalent to position 212 of the whole A. variabilis sequence shown in Fig. 1), which is conserved in all species except T. gondii, the green algal and the root isofoms, against either negatively charged or uncharged residues (e.g. Ala232 in the whole T. gondii sequence in Fig. 1). Lys75 was shown by site-directed mutagenesis to be essential for Fd binding (37). In addition, there is perfect conservation of the preceding A. variabilis Lys75 (residue 209 in Fig. 1), which is also implicated in Fd contact, between all other FNRs (Fig. 1) except T. gondii, which displays again an alanine at that position. It is, however, important to note that all of these studies were done with photosynthetic active FNR and Fd (see “Discussion”). Nevertheless, these differences let us assume that differences in catalysis and/or binding of Fd might exist.

Functional Expression of TgFNR and the Size of the Native Protein—To prove that the TgFNR sequence encoded a functional enzyme, the cDNA was expressed in E. coli as a fusion protein with 6His-tagged yeast ubiquitin. Because the exact cleavage site of the transit peptide sequence from TgFNR is at present unknown (but see below), the start of the mature enzyme at residue 148 was somewhat arbitrarily chosen, taking the cleavage site of mature A. variabilis FNR as reference. It is known that the N terminus of plant FNRs is not critical for activity (38). The resulting fusion protein was found to be mostly soluble upon induction. It could be purified under native conditions by means of Ni2+-chelate affinity chromatography (Fig. 3A). NADPH-dependent diaphorase activity of the purified fusion protein by DCPIP reduction assays were conducted and showed the expected activity (Fig. 3C), which could be partially blocked by diphenylene iodonium chloride and, to a lesser extent, by iodoniunmiphenthyl HCl, two known inhibitors of FAD-dependent enzymes (39), but not by N-ethylmaleimide, a sulfhydryl-modifying agent. This inhibition profile is consist-
reactive bands of higher molecular masses is unclear, but presumably they are adducts of the monomeric form. Despite considerable differences in primary amino acid sequences, TgFNR is sufficiently conserved to react with an antibody raised against the plant enzyme.

To investigate the size of the native protein in whole lysates from RH strain tachyzoites separated by nonreducing SDS-polyacylamide gel electrophoresis, the polyclonal anti-GST-FNR serum was used in a Western blot analysis. While no specific signals were detectable with the preimmune serum, two faint bands of approximately 55 and 50 kDa were observed with the anti-GST-FNR serum (Fig. 3D). The slower migrating band at 55 kDa is in good agreement with it being the unprocessed FNR still containing the uncleaved transitpeptide, while the more prominent, faster migrating band is probably the mature form. Detection of unprocessed and processed forms of apicoplast-targeted enzymes has been described previously (33, 42). Because at present the exact cleavage site for the mature form is not known, and no N-glycosylation sites are present in the primary sequence, a size of 50 kDa would mean that the mature TgFNR is unusually large compared with all other known FNRs.

The \textit{P. falciparum} Genome Contains a Transcribed Coding Sequence for a Typical [2Fe-2S] Ferredoxin—The presence of FNR in \textit{T. gondii} infers that the intimate redox partner, ferredoxin, must be present in the parasite and would also be expected to be localized in the apicoplast. Therefore the different \textit{Plasmodium} genome data bases were searched for such a homologue (currently there is no advanced \textit{T. gondii} genome project). Indeed, a genomic fragment encoding an open reading frame of 194 aa (contained within contig MAL13_05317, obtained from The Sanger Center website) was identified (starting at aa 97, corresponding to residue 1 in Fig. 4). It shows a predicted signal peptide cleavage site between Thr19 and Tyr20 and contains 16% of Ser (9) and Thr (3) residues and 18% of basic aa in its putative 76-aa transit peptide (data not shown). All four Cys required for [2Fe-2S] cluster formation are conserved (41) as well as most of the residues implicated in the direct contact with plant or cyanobacterial FNR (42, 43). RT-PCR with PfFd-specific primers proved that it is transcribed in the parasite (see Fig. 6B). PfFd starting from nt 108 was expressed in the same way as TgFNR as a fusion with 6His-ubiquitin, however, in the presence of Ub1 from yeast, which leads to cotranslational cleavage of the ubiquitin moiety (25), resulting in the release of mature PfFd. Native gel electrophoresis of such cultures and subsequent staining with iron-specific stains showed that PfFd contained bound iron, indicating that it has an assembled iron-sulfur cluster (data not shown).

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\footnotesize{4 A. Aliverti and G. Zanetti, personal communication.}
Phylogenetic Relationship between TgFNR and Other Plant-type Isoforms—Phylogenetic analysis of all FNR sequences found in various data bases corroborated the closer relationship of TgFNR to the green algal and root isozymes compared with the other FNRs from photosynthetic active plastids (termed ps and n-ps in the following sections for photosynthetic and non-photosynthetic).

