Parasite plastids: maintenance and functions


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Malaria and related parasites retain a vestigial, but biosynthetically active, plastid organelle acquired far back in evolution from a red algal cell. The organelle appears to be essential for parasite transmission from cell to cell and carries the smallest known plastid genome. Why has this genome been retained? The genes it carries seem to be dedicated to the expression of just two ‘housekeeping’ genes. We speculate that one of these, called \( \text{ycf24} \) in plants and \( \text{supB} \) in bacteria, is tied to an essential ‘dark’ reaction of the organelle—fatty acid biosynthesis. ‘Ball-park’ clues to the function of bacterial \( \text{sup} \) genes have emerged only recently and point to the areas of iron homeostasis, [Fe–S] cluster formation and oxidative stress. We present experimental evidence for a physical interaction between \( \text{SuB} \) and its putative partner \( \text{SuC} \) (\( \text{ycf16} \)). In both malaria and plants, \( \text{SuC} \) is encoded in the nucleus and specifies an ATPase that is imported into the plastid.

Keywords: apicomplexa; plastid function; SufB; SufC

1. INTRODUCTION

Until recently, secondary symbiosis played an unsuspected part in the evolution of apicomplexan parasites such as malaria. A sea change in the comparative functional genomics of these parasites came with the discovery of an extrachromosomal plastid DNA in the phylum (Wilson & Williamson 1997). This genome is carried in a vestigial multi-membraned plastid organelle (McFadden & Waller 1997; Kohler et al. 1997), strongly suspected to be of red algal origin and acquired by secondary endosymbiosis (Wilson et al. 1994; Fast et al. 2001). A number of laboratories, notably those of G. McFadden (Melbourne) and D. Roos (Philadelphia), have shown that the primary functions of the plastid are controlled by ‘hundreds’ of nuclear genes whose products are imported into the organelle (Waller et al. 1998; Roos et al. 1999). Using a new algorithm to PATS, it was recently estimated that ca. 8% of \( \text{Plasmodium falciparum} \) nuclear genes specify proteins destined for import into the plastid organelle (Zuegge et al. 2001). Biosynthetic pathways for type II (bacterial) FA biosynthesis (Waller et al. 1998; McLeod et al. 2001) and isoprenoid biosynthesis (Jomaa et al. 1999; Weisner et al. 2000; Rohdich et al. 2001) are localized to the plastid. There is preliminary evidence that some aspects of haem biosynthesis might also take place there (Sato & Wilson 2002). Genetic work, coupled with inhibitory studies with antibiotics, indicates that the parasite can grow and divide without a fully functional plastid, but the organelle plays an essential part when parasites establish themselves in a newly invaded host cell (Fichera & Roos 1997; He et al. 2001; Camps et al. 2002).

2. THE RELICT PLASTID GENOME

Too few species of apicomplexan parasites have been examined for one to be dogmatic about the level of conservation of the vestigial plastid genome, but the examples available show considerable similarity in gene content and arrangement. In each case, the genome is pared down to vanishing point in terms of size and complexity. Why is it maintained at all when the primary functions are controlled from the nucleus? This question echoes earlier exhaustive discussions of why chloroplasts and mitochondrial organelles in general retain relics of their eubacterial genomes (Palmer 1997; Race et al. 1999). Initial explanations resting on hydrophobicity of certain organellar proteins, or their possible toxicity in the cytosol, have been discarded in favour of the central theme of this meeting—maintenance of redox balance in bioenergetic membranes (Allen 1993a,b; Allen & Raven 1996). But can retention of the vestigial genome of the non-photosynthetic secondarily acquired plastid of apicomplexan parasites also be explained in this way?

At first glance, the issue seems readily resolvable as the parasite plastid genome teeters on the edge of oblivion—a mere 35 kb of DNA in \( \text{P. falciparum} \) carrying approximately 60 genes, only two of which specify recognizable proteins outside the machinery of protein synthesis (Wilson et al. 1996). Of these two proteins, one hardly fits the criteria we are looking for—ClpC is a regulatory ATPase of the Hsp100 family. It is typical of plastids, probably with a role in the import and/or folding of imported proteins (Nielsen et al. 1997). This only leaves for consideration the ORF of unknown function specifying a putative protein of ca. 55 kDa, designated ORF470, in \( \text{P. falciparum} \) (Wilson et al. 1996).

