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MicroReview

Glycosomes: parasites and the divergence of peroxisomal purpose

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Summary

Peroxisomes are membrane-bounded organelles that compartmentalize a variety of metabolic functions. Perhaps the most divergent peroxisomes known are the glycosomes of trypanosomes and their relatives. The glycolytic pathway of these organisms resides within the glycosome. The development of robust molecular genetic and proteomic approaches coupled with the completion of the genome sequence of the pathogens *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania major* provides an opportunity to determine the complement of proteins within the glycosome and the function of compartmentation. Studies now suggest that regulation of glycolysis is a strong driving force for maintenance of the glycosome.

Introduction

Trypanosomatids are fascinating and important organisms for two major reasons: their pathogenicity and divergent biology. Among others, trypanosomatids include African trypanosomes (*Trypanosoma brucei*), American trypanosomes (*Trypanosoma cruzi*), and *Leishmania* spp., which cause chronic, often fatal diseases of humans. Vector and animal reservoirs, efficient immune evasion strategies, lack of effective vaccines, poor drugs and emerging resistance make combating these protozoan pathogens an ongoing challenge. The parasites' extensive specialization after divergence from others in the eukaryotic lineage is reflected by numerous unusual features at the genetic, biochemical and cytological level. For example, trypanosomatids extensively decode their mitochondrial transcripts through RNA editing, regenerate thiols using trypanothione (a glutathione-spermidine conjugate) rather than glutathione, and possess novel cytological structures such as the paraflagellar rod. The unique compartmentation of glycolysis within unusual peroxisomes called glycosomes provides another example of functional divergence. As reviewed here with primary focus on the major human pathogens, the joint results from biochemical analyses, database mining, and molecular genetics are leading to an understanding of the role of glycosomal compartmentation in trypanosomatids.

Glycosomes (Fig. 1), the cytosol, and the mitochondrion cooperate in the energy metabolism of trypanosomatids. The case of the African trypanosome, *T. brucei,* shows how the different nutritional environments faced during parasite development are reflected in metabolism and organelle function. *T. brucei* is bathed in constant millimolar levels of glucose as it lives extracellularly in the bloodstream and cerebrospinal fluid of its mammalian host. All ATP is generated through glycolysis and the major proteins in the glycosome are glycolytic enzymes (Hart *et al*., 1984). In the insect host, conditions change. Tsetse flies feed every few days on blood, their only food. Glucose in the digesting bloodmeal is rapidly metabolized, leaving amino acids as the primary nutrients for the gut-dwelling parasites. Glycosomes remain numerous but most of the glycolytic enzymes decrease in abundance, and other glycosomal enzymes are induced. Oxidative phosphorylation is active, but substrate level phosphorylation is critical to parasite survival (Bochud-Allemann and Schneider, 2002; Coustou *et al*., 2003). The literature indicates that the carbohydrate metabolism of *T. cruzi* and *Leishmania* species probably more closely resembles that of *T. brucei* procyclic as opposed to bloodstream forms.

What is the basis for calling glycosomes 'divergent peroxisomes'? Like peroxisomes, glycosomes are bounded by a single membrane and have a protein-dense matrix. Early studies showed that at least some pathways or enzymes commonly found in peroxisomes are also present in the glycosomes of some species (Opperdoes, 1984; Wiemer *et al*., 1996). For example, catalase, the prototypic peroxisomal marker, is found in the glycosomes of the non-pathogenic trypanosomatid *Crithidia* (Soares and De Souza, 1988). Evidence for a close evolutionary relationship of peroxisomes and glycosomes was bol-

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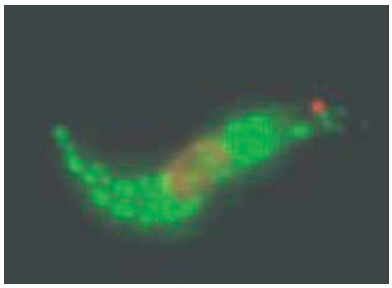

Fig. 1. Numerous glycosomes of *T. brucei*. Immunofluorescence analysis using an anti-glycosome serum (green) shows the glycosomes scattered throughout the cell, in this case a procyclic form. The DAPI-stained nucleus and kinetoplast (mitochondrial DNA) are pseudocolored red.

