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The extraordinary energy metabolism of the bloodstream *Trypanosoma brucei* forms: a critical review and hypothesis

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Summary

The parasite *Trypanosoma brucei* is the causative agent of sleeping sickness and involves an insect vector and a mammalian host through its complex life cycle. *T. brucei* mammalian bloodstream forms (BSF) exhibits unique metabolic features including: (*1*) reduced expression and activity of mitochondrial enzymes; (*2*) respiration mediated by the glycerol phosphate shuttle (GPSh) and the Trypanosome alternative oxidase (TAO) that is intrinsically uncoupled from generation of mitochondrial protonmotive force; (*3*) maintenance of mitochondrial membrane potential by ATP hydrolysis through the reversal of F_1F_O-ATP synthase activity; (*4*) strong reliance on glycolysis to meet their energy demands; (*5*) high susceptibility to oxidants. Here, we critically review the main metabolic features of BSF and provide a hypothesis to explain the unusual metabolic network and its biological significance for this parasite form. We postulate that intrinsically uncoupled respiration provided by the

GPSh-TAO system acts as a preventive antioxidant defense by limiting mitochondrial superoxide production and complementing the NADPH-dependent scavenging antioxidant defenses to maintain redox balance. Given the uncoupled nature of the GPSh-TAO system, BSF avoids cell death processes by maintaining mitochondrial protonmotive force through the reversal of ATP synthase activity using the ATP generated by glycolysis. This unique "metabolic design" in BSF has no biological parallel outside of trypanosomatids and highlights the enormous diversity of the parasite mitochondrial processes to adapt to distinct environments.

1. Sleeping sickness and the *Trypanosoma brucei* life cycle

Trypanosoma brucei is the etiologic agent of sleeping sickness, also known as Human African Trypanosomiasis (HAT). The infection affects the central nervous system and causes severe neurological disorders, leading to coma and, if left untreated, death (Rodgers 2010; Kennedy, Rodgers 2019; Kennedy 2013). Throughout its complex life cycle, *T. brucei* transits between invertebrate hosts of the genus *Glossina* (tsetse flies) and mammals (Schuster et al 2021; Marchese et al 2018). At each stage of its life cycle, the parasite undergoes differentiation processes and faces several physical, chemical, and nutritional challenges in the distinct host environments (Marchese et al 2018). To adapt to these remarkable environmental variations, the parasite alters not only its morphology, gene expression and signaling pathways but also its metabolism. Indeed, the metabolic rewiring observed along the transition from the insect forms (procyclic, PCF) to the mammalian forms (bloodstream, BSF) is impressive and critical for parasite survival and proliferation (Ziková 2022; Zíková et al 2017; Butter et al 2013; Matthews 2005).

The mechanisms involved in energy provision may change in different cell types to meet their energy demands. In this sense, the energy metabolism network of trypanosomatids starkly contrasts with the predominant paradigm for other eukaryotes. For example, in BSF the dominant mechanism of energy provision is mediated by substrate-level phosphorylation through glycolysis that takes place in a unique peroxisome-derived organelle (glycosomes; Opperdoes et al 1977; Visser, Opperdoes 1987; Visser et al 1981; Creek et al 2015). Glycosomes are found in trypanosomatids along their distinct life cycles. In contrast to *T. brucei* BSF, the key mechanism of PCF ATP production relies on oxidative phosphorylation (OXPHOS) which takes place within the parasite single mitochondrion (Dewar et al 2022).

Considering the unique energy metabolic pathways in BSF, we critically revise the knowledge framework on glycosomal, mitochondrial, and redox metabolism to provide readers a more complete picture of the enormous complexity of the BSF metabolic network. We propose a hypothesis to explain the metabolic signatures of BSF mitochondria as a preventive antioxidant mechanism to complement the classical scavenger redox defenses. Importantly, we discuss the potential consequences of this

unusual "metabolic design" at the cellular level in BSF that might be explored for future therapeutic interventions and key questions to be explored.

1.1. Glycosomes: peroxisomes turned into sugar-fueled metabolic powerhouses

Glycosomes are electron-dense structures enclosed by a single membrane that constitute approximately 4 % of the cell volume in trypanosomatids (Opperdoes, Borst 1977; reviewed in Allman, Bringaud 2017). When glycosomes were first evidenced in BSF, they were described as microbody-like organelles, bounded by a single lipid-bilayer membrane and heterogeneous morphology. In 1977, Opperdoes and Borst demonstrated in *T. brucei* the compartmentalization of the enzymatic activity of the first seven glycolytic enzymes (from hexokinase, HK, to phosphoglycerate kinase, PGK) and the other two enzymes involved in glycerol metabolism (glycerol-3-phosphate dehydrogenase G3PDH, and glycerol kinase GK) (Opperdoes, Borst 1977). Interestingly, glycosomes were proposed to be peroxisome-like organelles, although the *T. brucei* glycosomes are devoid of catalase or oxidase activity involved in hydrogen peroxide (H_2O_2) metabolism, the hallmark of peroxisomes. When catalase activity was found in glycosomes in other kinetoplastids such as the bodonid flagellate *Trypanoplasma borelli*, the evolutionary origin of glycosomes from peroxisomes was sealed (Deschamps et al 2011; Opperdoes et al 1988). Subsequent studies demonstrated the presence of this organelle in other Kinetoplastida organisms and in Diplonema, but not in Euglena. This indicates that glycosome development was a feature that arose in one of the common ancestors of the two Euglenozoa subclades, but after the separation from Euglenida (reviewed by Gualdrón-López et al 2012; Gabaldón et al 2016).

Although the *T. brucei* glycosomes are authentic peroxisomes, they have specific peculiarities (Gabaldón et al 2016; Gualdrón-López et al 2012). Glycosomes compartmentalize key metabolic pathways such as most reactions of glycolysis, the pentose phosphate pathway (PPP), purine salvage pathway, and sugar-nucleotide biosynthesis, which contrasts with most eukaryotic species (reviewed by Michels et al 2021; Allmann, Bringaud 2017; Gualdrón-López et al 2012; Opperdoes 1987). Compartmentalization of the initial glycolytic reactions in glycosomes resulted in the loss of HK and phosphofructokinase (PFK) regulation by their reaction end-products (Bakker et al 2000). In eukaryotes where glycolysis occurs in the cytosol, the lack of regulation of these two enzymes implies an acceleration of glycolytic flux by feedback occurring when ATP produced as an output is used as a substrate in the same pathway. This leads to an accumulation of phosphorylated intermediates of glycolysis, which affects cell physiology in two ways: (*1*) restricting access to the energy invested in these high-energy bonds (HEB) for other cellular energy demands; (*2*) causing osmotic disturbances generated by the accumulation of such intermediates. This type of buildup is known as the "turbo design" of glycolysis (Haanstra et al 2008). However, this risk is mitigated in trypanosomatids by splitting the glycolytic pathway into two distinct compartments: glycosomes and cytosol. Glycosomal membranes are impermeable to solutes with molecular mass above 400 Da (e.g. NAD^+/NADH and adenylates; Hammond et al 1985, Quiñones et al 2020), thus no free ATP/ADP/AMP exchange occurs between the glycosome and the cytosol. Since in glycosomes two mol ATP are invested per mol glucose from HK to PFK reactions and only two mol ATP are produced, the net ATP production is zero. The other two mol ATP from glycolysis are produced in the cytosol by the pyruvate kinase (PK) reaction. As a result, equilibrium of ATP consumption and production is

established in glycosomes and the glycolytic reaction rate is limited by the glycosomal ATP pool, preventing accumulation of HEB intermediates due to the turbo glycolysis (Bakker et al 2000; Clayton, Michels 1996; Opperdoes 1987; Visser et al 1981).