In an effort to find clues to the function of this gene, we first linked it (without illumination) to an orthologue of unknown function in red algal plastids (\( \text{ycf24} \)).
(Williamson et al. 1994). However, more recently we realized that it corresponds to a bacterial orthologue known as sufB (Ellis et al. 2001). In its most complete form the suf operon comprises six genes (sufA, -B, -C, -D, -S, -E) (Patzer & Hantke 1999). Intriguingly, deletion experiments in bacteria suggest that sufC, sufD and sufS have roles in iron homeostasis/oxidative stress/[Fe–S] cluster formation (Patzer & Hantke 1999; Nachin et al. 2001). Deletion or disruption of these individual members of the suf operon resulted in an increased concentration of intracellular iron, hypersensitivity to oxidative stress, and altered responses dependent on the [Fe–S] cluster regulatory protein SoxR (Hidalgo et al. 1997). A complementation study using isc mutants of Escherichia coli confirmed that the suf operon participates in an isc-independent minor pathway for the assembly of [Fe–S] clusters (Takahashi & Tokumoto 2002).

Genomic transcriptional analysis of E. coli, following exposure to H$_2$O$_2$, showed prominent upregulation of sufA, sufB and sufC amongst other genes well known for their role in the oxidative stress response (Zheng et al. 2001). At the National Institute for Medical Research, we have found a similar upregulation of orthologous genes in the pathogen Mycobacterium tuberculosis after treatment with cumene hydroperoxide (figure 1). This bacterium encounters oxidative stress during phagocytosis by the macrophage, and following macrophage activation during an acquired immune response, but it appears to have a different mechanism of regulating the response to oxidative stress since it lacks soxS/soxR and also the oxyR regulatory systems of E. coli. The suf genes and their function have therefore been conserved in quite disparate species of bacteria occupying greatly different ecological niches.

Could expression of sufB in the apicomplexan plastid be tied to an oxidative response mechanism and explain retention of the vestigial genome?

### 3. Suf PROTEINS

From the distribution of suf genes across approximately 40 completed bacterial genomes, Ellis et al. (2001) noted that only sufB and sufC invariably occur together. This seems to be true of red algal genomes as well (Kowallik et al. 1995; Douglas & Penny 1999). In P. falciparum, sufB stands alone on the plastid genome, and sufC (ycf16), with a putative plastid targeting sequence, is carried on chromosome 14. Genes for other members of the suf operon (sufA, -D, -S) also occur in the Plasmodium genomic databases, some with, and others without, putative plastid targeting sequences. Our investigations, to date, have been limited to the possibility that the products of sufB and sufC might interact in the organelle.

SufB is annotated in the databases as the ‘membrane subunit of an ABC type transporter’. Whether this is its actual function in bacteria or plastids remains unclear. Several authors have noted that secondary structure predictions do not suggest that SufB is an integral membrane protein (e.g. Patzer & Hantke 1999). No other structure in the protein databases relates clearly to it (a nuisance or a challenge depending on how you look at it). The amino-acid sequence of the C-terminal half is conserved relatively well across orthologues (figure 2) and is predicted to form an extensive set of short $\alpha$-strands followed by three small terminal $\alpha$-helices. This kind of structure can be found in $\beta$-solenoid proteins (Kobe & Kajava 2000), but we have found no repeat motifs that would support this sort of model for SufB. In the realm of transporters, the membrane segments of porins are formed from $\beta$-barrels. Whilst these are still difficult to predict, a new algorithm (Wimley 2002) applied to the genomic database of E. coli predicts that none of the Suf proteins comprise $\beta$-barrels.