stered by the commonality of targeting sequences and unequivocally substantiated by the significant sequence similarity in proteins required for matrix protein import in trypanosomatids, yeast and humans. However, the early finding that most *T. brucei* glycolytic enzymes are glycosomal (hence the name) established that these organelles are indeed unique. The glycosome may reflect another unusual evolutionary twist: recruitment of proteins from a photosynthetic organism through lateral gene transfer. Whether this gene transfer is a result of endosymbiosis (Hannaert *et al*., 2003a) or phagocytosis (Waller *et al*., 2004) remains open. Nonetheless, the signature of the donor is traced in trypanosomatid enzymes and pathways that are related to those of algae, plants and chloroplasts, implying that the genetic partner was an alga (Hannaert *et al*., 2003a). Interestingly, several plastid-like proteins are present in the glycosome.

How functional glycosomes and peroxisomes are formed

Numerous studies in yeast and humans have shown that a large set of peroxins (PEX) are required for the formation of a functioning peroxisome. Over 20 PEX proteins participate in the import of matrix proteins, and a handful are required for the formation of a functional peroxisomal membrane (including insertion of the membrane proteins) (Purdue and Lazarow, 2001). The first step in import is binding of the peroxisomal targeting sequence (PTS) to a soluble receptor (either PEX5 or PEX7). The receptor-bound cargo docks to a PEX protein complex that allows import. Many PEX proteins are not part of this complex, but are still required for the import of matrix proteins. The mechanism of protein import is truly unique in peroxisomes. The import machinery shows no relationship to those that participate in chloroplast or mitochondrial protein import. Nor do PEX proteins appear related to proteins participating in nuclear or ER import. Moreover, the physical process of import appears to be quite different from chloroplasts or mitochondria, which require protein unfolding. Despite the absence of any apparent pores in the peroxisomal membrane upon electron micrographic analysis, proteins can be imported into the peroxisome fully folded and can even drag along other proteins that lack a targeting sequence (Titorenko *et al*., 2002)! Indeed, property has been exploited to test for protein–protein interactions: a PTS added to one partner can localize the other partner to the peroxisome (Nilsen *et al*., 2003). In many organisms, peroxisomal proliferation is controlled by nuclear hormone receptors and transcription factors, often regulated by fatty acids. There is not any evidence for this type of regulation of glycosomes in trypanosomatids.

Several years ago, our laboratory used a genetic screen to identify an *L. donovani* gene involved in glycosome biogenesis. To our satisfaction, the encoded protein showed low, albeit significant, similarity to yeast and human PEX2 (Flaspohler *et al*., 1997), which is required for peroxisomal matrix protein import. Since then, several other PEX homologues have been identified in African trypanosomes and several species of *Leishmania*. In most cases the level of sequence identity with PEX proteins in other eukaryotes is rather low and conclusive identification has required corroborating evidence such as localization to the glycosome. In contrast, once a PEX gene has been identified in one trypanosomatid, the homologue in other trypanosomatids can be readily identified from the genomic sequence. The involvement of several PEX homologues in glycosomal function has been directly demonstrated (e.g. Lorenz *et al*., 1998; Furuya *et al*., 2002; Guerra-Giraldez *et al*., 2002). As discussed below, the identification of PEX genes and proteins, together with advances in genetic technologies in trypanosomatids, provides an opportunity to assess the functional importance of the glycosome.

Peroxisomal and glycosomal metabolic pathways

Experimental biology has elucidated much of what we know about the metabolic pathways contained in peroxisomes and glycosomes, but database mining and sequence analysis have recently suggested the organellar location of additional proteins and pathways. A study examining eight eukaryotic genomes ranging from yeast to humans identified 430 predicted proteins bearing a probable type 1 PTS (Emanuelsson *et al*., 2003). Database analysis searching for proteins bearing the less well-defined PTS2 will undoubtedly identify numerous additional candidate peroxisomal proteins. The presence of a PTS does not ensure that the protein is predominantly glycosomal. For example, trypanothione reductase terminates in a PTS1, but is predominantly cytosolic (Smith *et al*., 1991). Furthermore, not all known peroxisomal/glycosomal proteins bear a recognizable PTS. Hence, data-