In *T. brucei,* glycosomes have different enzymatic contents and play distinct roles along the parasite's life cycle (Hart et al 1984). For example, in PCF the first six reactions of glycolysis take place within the glycosomes, while in BSF the first seven enzymes including the ATP producing phosphoglycerate kinase (PGK) are found within this organelle (Misset et al 1986; Hart et al 1984). As a consequence of the glycosomal heterogeneity throughout the parasite's life cycle, the main metabolic endproducts are different in distinct parasite forms. While BSF excretes pyruvate as the main endproduct and maintains redox balance (NAD^+/NADH) using the glycerol-3-phosphate shuttle (GPSh) and ATP/ADP balance using PGK, PCF metabolizes glucose to succinate in glycosomes and to acetate and alanine in mitochondria. Glycosomal succinate fermentation is a consequence of specific fumarate reductase activity found in glycosomes to recycle NAD^+ to sustain glycolysis (Besteiro et al 2002) and phosphoenolpyruvate carboxykinase (PEPCK) activity to replenish the ATP pool. Although fumarate reductase activity was postulated to exist only in PCF (Besteiro et al 2002), a small but consistent succinate production from glucose metabolism was reported in BSF (Mazet et al 2013). Although PEPCK activity in BSF is quite low compared to PCF (Durieux et al 1991; Hart et al 1984), surprisingly it is essential to this parasite form as silencing PEPCK causes BSF growth arrest (Creek et al 2015). Nevertheless, massive production and excretion of pyruvate as the metabolic product of glycolysis strengthens the critical role of the GPSh shuttle to regenerate glycosomal NAD^+ in BSF as will be discussed later.

1.2. Mitochondrial metabolic rewiring to support glycosomal redox balance

Over the years, the classical paradigm of mitochondria as the key organelles in providing cellular ATP has been extended to a myriad of other processes beyond energy metabolism (Kowaltowski, Oliveira 2020). Today, mitochondria are seen as key organelles directly involved in a variety of cellular events including differentiation (Chen et al 2003), growth (Son et al 2013), and signaling (Chandel 2015; Martínez-Reyes, Chandel 2020; Spinelli, Haigis 2018; Zhang et al 2010), and in the pathogenesis of numerous human diseases (Betarbet et al 2000; Narendra et al 2010). For example, superoxide and other reactive oxygen species (ROS) play central roles in cell physiology as natural by-products of mitochondrial metabolism (Brand 2020; Sies, Jones 2020; Boveris, Chance 1973; Boveris et al 1972).

The body of knowledge generated over the years on *T. brucei* mitochondria is quite extensive and comprises 1151 original papers from 1964 to 2021 (Figure 1). This represents ~49 % of papers published on all trypanosomatid species, indicating that *T. brucei* is considered a true model organism for mitochondrial research in trypanosomatids. However, the share of papers dealing with BSF stages falls to only 299, representing ~26 % of what is known about the mitochondria of *T. brucei*. Despite this, the knowledge accumulated so far provides us with an interesting scenario that challenges our understanding of energy metabolism in these exquisite parasite forms.

As the natural diversity of mitochondrial morphology, functions, and interactions with other organelles is enormous, some aspects of trypanosomatid mitochondria are unique or are much more represented than in other organisms, including: (*1*) The presence of a single mitochondrion undergoes striking remodeling of its structure and

activity along the parasite's life cycle (Bílý et al 2021; Hecker et al 1972). (*2*) The kinetoplast is a structure that holds the mitochondrial genome comprising a compacted network of the so-called kinetoplastid DNA (kDNA). The kDNA consists of a series of 25-30 circular DNA pieces of 25-50 kbp, and about 30 000 circular DNA pieces of about 1 kbp. The mitochondrial genome codes for some mitochondrial proteins and rRNA (Gluenz et al 2011; Ogbadoyi et al 2003). (*3*) The mechanism of mitochondrial mRNA editing adds another layer of regulation of mitochondrial gene expression (Benne et al 1986). (*4*) The absence of tRNA-coding genes in mitochondrial genomes requires the mitochondrial import of tRNA (Hancock, Hajduk 1990). (*5*) There is a remarkable heterogeneity of protein complexes involved in the electron transfer system (ETS) and ATP synthesis (Ziková 2022; Surve et al 2012; Clarkson et al 1989).

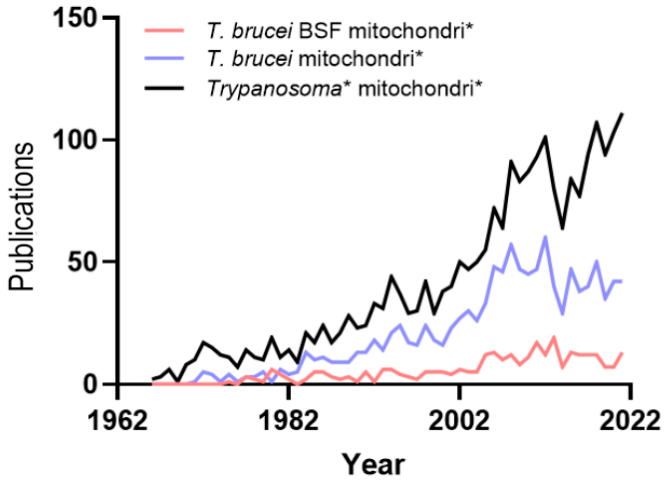

Figure 1. Evolution of knowledge on mitochondrial processes in trypanosomatids and *T. brucei*. Timeline of original publications on mitochondria between 1964 and 2021 in trypanosomatids (black line), *T. brucei* (blue line), and BSF (red line).

Fundamental mitochondrial functions are drastically altered throughout trypanosomatids life cycle, including the activities of the tricarboxylic acid (TCA) cycle, the ETS which directly impacts the protonmotive force (*pmF*), Ca^{2+} metabolism, and ATP synthesis (Docampo, Vercesi 2022; Gonçalves et al 2011; Zíková et al 2017; Priest, Hajduk 1994; Bringaud et al 2021). For example, in *T. cruzi* epimastigotes a fully functional TCA cycle and ETS maintains *pmF* to allow mitochondrial ATP production (Gonçalves et al 2011, Barisón et al 2017, Alencar et al 2020). The assessment of ROS production in *T. cruzi* mitochondria revealed that in epimastigote forms, the oxidant production is low compared to bloodstream trypomastigotes. Importantly, the mitochondrial ETS remodeling during the *T. cruzi* bloodstream trypomastigote differentiation favors electron leakage and production of mitochondrial H_2O_2 making these parasite forms more resistant to redox challenges than epimastigotes (Gonçalves et al 2011).

Regarding *T. brucei*, it is long known that BSF exhibits remarkable alterations of mitochondrial functionality and morphology compared to PCF (Vickerman 1965; Bílý et al 2021). In this regard, high-resolution 3D reconstruction of *T. brucei* revealed that mitochondria in PCF are reticulated structures with disk-like cristae that occupy a higher volume than in tubular-shaped organelles in BSF (Bílý et al 2021). In addition, BSF mitochondria have small cristae which occupy ~10 times less volume than cristae from PCF mitochondria (Bílý et al 2021). Considering that cristae represent the fundamental bioenergetic unit of mitochondria (Wolf et al 2019), cristae ultrastructure indirectly

reflects mitochondrial energy metabolism. In any case, the mitoproteome between the two parasite forms shows that the BSF mitochondria are similarly complex to the PCF mitochondria, a remarkable observation considering the morphological and metabolic rewiring of the BSF mitochondria (Ziková et al 2017).