Turning to SufC, both the sequence and predicted secondary structure of SufC are strikingly similar to those of HisP, the ATP-binding subunit of the bacterial histidine permease. This encouraged us to examine whether the permease complex HisJ-QMP2 (Ames et al. 2001) would serve as a model for SufC and its partner subunits. Attempts to overexpress recombinant forms of SufC from P. falciparum and E. coli were foiled by the insolubility of the expressed proteins. However, we successfully exploited the stability of SufC from the thermophilic bacterium Thermotoga maritima to produce a soluble monomeric His-tagged recombinant protein (Rangachari et al. 2002). Use of this protein as a model for other SufC orthologues can be justified by the high level of conservation between them, though there are undoubtedly other limitations related to the physiological adaptations of the thermophilic version of the protein (e.g. in the kinetics of hydrolysis; J. Eccleston, unpublished data). Based on HisP...
Figure 2. Alignment of orthologues of SufB with secondary structures predictions using PHD (Rost et al. 1994). α-helices (dark grey), β-strand (light grey). The Plasmodium falciparum sequence (Swiss Protein identifier p470.FPLA) was aligned with Cyanophora paradoxa (YC24.FCYAP), Synechocystis spp. (YO74.FSYNY), Odontella sinensis (YC24.FODOS), Porphyra purpurea (YC24.PORP), Guillardia theta (YC24.GUIT), Cyanidium caldarum (YC24.CYAC) Escherichia coli (SufB.FECOL).

from Salmonella typhimurium, which was solved to a resolution of 1.5 Å (Hung et al. 1998), we built a model of SufC from T. maritima (Nelson et al. 1999). The structure of HisP was obtained from the Protein Data Bank (Sussman et al. 1998) and the modelling software used was QUANTA96 running on a Silicon Graphics INDIGO2 workstation under the Unix operating system. The alignment between SufC and HisP ... of the template structure and its sequence similarity to SufC give us a high degree of confidence in the model.

4. PROPERTIES OF RECOMBINANT SufB AND SufC PROTEINS

The recombinant version of SufC from T. maritima hydrolyses ATP constitutively in vitro, whereas SufB does not (Rangachari et al. 2002). These data suggest that SufC could act like a typical ABC-transporter ATPase. But with what partner subunits does it associate? Overexpression of SufB from T. maritima gave an insoluble His-tagged protein that was solubilized in 6 M urea, purified, and refolded after dialysis. Although soluble, the protein revealed oligomeric structures when examined by analytical ultracentrifugation and electron microscopy. Whether this was due to misfolding or to some other property of the molecule is, at present, unclear. Despite this complication, we have measured interactions between the T. maritima forms of SufC and SufB in several ways.

First, we carried out stopped-flow fluorescence-anisotropy experiments with a fluorescent analogue of ATP, mantATP (Jameson & Eccleston 1997). As shown in figure 3a, the binding of mantATP to SufC resulted in an increase in fluorescence anisotropy to approximately 0.09 after 50 s. This increase is expected since the rotational motion of the fluorophore would decrease on binding to the protein. However, the data did not show a simple second-order binding process since they do not fit well to a single exponential process expected in these pseudo-first-order conditions. This indicates a more complex binding mechanism. In the presence of SufB alone, there was a negligible increase in fluorescence anisotropy. When the experiment was repeated using both SufC and SufB in solution (figure 3b) the anisotropy increased to about 0.14 after 50 s. This suggests mantATP is binding to a complex of SufB and SufC. This was further confirmed by stopped-flow fluorescence experiments with a fluorescent analogue of ATP (Jameson & Eccleston 1997). As shown in figure 4, when a solution of SufC was titrated into a solution of SufB, the data showed that the binding of the

two proteins is an exothermic process. When fitted to a model in which SufC and SufB bind in a stoichiometric manner the data gave a $K_d$ for the interaction of 2.1 $\mu$M.