base analysis must be supplemented with experimental biology to identify peroxisomal/glycosomal proteins. The stage-specific metabolism described above means that a full understanding of glycosomal metabolism requires an analysis of expression in addition to sequences. Proteomics offers an opportunity to identify candidate glycosomal proteins and to examine their expression during development. This technology has been applied to peroxisomes of plants, yeast and animals, allowing the identification of novel proteins, including those likely to function in regulation such as protein kinases (Fukao *et al*., 2002). The completion of trypanosomatid genome sequences coupled with proteomics may allow a definition of the full constellation of glycosomal proteins in the near future.

One theme that has emerged over the years is that peroxisomes are organelles whose specialization reflects the peculiarities of each species. While in most species, peroxisomes compartmentalize ether-lipid synthesis and β-oxidation of fatty acids, in other species they additionally compartmentalize some steps of isoprenoid synthesis, aromatic amino acid synthesis, purine catabolism or other oxidative pathways. Similarly, glycosomes contain many of the hallmark peroxisomal activities, plus specialized processes. At this juncture, there is little data to suggest major differences in the complement of enzyme pathways contained in glycosomes of different trypanosomatids. Like peroxisomes, *T. brucei* glycosomes contain several enzymes in pathways of ether-lipid biosynthesis (dihydroxyactetone-phosphate acyltransferase, 1-acyl glycerol-3-phosphate: NADP oxidoreductase, and acyl-coA reductase) (Opperdoes, 1984) and at least two activities of β-oxidation (2-enoyl coA hydratase and hydroxyacyl-dehydrogenase, encompassed in a single protein) (Wiemer *et al*., 1996). The presence of a PTS-1 on *T. brucei* and *T. cruzi* carnitine acetyl transferase, catalysing the last peroxisomal step in fatty acid oxidation (it is required for the transmembrane transport of the product, acetyl coA), suggests that the full peroxisomal pathway could be present in glycosomes at some stage of development. Although mammals possess specialized fatty acid oxidation systems in mitochondria and peroxisomes, little oxidation of fatty acids was observed in the mitochondrial fraction of *T. brucei* (Wiemer *et al*., 1996). These data suggest that the glycosomal pathway is required for utilization of fatty acids as a carbon and energy source. Although *Leishmania mexicana* amastigotes show higher activity than promastigotes (Coombs *et al*., 1982), the importance of this pathway to parasite survival remains unclear.

Peroxisomal β-oxidation generates hydrogen peroxide, which is broken down by peroxisomal catalase. Interestingly, this enzyme is absent in *T. brucei* and *Leishmania*. However, *T. cruzi* possesses a glycosomal glutathione-dependent peroxidase that could remove hydrogen peroxide (Wilkinson *et al*., 2002), and the *T. brucei* predicted protein contains a PTS1 (Hillebrand *et al*., 2003). A subset of *Leishmania chagasi* superoxide dismutases are also reported to be glycosomal (Plewes *et al*., 2003).

A few reports suggest that the pentose phosphate pathway is peroxisomal in some organisms. In *T. brucei*, this pathway provides an important example of dual localization effected by partial targeting. Single copy genes encode the enzymes, but at least the first activities of the pathway (glucose-6-phosphate dehydrogenase and 6-phosphogluconolactonase) are partitioned between the glycosome and cytosol (Duffieux *et al*., 2000). Several enzymes which catalyse subsequent steps bear PTS1 sequences. Barrett suggested that the cytosolic pathway may fulfil both reductive and biosynthetic functions (phosphorylated sugars for nucleotide salvage and biosynthesis and the building blocks for aromatic amino acid synthesis), while the glycosomal pathway may primarily serve to regenerate reducing equivalents (Barrett, 1997). The recent finding of some purine salvage enzymes in the glycosome (see below) broadens this view, because the glycosomal pentose phosphate pathway would produce the ribose-5-phosphate required for the salvage reactions. In any case, the NADPH regenerated by the pentose phosphate pathway can be used in biosynthetic reactions or to reduce glutathione (or trypanothione) to combat oxidative stress. Among the more surprising proteins that may be associated with this pathway is a Calvin cycle enzyme heretofore considered to be plant-specific: sedoheptulose-1,7-bisphosphatase. The *T. brucei* predicted protein bears a PTS1 and may be a relict of an ancient endosymbiosis (Hannaert *et al*., 2003a) or perhaps an algal lunch (Waller *et al*., 2004).