A remarkable feature of BSF mitochondria is the profound changes in the composition and activities of many enzymes, including those involved in the TCA cycle and the canonical ETS complexes (Priest, Hajduk 1994; Njogu et al 1980, Clarkson et al 1989; Doleželová et al 2020; Smith et al 2017; Markos et al 1989). Noteworthy is the fact that the inability of BSF to perform OXPHOS is not due to the absence of ETS but to a remarkable remodeling of its function. Indeed, ETS in BSF is essentially carried out by a reduced form of electron transfer which does not involve proton-pumping and cytochrome-containing complexes but two other critical components: (*1*) the glycerol phosphate shuttle (GPSh) composed of glycosomal and mitochondrial glycerol-3-phosphate dehydrogenases (G3PDH) (Škodová et al 2013; Opperdoes et al 1977); (*2*) the Trypanosome alternative oxidase (TAO), which bypasses electron transfer through the Complex III-cytochrome *c*-Complex IV pathway (Clarkson et al 1989; Chaudhuri et al 1995). The GPSh-TAO system thus allows complete oxygen reduction to water through the redox cycling of ubiquinone without contributing to hydrogen ion translocation across the mitochondrial inner membrane mtIM (Clarkson et al 1989; Opperdoes et al 1977).

A key aspect regarding BSF mitochondria is their apparent inability to generate oxidants compared to PCF. Early observations demonstrate that PCF mitochondria can produce H_2O_2 at fairly high rates which strikingly contrast with low (or even undetected) oxidant generation in BSF (Penketh, Klein 1986; Fang, Beattie 2003; Turrens 1987). As will be described below, TAO activity plays a key role in regulating cellular redox metabolism since superoxide production in both parasite forms was boosted when TAO was pharmacologically inhibited (Fang, Beattie 2003).

As the energy from redox reactions of the ETS through the GPSh-TAO system is not conserved as *pmF* (mitochondrial membrane potential, $\Delta\Psi_{mt}$ and ΔpH), this represents the case of respiration intrinsically uncoupled from *pmF* generation. Intrinsically uncoupled respiration is different from inducibly uncoupled respiration, as seen in brown adipocytes under thermogenic stimuli. Inducibly uncoupled respiration in brown fat is a reversible phenomenon that is mediated by uncoupling protein 1 (UCP-1) which under certain signals divert the energy of *pmF* from the ATP synthase causing massive proton leak, reduction in OXPHOS efficiency, and thus stimulation of oxygen consumption and heat dissipation (Hittelman et al 1969; Heaton et al 1978). However, brown adipocytes do not uncouple their mitochondria all the time, and under basal (non-thermogenic) conditions, mitochondria can use the *pmF* to synthesize ATP at fairly high rates (Benador et al 2018). Thus, the energy from *pmF* can be used either to generate ATP (coupled) or heat (uncoupled) depending on the stimuli and UCP-1 activation. In this regard, intrinsically uncoupled respiration, as is the case of TAO, cannot harness energy from electron transfer to hydrogen ion translocation due to the inherent structural nature of its components.

Given the non-conservative nature of respiration in BSF, these parasites maintain $\Delta\Psi_{mt}$ not by ETS activity, but rather by the reversal of F_1F_0-ATP synthase activity (Nolan, Voorheis 1992). To accomplish this, F_1F_0-ATP synthase hydrolyses ATP to allow hydrogen ion translocation across the mtIM towards the intermembrane space. The *pmF* generated

by reversal of F_1F_0-ATP synthase is close to 130-150 mV, (Vercesi et al 1992; Nolan, Voorheis 1992), which allows the transport of ions, metabolites, and nuclear-encoded proteins across the mtIM (Bertrand, Hajduk 2000). Therefore, given the unique features of ETS and F_1F_0-ATP synthase in BSF, we will describe each of these components in more detail below.

Although the discussion on cell death (CD) in unicellular organisms remains open, there is no doubt about the role of mitochondria as one of the main actors in the CD process. Trypanosomatids do not have the classic caspases described in the 1990s in mammals (Tsuji et al 1977; Thornberry et al 1992; Yuan et al 1993). However, these parasites have a group of proteins called metacaspases, described as ancestors of the caspases of multicellular organisms, preserving typical domains found in caspases and similarity in tertiary structure (Minina et al 2017; Welburn et al 2006; Ameisen et al 1995; Meslin et al 2011; Kaczanowski et al 2011). Despite the pointed differences between the CD processes in pathogenic trypanosomatids and animals, similarities such as the involvement of typical markers of classical CD processes can be noted. Indeed, trypanosomatids show DNA fragmentation, phosphatidylserine externalization, loss of $\Delta\Psi_{mt,}$ cytochrome *c* release, and formation of the mitochondrial transition pore (Ameisen et al 1995; Debrabant et al 2003; Das et al 2001; Duszenko et al 2006; Menna-Barreto 2019; Morciano et al 2021; Dewar et al 2018; Bustos et al 2017). Therefore, there is sufficient evidence for a key role of mitochondria in the CD process in trypanosomatids.

1.2.1. Glycerol phosphate shuttle as a redox bridge linking glycosomes and mitochondria

The crucial importance of GPSh for BSF deserves a closer look at the general roles of this metabolic pathway in other organisms. In most eukaryotic cells, GPSh directly regulates cellular redox balance in two interconnected ways by: (*1*) providing a mechanism for cytosolic NADH oxidation to NAD^+ to maintain glycolysis and serine biosynthesis; (*2*) transferring cytosolic NADH to mitochondria. GPSh is a quite simple system composed of a cytosolic (NAD-dependent, cG3PDH) and a mitochondrial (FAD-dependent, mtG3PDH) glycerol-3-phosphate dehydrogenase (Mráček et al 2013). The activity of GPSh involves the cytosolic reduction of dihydroxyacetone phosphate (DHAP) to glycerol-3-phosphate (G3P) by cG3PDH using NADH as the electron donor and generating NAD^+. Then, G3P is oxidized back to DHAP by mtG3PDH transferring the electrons to FAD and ubiquinone in the mtIM. In this regard, GPSh represents a metabolic hub that interconnects glucose and lipid metabolism, as well as respiration and mitochondrial ATP production. Indeed, glycolysis is one of the key metabolic sources of DHAP for GPSh, and G3P can be converted to DHAP by mtG3PDH, which can be used as a precursor for gluconeogenesis. Given that glycolysis produces G3P, which can be utilized to support phospholipid and triacylglycerol synthesis, glucose and lipid metabolism are linked through G3P and ultimately by GPSh (Mráček et al 2013).

Mammalian mtG3PDH has been known for decades (Green 1936); it is ~74 kDa and is one of the simplest components of the entire ETS (Mráček et al 2013). The activity of mtG3PDH is directly linked to respiration and mitochondrial ATP synthesis, which mediates electron transfer independent of the TCA cycle and Complex I. GPSh is mostly regulated at the mtG3PDH level by allosteric regulation by specific signals including free fatty acids and Ca^{2+} (Wernette et al 1981; Bukowiecki, Lindberg 1974). However, mitochondrial G3P oxidation is linked to other ETS components and can be indirectly

controlled by their respective regulators. For example, in *Aedes aegypti* mosquitoes, mitochondrial G3P oxidation is indirectly controlled by ATP through allosteric regulation of cytochrome *c* oxidase (Complex IV, CIV) activity by adenylate levels (Gaviraghi et al 2019).