For construction of the phylogenetic trees shown in Fig. 5 the sequence alignment starting with aa 138 of T. gondii (see Fig. 1) was chosen, assuming that the conserved block in the root isozymes denotes the processing site of the mature form (15). However, similar results were obtained when the sequences starting at the first FAD binding motif (Arg264 of T. gondii), leaving out the 31-aa insertion, were compared (data not shown). From this analysis an interesting aspect emerged. Although the topology of the inferred trees are somewhat different with the three methods used, they all lead to the same clustering of T. gondii, green algal and n-ps FNRs (Fig. 5, shaded parts). Although it is not surprising that TgFNR is most similar to green algal FNRs (Chlamydomonas and Volvox), it is intriguing to note that TgFNR seems to be closer related to n-ps FNRs from roots than to the cyanobacterial proteins. Clustering of n-ps FNRs with the two green algae has been noted earlier (33) but is substantiated here by including 13 new FNR sequences, adding additional weight to the analysis. Likewise, the n-ps plant isofoms are more similar to TgFNR than they are to their respective counterparts from the photosynthetic active organelles. In this respect it is also worthwhile to consider the above mentioned two insertions common only to T. gondii, the n-ps and green algal FNR as sequence signatures (44), being remnants of a common ancestor. This notion is corroborated by the following findings. Searching the data bases for a P. falciparum FNR homologue revealed only two unfinished, overlapping DNA sequences, resulting in the 213-aa-long open reading frame shown in Fig. 6A, covering the region of TgFNR aa 145–373. It possesses significant differences compared with the T. gondii enzyme, and even more so to the other FNRs, but good conservation in the FAD and NADP(H) binding motifs present in this partial sequence. More important, however, is the fact that it shows perfect length conservation of the 31-aa insertion. Because other FAD and NADP(H) binding enzymes could also have similar cofactor binding motifs (with cytochrome P450 reductase and adrenodoxin reductase the most relevant proteins in this respect), we searched the data bases for these two and found very good matches for them, which are, however, clearly distinct from the PfFNR-like sequence shown in Fig. 6A, and without the 31-aa insertion at the relevant sites (data not shown). While at present we do not know if this FNR-like polypeptide belongs to a functional enzyme in P. falciparum, its gene is actively transcribed in the parasite as shown in Fig. 6B. RT-PCR yielded a specific band of identical size to the genomic product (460 bp, lane 6) in the presence, but not in the absence, of mRNA (lanes 4 and 5). As control served Fd, which also gave a mRNA-dependent signal (lanes 1 and 2) with respective primers being of the same size as the genomic amplicon (310 bp, lane 3).

Phylogenetic analysis of PfFd and a subset of 34 plant-type Fds representing all classes of organisms for which these sequences are available indicated that PfFd, like TgFNR, showed a tendency to cluster with n-ps proteins, albeit less signifi-

Fig. 5. Phylogenetic analysis of all 26 FNR sequences shown in Fig. 1. The trees were obtained either by distance matrix/neighbor joining (DM), maximum parsimony (MP), or maximum likelihood/quartet puzzling (ML) methods. Clustering of TgFNR with algal and n-ps FNRs is shaded.
cantly than in the case of TgFNR (data not shown).

We have presented data that show that apicomplexan parasites possess genes for plant-type FNR as well as for its intimate redox partner, Fd. The rationale for the initial cloning of the gene corresponding to a T. gondii EST with similarity to the C terminus of plant-type FNRs was that in plants this enzyme is found exclusively in plastids. Thus, our assumption was that TgFNR should be localized in the apicoplast. Indeed, both TgFNR and PfFd possess a N-terminal bipartite targeting domain, a prerequisite for transporting a protein to the apicoplast (36, 45), and which are sufficient to target a GFP reporter into it (58). Both TPS show an overall net positive charge and a high proportion of Ser and Thr. Enrichment for these residues is a consistent finding within plant TPS (46). However, in contrast to T. gondii, the plasmoidal proteins examined by Waller et al. (36) did not show a high content of hydroxylated residues, and a recent study in P. falciparum confirmed that the only two Thr present in the TPS are dispensable for proper targeting of a FabH TPS-GFP fusion to the plastid of this parasite (45). This contrasts with the Fd TPS, having 16% of Ser (9) and Thr (3) residues and 18% of basic aa in its putative 76-aa transit peptide. Thus, despite overall functional similarity, which has been reported to allow experimental swapping of plastid-targeting domains between T. gondii and P. falciparum (3), it is possible that “fine tuning” of the targeting or processing requires different physico-chemical properties of the transit peptide for different proteins in the two organisms.

In contrast to P. falciparum Fd, which shows no unusual sequence features compared with plant-type Fds, the TgFNR sequence is distinct in several respects compared with all other known FNRs, possibly with functional consequences. The most prominent difference is the large insertion of 31 aa, located immediately N-terminal of the FAD binding site, and the replacement of the positively charged Lys, which is conserved in all species except T. gondii, the green algal and the root isoforms. Lys$^{76}$ was shown by site-directed mutagenesis to be essential for Fd binding (37), whereby neutral residues showed a 50–100-fold increase in $K_{b}$ for Fd, and charge reversal led to total loss of Fd binding. It is important to note that all of these studies were done with FNR and Fd isoproteins involved in the photochemical pathway (i.e oxidation of Fd) and that the isoforms from n-ps tissues also do not possess this Lys residue. Only recently has there been interest in the biochemical and structural differences between ps and n-ps FNR isoforms (15, 47), and T. gondii, because of its ease of genetic manipulation (16), might offer an attractive in vitro system to study the functional consequences of this and other crucial FNR residues by mutational analysis.