The localization of the trypanosomatid enzymes involved in sterol and isoprenoid metabolism have been little studied, although some of them bear predicted PTSs.

The molecular signals for targeting membrane proteins to peroxisomes are not well-defined. The low permeability of peroxisomes to small molecules such as NAD/NADH, ATP, and acetyl coA, as deduced from studies of yeast peroxisomes (Van Roermund *et al*., 1995) clearly indicate that specific carriers are required to transport substrates and products across the organelle membrane. Some transporters have been defined at the molecular level for peroxisomes, such as those involved in uptake of activated fatty acids, but they are not yet known for glycosomes. The low permeability for small molecules also indicates that the organelle must contain activities to regenerate cofactors and ATP so that metabolism can continue.

Pathways unique to glycosomes

The most unusual pathway detected in glycosomes is the

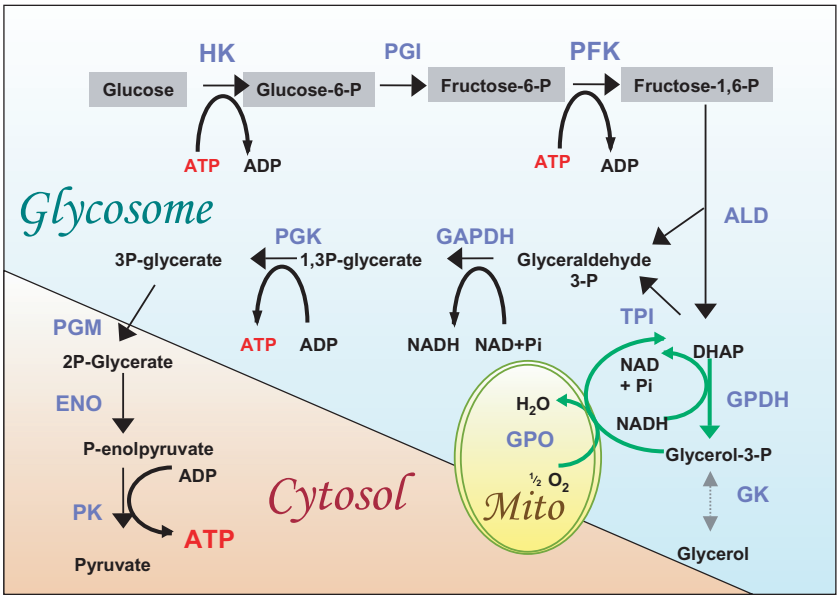

Fig. 2. Glycolysis in bloodstream form *T. brucei*. Glycolysis requires cooperation of three cellular compartments: the glycosome, the cytosol and the mitochondrion. The black arrows show the predominant flow of metabolites of glucose. Note that two ATPs are required to start the process. The six carbon sugars (grey background) are eventually broken into two trioses, each of which can generate an ATP. Thus ATP is balanced within the glycosome. Net ATP is generated in the cytosol. A small proportion of trioses are used in the glycerophosphate shunt in collaboration with the mitochondrion. Here, dihydroxyacetone phosphate is cycled to glycerol-3-phosphate and back (green arrows). This allows the glycosome to maintain the NAD/NADH balance. Enzymes are in blue font, with hexokinase (HK) and phosphofructokinase (PFK) emphasized, because they lack the normal regulation seen in other organisms. Other enzymes in order of action are: PGI, glucose phosphate isomerase; ALD, aldolase; TPI, triose phosphate isomerase; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; ENO, enolase; PK, pyruvate kinase; GDH, glycerol-3-phosphate dehydrogenase; GPO, mitochondrial glycerol phosphate oxidase complex composed of glycerol-3-phosphate dehydrogenase (FAD), alternative oxidase, and ubiqinone; GK, glycerol kinase.