In a separate approach, we found that SufB’s interaction with SufC partially protects both proteins from certain conditions of tryptic digestion. Conditions that cleaved SufC or SufB individually into small fragments yielded two large fragments of SufC when the two proteins were premixed (see A and B in figure 5). An irrelevant protein (bovine serum albumin) mixed with SufC was not protective. N-terminal sequencing and size determination of the tryptic fragments A and B indicated that cleavage was confined to the extreme C-terminus of SufC—the C-terminal His-tag was removed at one cleavage site and the protein was clipped 36 amino acids in from the C-terminus at the other (see figure 7). Reference to the modelled structure of SufC shows an arginine residue at this last position (B in figure 8). This would be exposed in the monomeric state of the recombinant protein found in ultracentrifugation experiments (Rangachari et al. 2002). Minor differences in bands evident in figure 5, due to the presence or absence of ATP, have yet to be investigated.

Mixing SufB with SufC, however, also protected two internal cleavage sites of SufC that would otherwise give rise to a pair of small fragments (C and D in figure 5). These fragments contain individually the Walker A and B sequences, signatures of an ATP-binding site (Walker et al. 1982). The results of the various tryptic digests are summarized in diagrammatic form in figure 7.

Assuming SufC has a membrane disposition like that depicted for HisP, that is, it is partially inserted (Hung et al. 1998), our model shows that the protected cleavage sites lie on the outer membrane face of SufC. One cleavage site (D in figure 8) lies at the distal end of arm II (in the centre of the ABC signature sequence) whilst the other (C in figure 8) is in the region where arm I bends into arm II above the ATP-binding pocket. Thus, SufB binds across the ‘outer’ face of arm II of SufC in vitro and not at the end of arm II or in the ATP-binding pocket formed with arm I.

Turning to SufB, when it was mixed in increasing proportion to SufC, two fragments (12 and 15 kDa) were protected from further tryptic digestion (E and F in figure 6). N-terminal sequencing showed these two fragments span most of the region comprising $\beta$-strands of SufB (figure 7). We infer that this part of the molecule interacts with SufC rather than the termini.

These preliminary data suggest SufB does not interact with SufC as the two lobes of HisJ bind to HisP (Liu et
5. Fe–S CLUSTER FORMATION/OXIDATIVE STRESS

In returning to a consideration of the potential role of Suf proteins in [Fe–S] cluster formation and oxidative stress, the criteria demanded by Race et al. (1999) for retention of organellar genomes are worth recalling. These criteria are: (i) the presence of organellar ribosomal machinery to enable rapid synthesis of a protein subject to redox balance; (ii) redox regulation of the protein’s transcription; and (iii) the presence of membrane components that sense redox poise and activate the transcriptional control mechanism.

The first requirement is met by the vestigial genome of apicomplexans, as it is almost entirely dedicated to specifying components of the protein expression machinery: components not encoded on the organellar genome, such as the ribosomal protein S9, are specified in the nucleus and the product is imported into the organelle (Waller et al. 1998). The second requirement, redox regulation of expression, is met for Suf proteins in bacteria, as mentioned above, but no information is known to the authors concerning such regulation of Suf proteins in plastids, let alone in non-photosynthetic plastids.

We can still say little about plastid processes in malaria that are likely to involve iron dependency or redox sensitivity. One interesting protein likely to be in this category is the enzyme peptidyl deformylase that modifies N-terminal methionine residues of newly synthesized proteins (Meinnel 2000; Kumar et al. 2002). This enzyme has a catalytic Fe²⁺ ion that is highly sensitive to oxidation (Rajagopalan & Pei 1998). A role for plastidic Suf proteins in the maintenance of iron homeostasis also deserves consideration. Indications for this option can be inferred from a deletion (Δ) mutant of sufB (referred to as ΔatABC1) in Arabidopsis thaliana (Moller et al. 2001). This mutant accumulated the haem/chlorophyll precursor PPIX, the defect being ascribed to disrupted transport of PPIX between the plastid and cytosol. We suggested that a disturbance of iron homeostasis and coordinated haem biosynthesis might be an alternative explanation, because mutants of Bradyrhizob...
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Figure 7. Cleavage sites for the various tryptic fragments of SufB and SufC from Thermotoga maritima are indicated on the sequences as well as in diagrammatic form. Block sizes for SufB are scaled to one-half of those for SufC. Predicted α-helices (black), β-strand (light grey).