In mammalian cells, mtG3PDH protein levels are low compared to cG3PDH levels which limits the GPSh activity. However, the role of GPSh in mammalian brown adipose tissue (BAT) is particularly relevant, as mtG3PDH has the highest activity in BAT than any other tissue (Houstěk et al 1975; Ohkawa et al 1969). Importantly, cG3PDH and mtG3PDH in BAT have equivalent activities, a key requisite for a functional GPSh (Ohkawa et al 1969). Although the potential contribution of GPSh to BAT thermogenesis remains to be determined, the very nature of mtG3PDH activity might give some hints about this process. Considering that mGPDH activity is not coupled to hydrogen ion translocation across the mtIM, energy conservation through GPSh is expected to be low (Masson et al 2017; Syromyatnikov et al 2013; Miwa et al 2003; Gaviraghi et al 2019; Soares et al 2015). Indeed, a rat model resistant to diet induced obesity is strongly associated with increased liver mGPDH expression (Taleux et al 2009). Also, in bumblebee flight muscles mitochondrial G3P oxidation is intrinsically and poorly coupled to ATP production with a strong thermogenic role especially under cold exposure (Syromyatnikov et al 2013; Masson et al 2017). The broader picture across different organisms reveals a clear association between increased GPSh function and reduced mitochondrial energy efficiency triggered by nutritional and environmental stresses.

T. brucei codes for a single glycosomal NAD^+ dependent G3PDH (systematic number Tb927.8.3530 at https://tritrypdb.org) and surprisingly two FAD^+ dependent mitochondrial G3PDH sequences (Tb927.11.7380 and Tb927.1.1130), a feature only shared with *Leishmania major* (Škodová et al 2013). Tb927.8.3530 codes for a protein which is similar to human cG3PDH. On the other hand, both mitochondrial sequences are slightly shorter than mammalian mtG3PDH: Tb927.11.7380 codes for a 67 kDa protein with 603 amino acids, while Tb927.1.1130 codes for a 66.2 kDa protein with 617 amino acids. Importantly, even though both sequences have FAD-dependent oxidoreductase domains, a notable distinction between these and mammalian mtG3PDH is the apparent absence of the canonical Ca^{2+} binding motif at the C-terminus. It is long known that Ca^{2+} binds to the EF-hand domain of mammalian mtG3PDH, which increases its affinity for G3P and boosts mitochondrial superoxide production (Wernette et al 1981; Orr et al 2012). Conceivably, the absence of Ca^{2+} regulation renders *T. brucei* mtG3PDH insensitive to this cation as a possible mechanism to reduce mitochondrial superoxide production.

While the subcellular localization of Tb927.1.1130 remains to be determined, proteomic analyses and ectopic expression of Tb927.11.7380 support its mitochondrial localization (Guerra et al 2006). Glycosomal and mitochondrial G3PDH activities were first identified in *T. brucei* BSF (Opperdoes et al 1977), but a functional GPSh has also been demonstrated in *T. brucei* PCF establishing a functional redox link between glycosomal and mitochondrial metabolism (Guerra et al 2006). Although glucose is considered the main nutrient for BSF, glycerol can also be oxidized in a glycerol kinase and TAO-dependent manner and can replace glucose to support cell growth (Pineda et al 2018). In addition, gluconeogenesis and PPP are sustained by glycerol metabolism in BSF, especially under glucose shortage (Kovářová et al 2018). Interestingly, respiration supported by glycerol oxidation is insensitive to uncoupling agents or inhibition of F_1F_O-ATP synthase, suggesting that collapse of $\Delta\Psi_{mt}$ does not affect mitochondrial glycerol

metabolism (Pineda et al 2018). Regarding genetic disruption of GPSh, silencing of mtG3PDH impairs BSF growth while causing no apparent effect on PCF (Škodová et al 2013). Surprisingly, when the alternative rotenone-insensitive proxy of Complex I (NADH:ubiquinone oxidoreductase, NDH2) is depleted in PCF, a compensatory increase in mitochondrial G3P oxidation is observed (Verner et al 2013). However, the opposite is not true as mtG3PDH silencing causes no apparent effect on NADH-induced respiration in PCF (Škodová et al 2013). Nevertheless, GPSh plays a key metabolic role in supporting BSF growth.

1.2.2. Trypanosome alternative oxidase mediates respiration by shortcutting electron transfer

Alternative oxidases (AOX) are small enzymes that mediate respiration in many organisms from algae and bacteria, plants, nematodes, and even ascidians (May et al 2017). AOX belongs to the family of non-heme di-iron carboxylate protein, which is shared by many proteins including ribonucleotide reductase and others. In eukaryotes, AOX is a mtIM homodimer protein that faces towards the matrix side. Like CIV, AOX catalyzes the complete reduction of O_2 to water but through unique mechanisms: (*1*) AOX uses ubiquinol as an electron source rendering respiration cyanide-insensitive as a result of the alternative electron path (Chance, Hackett 1959; Clarkson et al 1989; Chaudhuri et al 1995); (*2*) the energy from electron flow is not conserved as *pmF*, and can be dissipated as heat as shown in thermogenic plants (Elthon, McIntosh 1987). The thermogenic role of AOX in plants has a key biological significance as the increase in temperature of the flowers induces the evaporation of compounds that attract pollinators (Wagner et al 2008); (*3*) AOX strongly associates with cellular redox balance since its expression and activity are regulated by and regulates cellular oxidant levels (Wagner 1995).

A remarkable metabolic feature of *T. brucei* life-stages is the change in respiratory mode. While in PCF reduction of O_2 to water is mediated by a cyanide-sensitive CIV, in BSF respiration is carried out by a cyanide-resistant and salicylhydroxamic acid-sensitive TAO (Chaudihuri et al 2002). *T. brucei* TAO is coded by two nuclear-encoded genes (Tb927.10.9760 and Tb927.10.7090) that are mainly expressed in BSF (Butter et al 2013). The mitochondrial localization of the protein products of Tb927.10.9760 and Tb927.10.7090 genes has been determined (Hamilton et al 2014). The first molecular structure for AOX was determined for the *T. brucei* enzyme. TAO has two iron pockets that bind O_2, which distinguishes it from classical cytochrome *a* binding at CIV (Shiba et al 2013).

Phylogenetic studies indicate that *T. brucei* TAO and fungi AOX sequences are phylogenetically related as they cluster in taxonomic distribution analyses (Luévano-Martínez et al 2020; Pennisi et al 2016). Importantly, adenylates were shown to regulate the activity of TAO and fungi AOX (Woyda-Ploszczyca et al 2009; Sakajo et al 1997; Luévano-Martínez et al 2020). Although the mechanism by which adenylates regulate TAO activity remains elusive, it is possible that it shares some similarities with the allosteric regulation of CIV by ATP/ADP (Sakajo et al 1997). Conceivably, *T. brucei* TAO might have specific allosteric binding sites for adenylates that reduce (ATP) or increase (ADP, AMP) its activity depending on energy availability and F_1F_O-ATP synthase activity (Hierro-Yap et al 2021). Inhibition of F_1F_O-ATP synthase by oligomycin reduces *T. brucei* TAO activity. Although direct evidence to explain this effect is missing, it is conceivable that reversal of F_1F_O-ATP synthase activity prevents accumulation of matrix ATP and

maintains TAO activity (Luévano-Martínez et al 2020; Hierro-Yap et al 2021). Oligomycin treatment of BSF raises intramitochondrial ATP levels, suggesting a regulatory axis between F_1F_0-ATP synthase and TAO (Williams et al 2008).