Embden–Meyerhof segment of glycolysis (Fig. 2). Glucose is imported into the parasite using specific transporters (often stage-specific) and then into the glycosomes via an unknown mechanism. The enzymes from hexokinase to phosphoglycerate kinase (or glyceraldehyde-3-phosphate dehydrogenase in the case of *T. brucei* procyclic forms) are present in glycosomes. Within the glycosome, no net synthesis ATP occurs as a result of glycolysis. Rather ATP is generated outside of the glycosome, when pyruvate kinase transfers the high energy phosphate from phosphoenolpyruvate to ADP.

Glycosomal glycolysis requires the regeneration of NAD^+, which is accomplished through a collaboration with the mitochondrial glycerol phosphate oxidase complex in bloodstream forms. As shown in Fig. 2 (green arrows), glycosomal glycerol phosphate dehydrogenase generates glycerol-3-phosphate and NAD^+. The glycerol-3-phosphate is reoxidized by the mitochondrial glycerol phosphate complex [which is comprised of glycerol phosphate dehydrogenase (FAD), a plant-like alternative oxidase, and ubiquinone], and returned to the glycosome for further cycling. Without such a cycle, glycerol-3-phosphate could not be diverted to glyceraldehyde-3-phosphate, sacrificing half of the net ATP synthesis per glucose. When this shuttle is disrupted through biochemical or genetic means, *T. brucei* bloodstream forms die because the glycolytic pathway is blocked.

Preliminary database mining has recently revealed the presence of a key enzyme for gluconeogenesis in *T. brucei*: fructose-1,6-bisphosphatase (Hannaert *et al.*, 2003b). The predicted protein contains both a PTS1 and PTS2, strongly suggesting that it is localized to glycosomes. Several other glycolytic enzymes could participate in gluconeogenesis, functioning in reverse. It is unclear how metabolism is regulated such that the two pathways do not result in futile cycling. A few other carbohydrate metabolizing enzymes are present in the glycosomes, with their primary function possibly being the recovery of oxidizing equivalents to spur glycolysis and perhaps β-oxidation of fatty acids.

Purine salvage occurs in glycosomes. Because the parasites do not synthesize purines *de novo*, the enzymes are of considerable importance. *L. donovani* hypoxanthine guanine phosphoribosyl transferase and xanthine phosphoribosyl transferase both contain PTS1 signals and have been localized to glycosomes (Zarella-Boitz *et al.*, 2004). Adenine phosphoribosyl transferase does not contain a recognizable PTS. The presence of these salvage enzymes in glycosomes is a mystery because they are cytosolic in other organisms. Xanthine phosphoribosyl

transferase is functional for purine salvage when expressed in the cytosol (Zarella-Boitz *et al*., 2004). A few steps of pyrimidine metabolism, typically cytosolic in other organisms, also occur in glycosomes. Orotate phosphoribosyltransferase and orotidine-5′-phosphate decarboxylase activities are mediated by a fusion protein bearing a PTS1 (Gao *et al*., 1999).

The role of peroxisomal compartmentation

The disorders of peroxisome biogenesis in humans provide some insights as to the consequences of loss of compartmentation. Although affected individuals survive to birth and their cells are viable in culture, those with the most severe disease, Zellweger's syndrome, die in infancy. These patients are deficient in oxidation of long-chain fatty acids and cannot use them as carbon and energy sources. Long-chain and very-long-chain fatty acids accumulate and may be toxic. Similarly, yeast mutants defective in peroxisome biogenesis cannot grow on fatty acids, although they are healthy when grown on other substrates. Zellweger's cells are also defective in ether-lipid synthesis. Defects in lipid metabolism may alter the levels of small molecule mediators that affect the development of the nervous system and possibly other processes. These data suggest that disruption of glycosomal biogenesis would likely result in the abrogation of these pathways, eliminating the ability to use fatty acids as carbon and energy sources and changing the cellular lipid profile.

The function of glycosomal compartmentation

Unlike the peroxisomes of yeast and humans, the glycosomes of *T. brucei* are essential for cellular survival. RNAi-mediated knockdown of PEX2 (Guerra-Giraldez *et al*., 2002) or PEX14 (Furuya *et al*., 2002) disrupts import of matrix proteins into the glycosome and kills both mammalian and insect stages. The inability to obtain PEX gene knockouts in *Leishmania* suggests that glycosomes are essential in this genus as well. We propose that as a result of the evolutionary history of the glycosome, multiple factors drive its maintenance.