bium japonicum with deregulated iron control of haem biosynthesis also accumulate PPIX when iron is limiting (Hamza et al. 1998). However, a final explanation for the phenotype of the \(D\)\(_{sufB}\) mutant in \(Arabidopsis\) still has to be established. Whilst some stages of \(de novo\) haem biosynthesis might take place in the parasite plastid (Sato & Wilson 2002; S. Sato, unpublished data), as in plants without chlorophyll (Howe & Smith 1991), we feel this is unlikely to involve Suf proteins for the following reasons. Although three of the newly discovered \(Plasmodium\) genes for haem biosynthesis encode enzymes sequential in the pathway (ALAD, PBGD and UROD) and appear to have N-terminal plastid targeting presequences (\(p \leq 0.9\) as judged by PATS), the last three enzymes in the pathway (CPO, PRO and FC) are likely to be cytosolic or mitochondrial. Since iron chelation is the final step of the pathway, this obviates a role for plastidic Suf proteins. Moreover, whilst \(hemB\), the gene encoding δ-aminolevulinic acid dehydratase in bacteria, is regulated by the availability of iron (Chauhan et al. 1997), this gene is nuclear in \(Plasmodium\) spp., ruling out a regulatory role for plastidic Suf proteins in this case.

In the context of redox reactions, perhaps the most interesting potential role for plastidic Suf proteins stems from the report that a ‘dark’ isoform of ferredoxin (Fd III) and a non-photosynthetic, plant-like ferredoxin NADP\(^+\)-reductase are imported into the plastid of apicomplexans (Vollmer et al. 2001). We suggest that Suf proteins might have a role in the formation of [Fe–S] clusters for conversion of imported apoferredoxin to the holoprotein (Ellis et al. 2001). This speculation built on the proposal by Vollmer et al. (2001) that a source of reduced ferredoxin might be required for FA biosynthesis in the apicomplexan plastid as acyl–acyl carrier protein desaturases of higher plants use molecular oxygen and reduced ferredoxin (see Cahoon et al. 1996). Because FA biosynthesis is a special, and probably primary, function of the parasite’s vestigial organelle, linking retention of \(sufB\) and the organellar genome to a supportive role for FA biosynthesis is an attractive proposal, and along the lines suggested by both Allen (1993a) and Palmer (1997) for retention of organellar genomes. However, evidence is still lacking for an important participant required by this proposal, namely a plastid-targeted NifU-like protein in \(Plasmodium\) spp. In bacteria, it is NifU that transfers a [2Fe–2S] cluster to apoferredoxin (Nishio & Nakai 2000). Regarding the general argument for retention of organellar genomes, it is noteworthy that both \(sufB\) (\(ycf24\)) and \(sufC\) (\(ycf16\)) are now carried in the nucleus of higher plants and have acquired plastid-targeting presequences (Martin et al. 1998). Clearly, it is not obligatory for plastid genomes to carry \(sufB\). This removes it from the category of genes that comprise a distinctive organellar requirement (Allen 1993a). It also favours the notion that \(sufB\) is retained on the parasite vestigial plastid genome to support a specialized function of the organelle. Interestingly, as in apicomplexans, the vestigial plastid genome of \(Epifagus virginiana\), a non-photosynthetic, parasitic higher plant, carries only five protein-coding genes besides those required for protein expression (Wolfe et al. 1992). One of the five is \(accD\) for the β- or carboxyl transferase subunit

Figure 8. Side view of SufC modelled on the structure of HisP (Hung et al. 1998). The thickness of arm II is \(ca. 25\) Å. α-helices are red and β-strands yellow. Arginine residues at the sites of tryptic digestion yielding the fragments mentioned in the text (B–D) are shown as ‘ball and stick’ representations.
of the four-subunit enzyme acetyl-CoA carboxylase which catalyses the first committed step in FA biosynthesis. Genic support for this pathway might be a common factor explaining retention of vestigial non-photosynthetic plastid genomes.