The non-conservative energy flux through the GPSh-TAO respiratory system in BSF raises a central thermodynamic question: does BSF dissipate energy by increasing the heat flux? If so, are there benefits by increasing the heat flux for a unicellular organism, or is it an unavoidable consequence of this particular "metabolic design"? A simple answer for these intriguing questions remains open and has not yet been directly addressed. Temperature regulation in unicellular eukaryotes is considered to be unlikely because of their microscopic size and the fast heat diffusion from cells to the environment (Jarmuszkiewicz et al 2010). However, we think that evidence collected to date suggests that at least part of the chemical energy made available via GPSh-TAO can be diverted to increase the heat flux, which consequently could be translated to an increase in temperature within mitochondria. Indeed, increased heat flux was already quantified by calorimetry not only in intact brown adipocytes, but even in isolated BAT mitochondria (Ricquier et al 1979; Bokhari et al 2021; De Meis et al 2012). Also, a recently developed temperature-sensitive fluorescent probe (MitoThermo Yellow, MTY) allows the assessment of mitochondrial temperature (Arai et al 2015). Although MTY was originally designed to sense intracellular temperature changes due to alterations in the extracellular milieu, MTY fluorescence can be used to quantify temperature increase/decrease by mitochondrial metabolism (Chrétien et al 2018). This was elegantly demonstrated by ectopically expressing AOX in human embryonic kidney 293 cells which caused no apparent effects on respiration and temperature when cells respire through CIV activity. However, when CIV-dependent respiration was blocked, both processes were preserved strongly indicating that the energy made available by the electron short-circuit provided by AOX is engaged in heat dissipation (Chrétien et al 2018). However, we must keep in mind that the rates of chemical reactions govern the changes in the molar enthalpy and thus heat flux. Since respiratory rates are quite similar between BSF and PCF (Markos et al 1989), it is likely that the molar enthalpy change in BSF would be increased given its non-conservative nature of energy transduction. In any case, without a direct assessment of mitochondrial heat flux by calorimetry parallel to oxygen consumption in *T. brucei* life forms, the possibility of GPSh-TAO could increase heat flux remains open.

Several lines of evidence suggest a key role of AOX in regulating mitochondrial superoxide production (Popov et al 1997; Maxwell et al 1999; Cvetkovska, Vanlerberghe 2012; Fang, Beattie 2003; El-Khoury et al 2013) and conferring tolerance to redox insults in different organisms (Giraud et al 2008). Despite TAO's minor role in PCF mitochondrial metabolism, when these parasite forms are stressed by redox challenges, TAO expression and activity increase (Fang, Beattie 2003). Importantly, pharmacological inhibition of TAO in BSF strongly induces superoxide production and protein oxidation, indicating that TAO activity has an antioxidant role (Fang, Beattie 2003). Structural studies revealed that TAO binds H_2O_2 stronger than O_2, and this interaction reversibly inhibits the enzyme at micromolar H_2O_2 concentrations (Yamasaki et al 2021). This indicates that redox imbalance may directly affect mitochondrial and glycosomal metabolism by inhibiting TAO activity.

Finally, *T. brucei* TAO is a potential target for HAT chemotherapy since it has no ortholog in mammals. Several TAO inhibitors were identified with different degrees of potency and specificity (Ebiloma et al 2019). Ascofuranone is the most potent TAO

inhibitor known capable of affecting BSF respiration, ATP production, and viability (Yoshisada et al 1997; Yabu et al 2003). Genetic modulation of *T. brucei* TAO expression revealed relevant phenotypes. For example, TAO silencing strongly reduced BSF growth and respiration while rendering parasites with increased sensitivity to glycerol (Helfert et al 2001). The mechanism of glycerol toxicity lies in its inhibitory effect on ATP production by glycerol kinase which partially sustains parasite energy demand especially under ETS blockage or glucose deprivation (Kovářová et al 2018; Pineda et al 2018). More importantly, overexpression of TAO in PCF causes no effects on parasite growth and as expected, increases the share of cyanide-resistant respiration two-fold (Walker et al 2005). Curiously, TAO overexpression strongly reduces the expression of the CIV subunit IV and cytochrome c_1 while upregulating the expression of the surface coat protein GPEET (Walker et al 2005).

1.2.3. F_1F_0-ATP synthase runs 'backwards' to avoid cell death

Another singular aspect of BSF mitochondria is the reversal of F_1F_0-ATP synthase activity, which transduces energy from ATP hydrolysis for *pmF* generation (Gahura et al 2021). In other eukaryotes, reversal of F_1F_0-ATP synthase activity is observed under conditions when electron transfer through the ETS is limited, either by hypoxia or activation of mitochondrial permeability transition (Rego et al 2001; Power et al 2014; Simbula et al 1997). However, in other eukaryotes, ATP hydrolysis is an acute effect to maintain reverse hydrogen ion pumping by F_1F_0-ATP synthase that, if prolonged, can deplete cellular ATP to critical levels (~40 % of cellular ATP; Ichikawa et al 1990; Campanella et al 2008; Leyssens et al 1996). Thus, short-term maintenance of *pmF* by means of ATP synthase reversal is limited by the availability of cellular ATP mainly supplied by glycolysis (Chalmers-Redman et al 1999). The uniqueness of reversal of F_1F_0-ATP synthase activity in BSF is that it operates chronically along this parasite life-form, which, to the best of our knowledge, has no parallel in nature.

In BSF, reversal of F_1F_0-ATP synthase activity generates a *pmF* higher than 190 mV and is classically regulated in many organisms by the inhibitory factor 1 (IF1) which impairs specifically the ATPase but not ATP synthase activity (Schnaufer et al 2005; Pullman, Monroy 1963; Nolan, Voorheis 1990; 1992). *T. brucei* expresses IF1 (TbIF1) only in PCF exhibiting specific inhibitory effects of the ATPase activity (Panicucci et al 2017). As expected, overexpression of TbIF1 in BSF collapses $\Delta\Psi_{mt}$ and promotes cell death, underscoring the critical role of reversal of F_1F_0-ATP synthase activity for BSF survival (Panicucci et al 2017).

The energy provision to sustain this $\Delta\Psi_{mt}$ is a critical aspect to consider, as many cellular processes compete for ATP. The likely ATP source to sustain the $\Delta\Psi_{mt}$ by F_1F_0-ATP synthase reversal is the cytosolic ATP pool generated by PK (reviewed in Michels et al 2021; Nolan, Vooheis 1992). Possible mechanisms for ATP transport into the mitochondrial matrix are the adenine nucleotide translocator (ANT) and the mitochondrial Ca^{2+}-dependent ATP-Mg^{2+}/P_i exchanger (SLC25A25). ANT seems to be the major route for cytosolic ATP to reach the F_1F_0-ATP synthase (Hierro-Yap et al 2021). An alternative source of ATP is the coupled activity between acetate:succinate CoA transferase (ASCT) with succinyl-CoA synthetase (SCS) which generates acetate and ATP in BSF via succinyl-CoA production within the mitochondrial matrix (Mochizuki et al 2020).