Although in most computational phylogenetic analyses amino acid insertions or deletions (indels) are ignored, they can, on the other hand, be very informative provided they are flanked by conserved regions, thus ensuring that they are not alignment artifacts (for review, see Ref. 44). Assuming that during evolution such “sequence signatures” are introduced only once and are unlikely the result of independent mutational events in already separated lineages, both the 31-aa insertion in TgFNR and the PfFNR-like protein, and the presumed remnants thereof in the green algal and root FNRs, as well as the 3-aa insertion at position 298 (Fig. 1) can be regarded as such sequence signatures, being remnants of a common ancestor. The differences in length for the 31-aa insertion between T. gondii (P. falciparum) and the others (31 versus 4 and 3 aa, respectively) might reflect the shaping of this region over time in the different organisms by adaptations of the enzymes to their physiological roles in the different plastids. Is the phylogenetic clustering of T. gondii, green algal, and root FNRs helpful for inferring the events leading to the diversification of ps and n-ps FNRs? It has been proposed that an ancient duplication event of the FNR gene prior to the separation of algal and plant groups, followed by the subsequent loss of the ps FNR in the algal lineage, led to the observed clustering (33). Assuming that TgFNR is a single copy gene (see Fig. 2), either the ancestor giving rise to the apicoplast was an alga that had already lost its ps FNR gene, or ps FNR gene loss must have occurred twice independently. Both scenarios are possible, as evidence for independent gene loss during transfer of apicoplast genes to the nucleus has been provided (10), and the debate about the nature of the secondary endosymbiont (red or green alga) giving rise to the apicoplast is still ongoing (3, 48). In this regard cloning of more algal FNRs, especially from red algae for which no sequences are available yet, could be very informative.

Plant-type FNRs/Fd are often referred to as the functional homologous of animal adrenodoxin reductases (AdxR) and adrenodoxin (Adx) (33) and can even substitute them in some in vitro assays, albeit much less efficiently (49). The P. falciparum data bases contain two sequences with significant homology to AdxR and Adx (data not shown). AdxRs are FAD-containing, NADPH-dependent enzymes intimately connected to the mitochondrial cytochrome P450 electron transfer systems and, in mammals, are involved in the biosynthesis of steroid hormones (50). Adxs are small Fd-related iron-sulfur proteins functioning as an electron carrier between AdxR and cytochromes P450 (51). Although the functionality of these two proteins in P. falciparum has still to be determined experimentally, it seems likely that in plants and apicomplexan parasites FNR/Fd and AdxR/Adx fulfill different tasks. This assumption is corroborated by the presence of homologues of both AdxR and Adx in the genome of Arabidopsis and the recent description of a yeast Adx (YAH1) being an essential component of the mitochondrial

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5 B. Striepen, personal communication.
iron-sulfur cluster assembling machinery (52), implying that AdxR/Adx are vital proteins for all eukaryotes, but whose physiological function is distinct from that of FNR/Fd.

Since there is no reason to believe that T. gondii or other apicomplexa have retained photosynthetic functions after acquiring the plastid precursor (3), and because of the close relationship between TgFNR and n-ps FNRs, it seems reasonable to suggest that the major physiological role of FNR in the apicoplast lies in the provision of reduced Fd for processes yet to be defined. In this regard it is interesting to note that in addition to PfFd and the PfIFNR-like sequence, the Plasma
dium genome data bases contain additional sequences corresponding to proteins with functional relations to FNR/Fd: (i) a partial sequence for a α-de
saturase, although sequence informa
tion from the N terminus is not available yet to determine whether it contains a putative TPS, and Fd (as opposed to NADPH) dependence is hard to infer from sequence data alone (13), and (ii) a complete sequence for glutamate synthase (Gen
dBank™ accession number Y17045). However, the encoded pro
ctein is clearly of the NADPH-dependent type, characterized by the lack of a 17-aa insertion typical for ferredoxin-dependent en
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crines are known to have a punctate targeting pattern. The exact bio
chemical role of this prototype of a plant electron transport system in protists needs to be determined. Nevertheless, it is reasonable to suggest, based on the presented data, that its main task lies in the provision of reduced Fd, whose most obvious function might be its involvement in fatty acid desaturation by Fd-de
dependent desaturases (54, 55). This would certainly be an es
tial function in the parasite, given the great number of mem
briels that it has to build up and to maintain under different environ
tmental conditions (vacuolar membrane, three-laminar mem
tes, various organellar membranes, plus the four plastid mem
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certainties about the manuscript. The sequence data for F. falci

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temperatures.

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