Perhaps most obviously, the lack of glycosomes could result in deficiencies of enzymes that are important at some particular stage of the life cycle. Here one would suspect the culprits to be enzymes of ether-lipid synthesis or β-oxidation of fatty acids, because compartmentation is required to prevent cytosolic degradation of these enzymes in other organisms. We are unaware of any tests of the requirement for oxidation of fatty acids in trypanosomatids. However, recently the role of ether-lipid biosynthesis was explored in *L. major*. In *Leishmania* promastigotes, the major surface glycoconjugate, lipophosphoglycan (LPG), is GPI-anchored through an ether lipid. Surprisingly, genetic blockade of ether-lipid formation does not affect the viability of promastigotes, even though LPG and several other major glycoconjugates are absent (Zufferey *et al*., 2003). However, the lack of ether lipids strongly impairs the ability of the parasites to transit the initial phases of macrophage infection. Once infection was established and amastigotes formed, these parasites retained their virulence. We propose that glycosomal compartmentation is required for biosynthesis of parasite ether lipids needed for effectively completing the *Leishmania* life cycle. With respect to the roles of ether lipids in other parasites, less is known. The major surface glycoconjugates of *T. brucei* are GPI-anchored through acyl lipids.

Glycolysis may be the most important driving force for maintenance of the glycosome. Early hypotheses suggested that compartmentation might provide for a higher efficiency of glycolysis through maintaining a high concentration of metabolites. However, comparison with Baker's yeast indicates that compartmentation is not required for a high glycolytic flux (Bakker *et al*., 2000; Hannaert *et al*., 2003a). Recent data suggest that compartmentation may be most important in preventing metabolic interference. Because of the low permeability of the peroxisomal (glycosomal) membrane, enzymes and metabolites in the cytosol and glycosome are segregated from one another. Expression of some glycolytic enzymes in the cytosol is toxic. For example, phosphoglycerate kinase is exclusively glycosomal in *T. brucei* bloodstream forms, and when it is expressed in the cytosol, the parasites die (Blattner *et al*., 1998). Because this experiment was performed on cells co-expressing wild-type levels of the glycosomal form, the glycosomal glycolytic pathway was still intact. These findings suggest that the mislocalized enzyme might be consuming cytosolic substrates or generating products at levels the cell cannot tolerate. Similarly, triose phosphate isomerase expressed in the cytosol is toxic to bloodstream forms (Helfert *et al*., 2001). This toxicity could result from the disruption of the glycerophosphate shunt.

Regulation by glycosomal compartmentation

Some catabolic pathways require an initial investment of ATP so that the following reactions, which generate ATP, can be thermodynamically favourable. Glycolysis is one of those pathways. Described by Westerhoff and colleagues as a 'turbo design', this early investment requires that glycolysis be kept under control (Teusink *et al*., 1998). In most organisms, control is accomplished by tightly regulating the early ATP utilizing steps catalysed by hexokinase and phosphofructokinase. However, purified hexokinase and phosphofructokinase from trypanosoma-

tids are not subject to the typical feedback regulation (Nwagwu and Opperdoes, 1982). Using knowledge of the kinetic parameters and enzyme concentrations of the glycolytic enzymes of bloodstream stage *T. brucei*, Bakker and colleagues generated a computer kinetic model that strongly suggested that glycosomal compartmentation was required to protect the parasite from ill-fated investments of ATP (Bakker *et al*., 2000). The low permeability of the organelle membrane to ATP and cofactors would normally limit the consumption of ATP for glycolysis to what is regenerated within the glycosome. As glycosomal ATP is depleted, hexokinase and phosphofructokinase activity would be curbed. The analysis indicated that when the glycosomal enzymes were freed into the cytosol, more ATP would be available and consumed, generating high levels of phosphorylated hexoses. The concomitant osmotic effects and depletion of phosphate pools would be catastrophic.