Regardless of the ATP source, the $\Delta\Psi_{mt}$ is required for the import of nuclear encoded mitochondrial proteins (Neupert 1997) and allows the transport of ions and metabolites including Ca^{2+} (Huang et al 2013; Docampo, Lukeš 2012; Lukeš, Basu 2015). For example, maintenance of mitochondrial respiration in BSF is essential for mtDNA stability and for the establishment of the parasite's life cycle. From a regulatory point of view, the reversed F_1F_O-ATP synthase function in BSF is responsible for maintaining a proper level of intramitochondrial ATP and modulating both G3PDH and TAO activities (Gahura et al 2021; Hierro-Yap et al 2021; Luévano-Martínez et al 2020).

1.3. Do PCF and BSF exhibit different redox susceptibilities?

Despite few studies addressing redox susceptibility of PCF and BSF, some evidence suggests that BSF are more sensitive to various oxidants than PCF (Rossi, Dean 1988; Meshnick et al 1977). For example, the trypanolytic effect of extracellular H_2O_2 exposure is more prominent in BSF than for PCF (Rossi, Dean 1988). Exposure of PCF to 100 μM H_2O_2 for 3 h caused 35 % of cell lysis, while the same effect was exerted by exposing BSF to only 1 μM for the same time (Rossi, Dean 1988). The differential redox susceptibilities of PCF and BSF were assessed by testing the effect of heme, the prosthetic group of hemeproteins which is a pro-oxidant and mediates the oxidation of lipids in the protein-free state, proteins, and nucleic acids (Ferreira et al 2018). Similar to extracellular H_2O_2 exposure, PCF were more resistant to free heme exposure than BSF (Meshnick et al 1977). Although the mechanistic basis for the BSF redox susceptibility is not fully understood, the lack of glutathione reductases, thioredoxin reductases, and catalase and reduced activity of iron superoxide dismutase may partly explain this trait (Kabiri, Steverding 2001; Tomás, Castro 2013; Fang, Beattie 2003).

A key missing aspect in *T. brucei* redox biology is a clear definition of the main cellular sources and the specific sites of mitochondrial ROS production during the parasite's life cycle. A critical limitation to address this issue is the absence of a systematic assessment of substrate preferences to sustain physiological mitochondrial superoxide production in different *T. brucei* life-forms. This is an important aspect as it has long been known that mitochondria represent the dominant source of cellular oxidants, which are generated at different sites (Boveris, Chance 1973; Wong et al 2017). The evidence available indicates that mitochondrial superoxide is produced at low levels in BSF especially when TAO is inhibited (Fang, Beattie 2003). Although direct comparisons of endogenous ROS production between BSF and PCF were not yet carried out, it seems that BSF produces less mitochondrial superoxide than PCF as determined by electron paramagnetic resonance studies (Fang, Beattie 2003). Regarding the topology of mitochondrial oxidant production, CI and NDH2 (Fang, Beattie 2002), and mitochondrial fumarate reductase (Turrens 1987), are potential sites to support superoxide generation in these parasites.

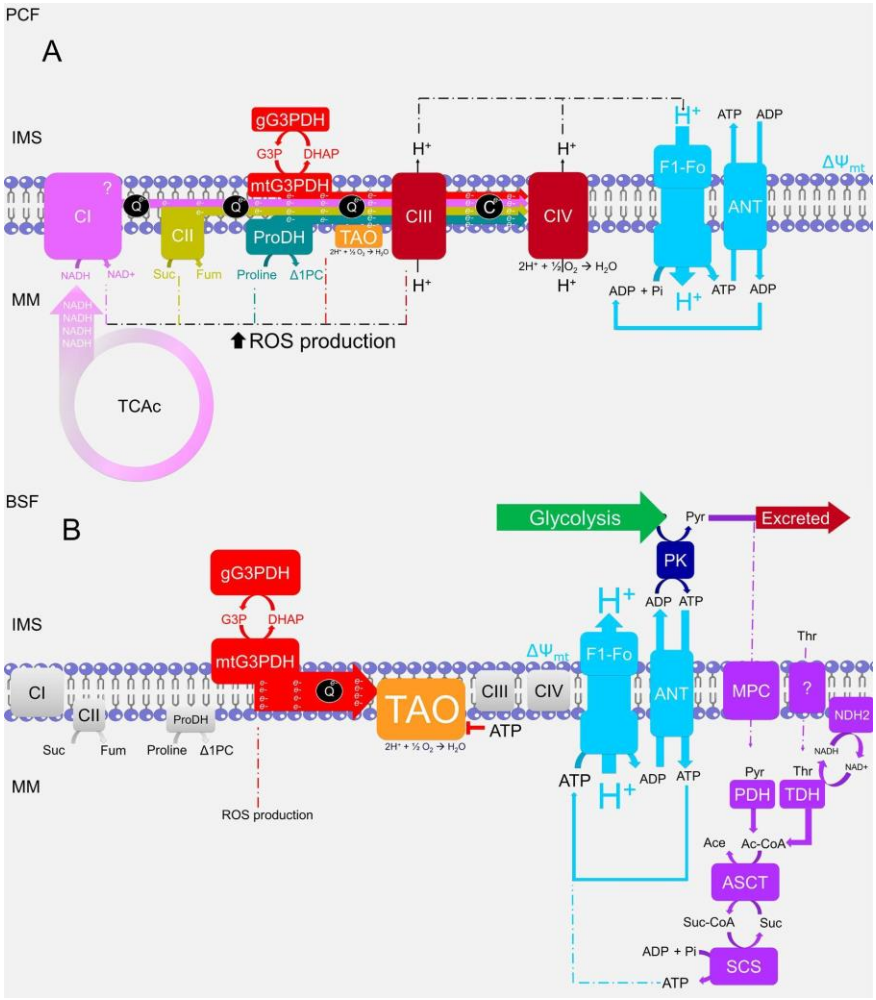

Figure 2. Scheme of mitochondrial electron transfer systems in PCF (A) and BSF (B). In PCF, mitochondrial metabolism is fully developed and comprises the reactions of the tricarboxylic acid cycle (TCAc), electron transfer system (ETS) and oxidative phosphorylation (OXPHOS). This contrasts with reduced mitochondrial metabolism in BSF. ETS reactions in PCF involve multiple dehydrogenases that channel electrons through the NADH dehydrogenase (CI, pink), succinate dehydrogenase (CII, light green), proline dehydrogenase (ProDH, dark green) and mitochondrial glycerol 3 phosphate dehydrogenase (mtG3PDH, red), as well as the electron-carriers ubiquinone (Q, black) and cytochrome *c* (C, black). Electron transfer mediated by mtG3PDH is directly linked to glycosomal G3PDH through the glycerol phosphate shuttle (GPSh). On the other hand, the ETS in BSF is mainly fueled by a single dehydrogenase (mtG3PDH) and GPSh. The fate of electrons flowing through the ETS is distinct between *T. brucei* forms, since in PCF most of the O_2 is reduced by cytochrome *c* oxidase (CIV) while in BSF it is reduced only by a *Trypanosoma* alternative oxidase (TAO, orange). Electron transfer in PCF is coupled to ATP synthesis by OXPHOS, which contrasts with BSF where electron transfer through the GPSh-TAO system is not coupled to hydrogen ion translocation. Thus, the energy from *pmF* in PCF is utilized by the "forward" reaction of the F_1F_0-ATP synthase to generate ATP and sustain cellular energy demands; in BSF, *pmF* is maintained by the "reverse" F_1F_0-ATP synthase activity that hydrolyses ATP to mediate hydrogen ion transport across the inner mitochondrial membrane. There are possibly two cellular sources of ATP to maintain "reversed" F_1F_0-ATP synthase activity: (*1*) the coupled activity of acetate:succinate CoA transferase (ASCT) with succinyl-CoA synthetase (SCS) in the mitochondrial matrix and (*2*) the pyruvate kinase (PK, dark blue), which represents the main source of ATP. The use of cytosolic ATP to maintain BSF *pmF* also requires the reversal of adenine nucleotide translocator (ANT) activity. Generation of mitochondrial superoxide and other ROS is a natural consequence of ETS activity and seems to be higher in PCF compared to BSF. Components of the OXPHOS "phosphorylation module" (F_1F_0-ATP synthase and ANT) are in blue, while those involved in the "oxidation module" (TCAc, Complexes CI, CII,CIII, CIV, ProDH, mtG3PDH) are in pink, dark red, light green, dark green, red, orange. The mechanisms involved in ATP production to support "reversed" F_1F_0-ATP synthase activity and the NAD^+/NADH mitochondrial balance are depicted in purple. Grey-shaded boxes in (B) represent components of the ETS which are detected by quantitative proteomic analyses but have no activity in BSF.

