Cell Metabolism
Article

Mitochondrial Dynamics, Biogenesis, and Function Are Coordinated with the Cell Cycle by APC/C^{CDH1}

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SUMMARY

Cell proliferation is associated with a high rate of aerobic glycolysis, which has been widely interpreted as a compensatory mechanism for suppressed mitochondrial function, despite reports of high respiration rates. The molecular mechanisms that link cell proliferation with mitochondrial metabolism, dynamics, and biogenesis remain obscure. Here, we show that proliferation is associated with an increase in both glycolysis and respiration, in conjunction with mitochondrial fusion and biogenesis. Changes in mitochondrial morphology and mass are due to accumulation of OPA1, MFN1, and TFAM, silencing any of which hinders cell proliferation. Moreover, the levels of OPA1, MFN1, and TFAM are regulated by the ubiquitin ligase APC/C^{CDH1}, which also controls the lysosomal degradation of key glycolytic, gluconeogenic, and cell-cycle proteins. Thus, we have identified an important component of the molecular mechanism that coordinates cell proliferation with activation of the mitochondrial metabolic machinery that provides the necessary energy and biosynthetic substrates.

INTRODUCTION

Cell proliferation is an energetically demanding process that requires enhanced metabolic activity to generate energy and intermediates for de novo biosynthesis of the macromolecules necessary to increase the cell mass. Eukaryotic cells generate energy (ATP) by glycolysis in the cytosol and through oxidative phosphorylation in the mitochondria. In most cell types the majority of cellular ATP is generated by the mitochondria (Saraste, 1999), and factors that compromise mitochondrial function have been shown to inhibit proliferation (Van den Bogert et al., 1988; DiGregorio et al., 2001). When mitochondrial function is impaired (by hypoxia, pharmacological agents, and/or by genetic means), glycolysis is enhanced to compensate for the decreased mitochondrial ATP generation, leading to increased accumulation of lactic acid, the principal by-product of glycolysis. Thus, when high levels of lactate production have been observed in proliferating cells, they have been interpreted as a sign of suppressed mitochondrial function (Guppy et al., 1993; Brand and Hermfisse, [illegible]; Ba[illegible] et al., 2004). Other studies have shown that the mitochondria of proliferating cells are functional, and that the increased rate of production in these cells is not linked to mitochondrial dysfunction (Weinhouse, 1976; Koppenol et al., 2[illegible]). Indeed, a significant increase in mitochondrial oxygen uptake has been observed during cell proliferation (Van den Bogert et al., 1988; Herzig et al., 2000; Schieke et al., 2008). Because of these contradictory reports, the role of mitochondria in cell proliferation remains controversial.

In addition to the generation of ATP, the mitochondria are also vital sources of intermediates for de novo synthesis of the macromolecules required to double the cell mass (Robbins and Morrill, 1969; DeBerardinis et al., 2008; Vander Heiden [illegible]09). These include essential steps in the synthesis of fatty acid, heme, and pyrimidine nucleotides in the cytosol. Factors that inhibit respiration suppress lipid, protein, and nucleotide synthesis and hinder cell proliferation (King and Attardi, 1989; Gattermann et al., 2004). Thus, it is reasonable to expect that the mitochondrial function of proliferating cells should be enhanced.

Mitochondrial function depends on mitochondrial mass, morphology, ultrastructure, and coupling efficiency (Hackenbrock et al., 1971; Arnoult et al., 2005; Chen et al., 2005; Gomes et al., 2011). Cells arrested in G1 have been reported to have defragmented mitochondria with reduced mass and function—a state that is reversed when cells are stimulated to proliferate (Neutzner and Youle, 2005). A strong link between cell proliferation and mitochondrial biogenesis has been reported in several cell types (D'Souza et al., 2007; Lee et al., 2007, 2011; Cormio et al., 2009), as has cell-cycle-dependent changes in mitochondrial morphology (Arakaki et al., 2006; Mitra et al., 2009). Although the proteins that control mitochondrial biogenesis, fusion, and fission have also been identified (Ekstrand et al., 2004; Detmer and Chan, 2007), the mechanisms that coordinate the expression level of these proteins with the cell cycle remain largely unknown (Aguilar and Fajas, 2010).

The increased requirement for energy and metabolic intermediates during cell proliferation suggests that regulation of metabolism (both glycolytic and mitochondrial) must be linked to that of the cell cycle. We have recently identified the molecular mechanism that leads to the increased utilization of glucose and glutamine during cell proliferation. Accordingly, the abundance of PFKFB3 (a key glycolytic regulator enzyme) and GLS1 (the first and rate-limiting enzyme of glutaminolysis) is controlled by the E3 ubiquitin ligase APC/C^{CDH1} (Almeida et al., 2010; Colombo et al., 2010; Tudzarova et al., 2011). Thus, PFKFB3 and GLS1

are released together with S phase cyclins at a nutrient-sensitive critical point in G1 known as the restriction point, allowing for coordination between cell-cycle progression into S phase and the metabolism needed to underpin this phase. These results prompted us to investigate whether the same mechanism may control mitochondrial dynamics and biogenesis during cell proliferation. We have used two types of primary murine cells—T cells and embryonic fibroblasts (MEFs)—that can be stimulated to proliferate by different mechanisms, to investigate whether proliferation is accompanied by increased mitochondrial activity and, if so, whether the mitochondrial proteins responsible for this increase are under the control of APC/C^{CDH1}.