6. CONCLUSION

The vestigial plastid genome of apicomplexans carries a sufB gene derived from a bacterial operon inferred to have a role in iron homeostasis/[Fe–S] cluster formation/oxidative stress. Despite the possibility that sufB has a subsidiary role in the maintenance of redox-related proteins, it is a nuclear gene in higher plants and so does not come into the category of essential genes retained by all plastids. It seems more likely that in apicomplexans sufB has a supportive role for one of the specialized functions of the vestigial plastid organelle, namely FA biosynthesis. The experimental results presented show that a recombinant form of SufB interacts, in vitro, with SufC, an ATPase similar to the nucleotide-binding subunit of ABC transporters. In apicomplexans, sufC is a nuclear gene with a putative plastid-targeting sequence and we conjecture that the two proteins interact in the plastid compartment. But exactly where the proteins are localized and what their functions are remain to be determined.

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Discussion

J. C. Gray (Department of Plant Sciences, University of Cambridge, Cambridge, UK). Can you explain how to fit Simon Møller’s mutant, the laf6 mutant, which accumulates protoporphyrin, and which is a ycf24 mutant, into your scheme of things?

R. J. M. (Iain) Wilson. Yes. This is a paper that perplexed us greatly. It concerns the deletion of suFB, i.e. ycf24, in Arabidopsis, with a detailed analysis of the phenotype. The authors tried to fit the results into a scenario where suFB was involved in a plastid–nucleus signalling mechanism now shown suFB is not an ATPase though it may be involved with an ABC transporter. It seems to me there is an alternative way of explaining some of their results. For example, levels of protoporphyrin IX are elevated when you have a defect in iron homeostasis, and it might be that which has caused this particular change in the tetrapyrrol pathway, rather than a signalling gene being knocked out. That is how I think we could explain it better.
membrane of the host cell it goes into; it actually enters a vesicle within the second cell. This vesicle is not just whole cell membrane but modified membrane. So one speculation is that the parasite is inserting things into this membrane, either lipids themselves, possibly isoprenoid-modified proteins, and this interface between the parasite and its host is made new each time. That is obviously very important for the survival of the organism. Maybe it is a defect in this second membrane that is formed in the invasion step; that is speculation.

J. A. Raven. Thanks.

W. Martin (Institute of Botany III, Heinrich-Heine Universität Düsseldorf, Düsseldorf, Germany). Does inhibiting the isoprenoid biosynthesis pathway kill the parasite and, if so, what is the mechanism of that killing?

R. J. M. Wilson. Yes, it does kill the parasite, and you are just touching on something that confuses me as well, because there are apparently really two kinds of death that occur if you start interfering with the plastid’s functions. One is this so-called ‘delayed death’ phenotype, where it is in the second cell where something happens. When you inhibit isoprenoid biosynthesis, the parasite actually dies in the first cell, and apparently quite rapidly. I do not think anyone understands why that is so, so it is not death entering the new cell, but death in the first cell. I can only give you the observation.

W. Martin. Quinones?

R. J. M. Wilson. It is possible.

T. Cavalier-Smith (Department of Zoology, University of Oxford, Oxford, UK). Is it possible that the plastid in the first cell is exporting lipids, so that the other ones in the cell can survive, but once a daughter enters another cell without any plastids, it dies because no more lipids can be exported to it?

R. J. M. Wilson. Yes, it is possible that there is some kind of exchange between parasites within one vacuole. Things could be floating around and being picked up by the other parasites. However, plastid-less parasites fail to grow in host cells co-infected with normal plastid-containing parasites. As these parasites die in different vacuoles, this is not quite the same thing as your point.

J. F. Allen (Plant Biochemistry, Lund University, Lund, Sweden). There are several things actually; I feel quite tied in a knot by this. The first is just to suggest that perhaps there is a function for chloroplast and mitochondrial genomes. Namely, that they permit the possibility of a loop of feedback from the electron transport chain of the mature, functional chloroplast or mitochondrion to the beginnings of its own assembly and transcription. That is testable. It is testable above all with DNA microarrays, which I am not against at all. My only objection to DNA microarrays is that I have not got one. So thank you for that reference. The point here is that I am surrounded by people upon whom the ceiling opens and money falls, and they go away and buy DNA microarrays and get results. Then they say, ‘Oh, what’s happening?’, and they do not know what the question was they were trying to answer.