Although trypanosomatids lack canonical scavenging antioxidant enzymes (e.g. catalase, glutathione reductase, thioredoxin reductase), they evolved unique mechanisms to cope with redox insults (Krauth-Siegel et al 2007). The Trypanosomatid-specific trypanothione (T(SH)2)-based system is a complex redox network that plays a key protective role against oxidative stress in these parasites. This system involves non-enzymatic antioxidants such as T(SH)2, tryparedoxin (Tpx) and ascorbate, but also antioxidant enzymes including trypanothione reductase (TR), non-selenium glutathione peroxidase-type enzymes (Pxs) and 2-Cys-peroxiredoxins (Prxs) (Krauth-Siegel et al 2007; Tomás, Castro 2013; Diechtierow, Krauth-Siegel 2011; Wilkinson et al 2003; Bogacz et al 2020). Under hydroperoxide exposure, Pxs and Prxs catalyze the decomposition of hydroperoxides to less reactive reduced alcohols. The oxidized Pxs and Prxs are then regenerated by the TR/T(SH)2/Tpx system in a NADPH-dependent way. Px and Prxs have distinct selectivity for ROS detoxification, since lipid hydroperoxides are preferentially detoxified by Pxs (Hillebrand et al 2003; Schlecker et al 2005; Wilkinson et al 2003), while H_2O_2 and peroxynitrite (ONOO-) are scavenged by Prxs (Tetaud et al 2001; Wilkinson et al 2003).

Compartmentation of antioxidant enzymes plays a role in cell protection against redox insults. Cytosolic Tpx (cTpx) content in BSF is 3-7 times higher than in PCF and its silencing strongly reduces parasite growth and increases sensitivity to extracellular H_2O_2 (Comini et al 2007). This apparently contrasts with the higher redox susceptibility of BSF to oxidants. However, we have to consider two key aspects: (*1*) the physiological effects caused by cTpx silencing were only observed when its expression was reduced to ~5 % of original levels and for long periods of time; (*2*) a similar scenario was observed in TR-silenced BSF, where arrest of cell growth and infectivity was only achieved in cells with >90 % of reduction of TR expression (Krieger et al 2000). This strongly suggests that mitochondrial scavenging antioxidant mechanisms do not play a key protective role in the survival of BSF. Indeed, evidence demonstrates that mitochondrial peroxidases play a minor role for BSF growth and viability (Wilkinson et al 2003; Diechtierow, Krauth-Siegel 2011; Bogacz et al 2020). Conceivably, lower mitochondrial ROS production in BSF relative to PCF might explain the apparent dispensable role of mitochondrial peroxidases for BSF but experimental evidence is needed to fully address this aspect.

In our view, it is quite plausible to assume that ETS in BSF mitochondria through the GPSh-TAO system has a limited capacity to generate mitochondrial ROS. However, the redox susceptibility of BSF might be strongly dependent on the source of oxidant challenge (extracellular, cytosolic, or mitochondrial). In this regard, evidence indicates that depletion of cytosolic antioxidant defenses render BSF more susceptible to extracellular oxidants (Diechtierow, Krauth-Siegel 2011; Comini et al 2007; Wilkinson et al 2003). Thus, the GPSh-TAO system would represent a “preventive” antioxidant defense to limit mitochondrial superoxide production in BSF (Figure 2).

2. Hypothesis: GPSh-TAO system acts as a preventive and complementary antioxidant defense in BSF

Given the unique — and apparently paradoxical — biochemical pathways in *T. brucei*, we propose a hypothesis to explain the complex mechanisms involved in energy and redox metabolism for BSF growth and survival. Our proposal aims at reconciling previous observations and re-interpretation of novel ones in the light of phenomena that have not yet been addressed in detail for BSF.