Our group has recently examined the effects of an RNAi knockdown of PEX14, a required component of the receptor docking complex, in light of this model (Furuya *et al*., 2002). Using procyclic forms, we demonstrated that despite the fact that this stage of the parasite life cycle does not require glycolysis for survival (they can generate ATP from amino acids), disruption of glycosomal import through knockdown of PEX14 is lethal. The cells lose their elongated morphology and balloon up before dying. When the procyclic forms were grown in the absence of glucose, they survived and proliferated in spite of the PEX14 knockdown. Thus glucose is toxic to cells deficient for glycosomal compartmentation. These data implied that the most proximal requirement for glycosomes in this stage is related to glycolysis or other carbohydrate metabolism. Because the knockdown (or wild-type) cells survive when glucose and other sugars are removed, it is clear that cell death is not a result of a lack of ATP generated by glycolysis. We suggest that cells are killed by a gain of a cytosolic function rather than the loss of a glycosomal function and hence even partial mislocalization of glycosomal proteins could be toxic. The same experiment cannot be done in bloodstream forms, because if glucose is removed they will die within an hour.

The mechanism of glucose toxicity in the absence of glycosomes in procyclic forms remains to be experimentally elucidated, although the findings are compatible with the computer model of bloodstream forms described above. From that model, one would predict that knockdown of hexokinase, required for entry of glucose into the glycolytic pathway, would suppress the effects of PEX14 RNAi. Such experiments are underway in our laboratory. Substrate-accelerated death has also been observed in mutant yeast lacking trehalose-6-phosphophosphate synthase, a regulator of glycolysis. When glucose is omitted, the yeast survive. Furthermore, downregulation of hexokinase activity was able to suppress lethality (Hohmann *et al*., 1993), compatible with a kinetic model showing that fivefold reduction alleviates toxicity (Teusink *et al*., 1998). In procyclic forms, hexokinase is naturally downregulated over 10-fold as compared to the levels found in bloodstream forms (Hart *et al*., 1984), which were used to generate the kinetic model.

More effort will be required to determine whether death is because of the generation of toxic levels of phosphorylated intermediates and consumption of ATP or a more complex mechanism. Measurements of the level of glycolytic intermediates could be helpful in this regard, although discounting other effects may be difficult. For example, it has been shown that removal of glucose or knockdown of hexokinase results in an alteration of the surface protein profile of procyclic form parasites (Morris *et al*., 2002). Hence, some metabolite of glucose may play an important regulatory role in the parasite life cycle. The dysregulation caused by disruption of glycosomal compartmentation could be more far-reaching than osmotic effects mediated by the accumulation of intermediates.

Could glycosomal compartmentation constrain other processes? Most other reactions that occur in the glycosome do not require glycosomal ATP. Even though β-oxidation of fatty acids requires an investment of ATP, this investment occurs in the cytosol. Whether glycosomal ATP is required to maintain other glycosomal pathways requires further examination. Another possibility is that compartmentation allows a different ratio or concentration of NAD and NADH to be maintained within the glycosome, thereby imposing an additional level of control in the organelle. Additionally, it will be important to expand studies from *T. brucei* to examine the role of the glycosome in the mammalian stages of *T. cruzi* and *Leishmania*.

Trypanosomatids cause some of the most intractable diseases of the world (African sleeping sickness, Chagas' disease and Leishmaniasis), and are also among the most cytologically and biochemically unique eukarytoes. Several unusual features of the parasite are related to their energy metabolism: the glycosome, RNA editing, plant-like pathways and enzymes. Could these features represent a target for attacking the parasite? For *T. brucei*, blocking glycolysis would be toxic in the mammalian stage. Although the parasites' glycolytic enzymes closely resemble those of the host (despite differences in regulation), some trypanosomatid-specific compounds have been synthesized based on crystal structures and have been shown to block parasite growth (Verlinde *et al*., 2001). More speculatively, compounds that disrupt the localization of proteins to the glycosome should also be toxic. Blocking some step in the cascade of protein interaction events required for matrix protein import could be

a means to this end. Although the challenges are considerable, recent progress in developing paradigms for the identification of small molecules that modulate protein interactions encourages further studies towards this goal (Berg, 2003; Gadek and Nicholas, 2003).

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