My other point, about the ‘vestigial’ plastid of non-photosynthetic organisms is really to say, let us seek a function for the maintenance of that genome. If I am correct, in the context of mature chloroplasts and mitochondria, maybe they are still doing some membrane-associated redox chemistry, which is so important that the organelle still needs to have its finger on the gene expression. It would not have this if the gene had been moved to the nucleus. Now, if I am right and you are right, the phylogenetic inference is that fatty acid biosynthesis in Plasmodium might be significantly different from that in higher plants that have lost the suFB gene from the plastid. Perhaps they do not need it any more because they are doing it a different way. So one might ask if there is a membrane-bound redox apparatus in this plastid participating in this job, which you obviously have considered very seriously, so that is the sort of position I am in. I mean, it is not decisive, actually.

R. J. M. Wilson. I think there are questions about the membranes within this organelle that still have to be properly answered. There is one report of an internal membrane complex that has attached, as well as of another membrane complex attached to the outside of the organelle. But no one knows what is going on in there. There are no markers whatsoever; it is a total black box.

J. F. Allen. Can you not just put them in a blender and isolate a test-tube full of plastids?

R. J. M. Wilson. I wish we could!

J. F. Allen. I would begin by doing a chlorophyll assay, of course.

R. J. M. Wilson. We tried that, but what we got were mitochondria.

J. F. Allen. And the other thing is are there any structural genes for proteins that do the real work lurking in this genome? I mean, can you say that there are no other real genes encoding real proteins than this one suFB?

R. J. M. Wilson. Not 100%. Actually, there are one or two small, unidentified open reading frames, which I suspect are probably very divergent ribosomal-protein-encoding genes. So it is true that there are one or two short stretches of DNA that are not totally accounted for. However, I would be surprised if they were the kind of protein you are talking about.

J. F. Allen. You are only saying that to be nice.

J. Tovar (School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey, UK). You alluded to the putative iron–sulphur assembly function of this organelle. My question is, what would be the relative contribution of this process in the apicoplast relative to the mitochondrion?

R. J. M. Wilson. It is true that in eukaryotes mitochondria make iron–sulphur clusters and export them into the cytosol for other proteins to utilize. But I think there is evidence that plastids also have the apparatus to make their own iron–sulphur clusters. There have been a number of papers on that subject using isolated plastids with various invitro manipulations to show they do contain the correct enzymes to make iron–sulphur clusters. For example, the import of apoferredoxin into the plastid would in fact utilize this endogenous, or plastid-made, iron–sulphur cluster, not one it had brought in with it. That would be a very difficult thing for it to do, I think, because it would have to transport the cluster when in an unfolded form, which is probably impossible. So we speculate that iron–sulphur clusters form in the organelle itself, quite separate from what goes on in the rest of the cell.

J. Tovar. But so far no direct evidence?

R. J. M. Wilson. Not in our system, but in higher plant plastids there is.
J. F. Allen. Yes, if it was once a red alga, of course. It might have quite a lot of things that the chlorophyte genomes do not have, including ferredoxin and so on. So there is something distinctive about that line. It comes back to this question of whether you believe in a monophyletic or polyphyletic origin of plastids, I suppose.

GLOSSARY

ALAD: δ-aminolevulinic acid dehydratase
CPO: coprogen oxidase

DTT: dithiothreitol
FA: fatty acid
FC: ferrochelatose
isc: iron–sulfur cluster
ORF: open reading frame
mantATP: 2′(3′)-O-N-methylanthraniloyl-ATP
PATS: predict apicoplast-targeted sequences
PBGD: porphobilinogen deaminase
PPIX: protoporphyrin IX
PRO: protogen oxidase
UROD: uroporphyrinogen decarboxylase