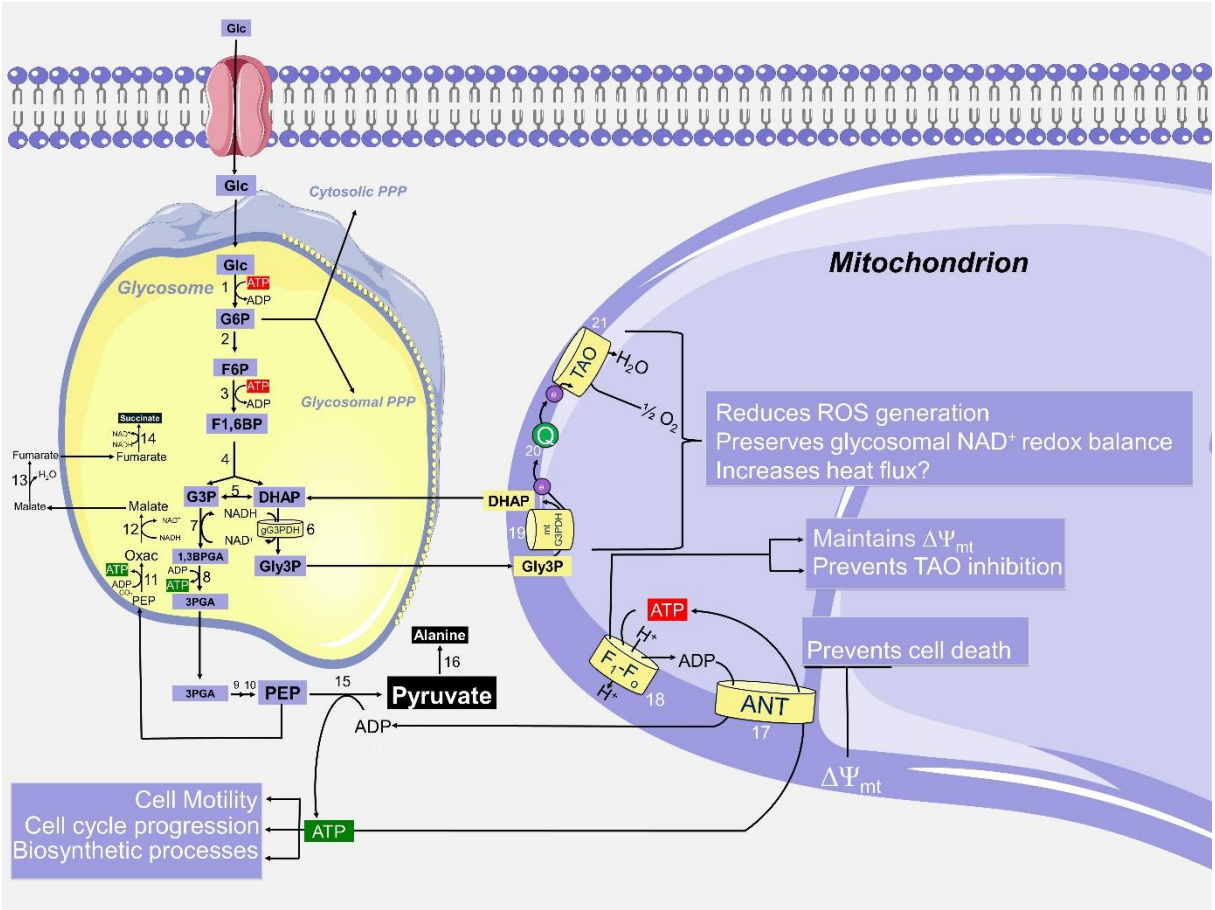

Figure 3. The complex energy metabolism of *T. brucei* BSF. Glycosome and mitochondrial metabolism are linked by GPSh-TAO system and F_1F_0-ATP synthase as a way to stimulate glycolysis and energy demand, limit superoxide production, and prevent CD. Biochemical and cellular outputs provided by the GPSh-TAO-F_1F_0-ATP synthase system are depicted in purple boxes within the mitochondrion. The cytosolic purple box indicates ATP generation by pyruvate, which is linked to motility, cell cycle progression and biosynthetic processes. $\Delta\Psi_{mt}$ - mitochondrial membrane potential; **1** hexokinase; **2** phosphoglucose isomerase; **3** phospho-fructokinase; **4** aldolase; **5** triosephosphate isomerase; **6** glycosomal glycerol 3 phosphate dehydrogenase; **7** glyceraldehyde 3 phosphate dehydrogenase; **8** phosphoglycerate kinase; **9** phosphoglycerate mutase; **10** enolase; **11** phosphoenol-pyruvate carboxykinase; **12** glycosomal malate dehydrogenase; **13** fumarase; **14** fumarate reductase; **15** pyruvate kinase; **16** alanine aminotransferase; **17** adenine nucleotide translocator; **18** F_1F_0-ATP synthase; **19** mitochondrial glycerol 3 phosphate dehydrogenase; **20** ubiquinone; **21** Trypanosome alternative oxidase. ATP production sites are depicted as green boxes while the ATP consuming sites are red boxes. The black boxes represent the main metabolic products excreted by BSF.

Figure 3 schematically depicts how GPSh-TAO and the F_1F_0-ATP synthase work in a concerted way not only to regulate glycolysis in glycosomes but also respiration and ROS production in mitochondria. In this regard, the use of the GPSh-TAO system has the following outcomes for BSF: (*1*) regenerates glycosomal NAD^+ required for glycolytic ATP production, which is the dominant mechanism for maintaining the cellular energy demand, (*2*) provides a safe mechanism for electron sink by preventing mitochondrial superoxide production, (*3*) complements the NADPH-dependent scavenging antioxidant

defenses to cope with cellular oxidants, (*4*) contributes to increased heat flux at subcellular level since the GPSh-TAO system is intrinsically uncoupled to hydrogen ion translocation across the mtIM. Conceivably, heat flux by the GPSh-TAO system would play a role to boost mitochondrial enzyme activities to optimal levels just like observed in mammalian cells (Chrétien et al 2018; El-khoury et al 2022).

On the F_1F_O-ATP synthase side, $\Delta\Psi_{mt}$ is maintained by reversing its activity, hydrolyzing ATP, and allowing hydrogen ion translocation across the mtIM. Given that eukaryotic cells in general undergo mitochondrial-dependent CD upon the collapse of $\Delta\Psi_{mt}$ (Narendra et al 2010; Liu et al 1996), we postulate that reversal of F_1F_O-ATP synthase activity in BSF represents a pro-survival mechanism avoiding CD and prevents TAO inhibition by avoiding accumulation of mitochondrial ATP content (Luévano-Martínez et al 2020).

3. Future perspectives and relevant open questions

Understanding how the GPSh-TAO system prevents mitochondrial ROS production and its contribution to energy dissipation are avenues for future research. The following questions are raised to better understand how knowledge of BSF energy metabolism can be exploited in basic research and innovative therapeutic interventions against HAT:

a) *What are the metabolic/energetic consequences if BSF regenerated glycosomal NAD^+ through fumarate reductase instead of GPSh?*

We hypothesize that forced regeneration of glycosomal NAD^+ using the glycosomal succinic fermentation pathway in BSF leads to significant ATP perturbations as glycosomal ATP content would increase, while cytosolic ATP content would decrease. Potentially, this directly affects cellular energy requirements and the maintenance of $\Delta\Psi_{mt}$ by F_1F_O-ATP synthase, ultimately leading to BSF death.

b) What are the metabolic/energetic consequences if PCF regenerates glycosomal NAD^+ through GPSh instead of fumarate reductase?

We postulate that if the GPSh system (gG3PDH and mtG3PDH) is overexpressed in PCF to an extent comparable to that of BSF, the excess of electrons fuels ETS, possibly leading to increased expression of TAO to avoid superoxide production. Glycosomal ATP balance would be maintained by pyruvate phosphate dikinase (PPDK) activity as a compensatory response in the presence of glucose as the main nutrient source.

c) What are the metabolic/energetic consequences if the PCF mitochondrial electron transfer system involves mainly TAO instead of Complex IV?

Previous studies have shown that overexpression of TAO in PCF has no effect on parasite growth but leads to increased cellular respiration and increased TAO-mediated respiration (Walker et al 2005). Curiously, expression of the COIV subunit of CIV is reduced, suggesting that PCF shifts electron transfer to TAO (Walker et al 2005). Regardless of nutrient availability, increased expression of TAO could lead to a reduction in mitochondrial superoxide because the TAO but not the CIII-CIV pathway is the dominant mechanism of oxygen reduction. In addition, overexpression of TAO could lead to a decrease in $\Delta\Psi_{mt}$ which promotes the reversal of F_1F_O-ATP synthase and ultimately leads to cell death due to ATP limitation of cellular energy demand.

Abbreviations

ACH	acetyl-CoA thioesterase	HK	hexokinase
AOX	alternative oxidase	kDNA	kinetoplast
ASCT	acetate:succinate CoA-transferase	mtIM	mitochondrial inner membrane
BAT	brown adipose tissue	OXPHOS	oxidative phosphorylation
BSF	Bloodstream forms	PCF	procyclic form
CIV	Complex IV, cytochrome *c* oxidase	PFK	phosphofructokinase
cPrx	peroxiredoxin	PGK	phosphoglycerate kinase
DHAP	dihydroxyacetone phosphate	PK	pyruvate kinase
ETS	electron transfer system	*pmF*	protonmotive force
G3P	glycerol 3 phosphate	PPP	pentose phosphate pathway
G3PDH	glycerol-3 phosphate dehydrogenase	Px	peroxidase-type
GK	glycerol kinase	ROS	reactive oxygen species
GPSh	glycerol phosphate shuttle	T(SH)2	trypanothione
HAT	Human African Trypanosomiasis	TAO	Trypanosome alternative oxidase
$\Delta\Psi_{mt}$	mitochondrial membrane potential	TCA	tricarboxylic acid
HEB	high-energy bonds	Tpx	tryparedoxin
CD	cell death	TR	trypanothione reductase
		UCP-1	uncoupling protein-1

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