RESULTS

Cell Proliferation Is Associated with Increased Rates of Respiration and Aerobic Glycolysis

To investigate the relationship between cell proliferation, respiration and aerobic glycolysis, we used murine spleen T cells and MEFs that can be induced to proliferate by antigen stimulation (anti-CD3 and anti-CD28 antibodies) or by serum refeeding, respectively. Naive T cells freshly isolated from mouse spleen are normally arrested in G0/G1 of the cell cycle (Figure 1A; 0 hr). Following antigen activation the cells began to proliferate, so that by 72 hr the cells had divided several times (Figure and the majority (53%) was in S phase (Figures 1A and 1C) rate of lactate production was initially low (0.01 μmol/hr/10^6 c but increased 8- and 60-fold after 24 and 72 hr of activati respectively, coinciding with the entry of cells in se (Fig ure 1D). Proliferation was also accompani by a amatic increase in the rate of both basal respirat and mi ondrial ATP synthesis by oxidative phospho latio sensitive respiration, Figures 1E an). Unco g the mitochondria of these cells with an op concentrati carbonyl cyanide 4-(trifluoromethoxy) ylh zone (FCC , 3 μM) increased respiration from approximat (0 hr) to 51 pmol O_2/s/10^6 cells after 72 activation (d not shown), indicating a significant i ase in the respirato y capacity (mitochondrial mass). T near r elation between the percentage of cells in S phase, produc and respiration rates (Figure 1G) s that c olifer is correlated with cellular energetic tab m, bot lytic and mitochondrial. We have a purifie D4$^+$ and C 8$^+$ T cells separately and investigated pr colysis, and respiration after antigenic sti on. We observed that the correlation between metabolism a proliferation is highly maintained and resembles that of the entire ell population (see Tables S1A and S1B available online).

In normally proliferating MEFs, 55%–60% cells were in S phase, 30%–35% in G1, and 5%–10% in G2/M (−72 hr, Figure S1A). Serum starvation for 72 hr resulted in the accumulation of cells in G1 (74%) with 19% in S phase (0 hr, Figure S1A). Within 24 hr of serum refeeding, proliferation was restored so that 59% of cells had reentered S phase; further incubation for 48 hr did not significantly change the cell-cycle profile (Figure S1A). Changes in lactate production and respiration were consistent with progression of the cell cycle (Figures S1B–S1D). After 72 hr of serum starvation, the rate of lactate production dropped dramatically (0 hr, Figure S1B), and both the basal and oligomycin-sensitive respiration rates (Figure S1C, 0 hr) declined significantly from those of proliferating MEFs prior to serum starvation (−72 hr). Within 24 hr of serum refeeding, there was a significant increase in the rate of lactate production and in the basal and oligomycin-sensitive respiration rates (Figures S1B and S1C). The FCCP-uncoupled maximum respiration of proliferating MEFs was 189 pmol O_2/s/10^6 cells (data not shown). After 72 hr serum starvation (0 h e declined to 118 and increased back to 195 p O_2/s/1 lls after 24 hr serum refeeding (data not sho further dem strating that respiratory capacity match s the liferative ate of the cell.

Cell Proliferation Is As ated th Mitochondrial Biogenesis

The increased res tion rate prolif ing cells suggested that they might ore active n ondria, more mitochondrial mass, o oth. s, we moni red the changes in mitochondrial mass after s lating naive T cells and MEFs to prolife e performed ltiparameter analysis, measuring the ount of mitochondrial DNA in the cells (mtDNA copy nu er), the mi hondrial matrix volume (citrate synthase [CS] zymatic ac y), the expression level of the mitochondrial trans on fac (TFAM) and of a representative mitochondrial pro x-iv) by western blotting, and by labeling of mitochondria with MitoTracker Green FM fluorescence. Non-p g (naive) T cells had 55 ± 7.3 mtDNA copies per cell, wh ich increased to 170 ± 13 within 72 hr of stimulation (Figure 2A). A similar trend was observed in pure CD4+ and CD8+ T cells (Tables S1A and S1B). Serum-starved MEFs had 188 ± 14 mtDNA copies per cell, which increased to 304 ± 39 within 24 hr of serum refeeding (Figure S2A), attaining a level similar to that before starvation (315 ± 27 copies per cell). CS enzymatic activity and expression of TFAM and Cox-iv also increased as proliferation progressed in both T cells (Figures 2B and 2C) and MEFs (Figures S2B and S2C). The amount of MitoTracker Green FM accumulated by mitochondria, and consequently its fluorescence intensity, is known to be determined by the mitochondrial mass. Stimulation of T cells to proliferate resulted in an increase in the amount of MitoTracker Green FM accumulation, as shown by both flow cytometry and confocal microscopy (Figures 2D and 2E). The median ± SD MitoTracker Green FM fluorescence intensity (assessed by flow cytometry) following stimulation of these cells to proliferate was 65 ± 9 a.u. (0 hr), 83 ± 7 a.u. (24 hr), 350 ± 25 a.u. (48 hr), and 490 ± 33 a.u. (72 hr). In serum-starved MEFs the median fluorescence of MitoTracker Green FM was approximately 295 ± 21 a.u. This increased to approximately 720 ± 59 a.u. after 24 hr serum refeeding, achieving a level similar to that before serum starvation (Figure S2D). These results indicate that cell proliferation is associated with mitochondrial biogenesis.

Cell Proliferation Is Associated with a Change in Mitochondrial Morphology

We then investigated mitochondrial morphology using confocal microscopy of MEFs stained with MitoTracker Green FM and Hoechst 33342. It was not possible to carry out this type of experiment in T cells because they are too small (especially the nonactivated ones) for detailed mitochondrial morphology