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Resource

Complementary RNA and Protein Profiling Identifies Iron as a Key Regulator of Mitochondrial Biogenesis

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SUMMARY

Mitochondria are centers of metabolism and signaling whose content and function must adapt to changing cellular environments. The biological signals that initiate mitochondrial restructuring and the cellular processes that drive this adaptive response are largely obscure. To better define these systems, we performed matched quantitative genomic and proteomic analyses of mouse muscle cells as they performed mitochondrial biogenesis. We find that proteins involved in cellular iron homeostasis are highly coordinated with this process and that depletion of cellular iron results in a rapid, dose-dependent decrease of select mitochondrial protein levels and oxidative capacity. We further show that this process is universal across a broad range of cell types and fully reversed when iron is reintroduced. Collectively, our work reveals that cellular iron is a key regulator of mitochondrial biogenesis, and provides quantitative data sets that can be leveraged to explore posttranscriptional and posttranslational processes that are essential for mitochondrial adaptation.

INTRODUCTION

Mitochondria are ubiquitous organelles that are essential for cellular energy generation and a range of key metabolic pathways. The production of mitochondria—termed mitochondrial biogenesis—is a complex process involving the orchestrated transcription, translation, and import of more than 1,000 proteins encoded by two genomes (Mick et al., 2011; Pagliarini et al., 2008; Scarpulla, 2008; Schmidt et al., 2010). Moreover, these organelles vary considerably in composition across tissues (Mootha et al., 2003a; Pagliarini et al., 2008) and remodel to meet cellular needs (Baltzer et al., 2010; Hock and Kralli, 2009), indicating that the mitochondrial biogenesis program is customizable and responsive to environmental conditions. Defects in this process are associated with a range of human disorders, including mitochondrial encephalomyopathy with ragged red fibers (MERRF), type 2 diabetes, and various cancers (Calvo and Mootha, 2010; DiMauro and Schon, 2003; Lowell and Shulman, 2005; Wallace, 2005).

During the past two decades, major progress has been made in deciphering the transcriptional networks that drive mitochondrial biogenesis. Of particular importance was the identification of peroxisome proliferator-activated receptor γ, coactivator 1 α (PGC-1α) (Puigserver et al., 1998). PGC-1α and related coactivators PGC-1β (Kressler et al., 2002; Lin et al., 2002) and PRC (PGC-1 related coactivator) (Andersson and Scarpulla, 2001) coordinate and activate the various transcription factors required for mitochondrial biogenesis (Scarpulla, 2008). Additionally, PGC-1α is activated by exercise, adaptive thermogenesis, changes in cellular redox state, and the availability of nutrients and growth factors (Hock and Kralli, 2009), helping to explain how mitochondrial content is responsive to changing cellular conditions.

Despite significant advancements in our understanding of PGC-1α and its corresponding transcription factors, important aspects of the cellular control of mitochondrial content remain unclear. These include posttranscriptional processes that control mitochondrial gene expression, mechanisms of active mitochondrial degradation and clearance, and extramitochondrial processes that help coordinate communication between mitochondria and the nucleus (Goldenthal and Marín-García, 2004). Posttranscriptional control mechanisms, such as upstream open reading frames (uORFs) (Calvo et al., 2009), iron-responsive elements (IREs) (Eisenstein and Ross, 2003), and microRNAs (Li et al., 2012), are already known to affect the expression of select mitochondrial genes, and the global discordance between cellular mRNA and protein levels suggests that these mechanisms are likely more widespread (Mootha et al., 2003a). Additionally, macroautophagy (i.e., mitophagy) is emerging as an important mechanism for eliminating damaged mitochondria (Youle and Narendra, 2011). However, identifying additional genes subject to posttranscriptional regulation, and spotlighting cellular processes that help synchronize the mitochondrial biogenesis program, would benefit from matched, cell-wide quantitative data of protein and mRNA abundance, which has largely been lacking.

Here, to produce such a resource, we performed parallel quantitative SILAC (stable *i*sotope *l*abeling by *a*mino acids in

cell culture)-based proteomics (Ong and Mann, 2006) and microarray analyses of PGC-1α-induced mitochondrial biogenesis in mouse muscle C2C12 cells. In doing so, we find that key proteins involved in maintaining cellular iron homeostasis are correlated with mitochondrial biogenesis. We further reveal that depriving various cell types of iron through chelation or active transport leads to a rapid and dose-dependent attenuation of mitochondrial transcript and protein levels that is fully reversible within 3–4 days. Together, our work demonstrates that iron deprivation results in an active and coordinated downregulation of mitochondrial gene expression, suggesting that the bioavailability of iron is a key parameter for establishing a set point of cellular mitochondrial activity. Because iron deficiency anemia is the most common nutritional disorder worldwide (McLean et al., 2009), this work has broad implications for understanding mitochondrial dysfunction in human health and disease. Additionally, our data serve as a resource for investigating genes subject to posttranscriptional regulation and for identifying additional auxiliary pathways that might be important for calibrating or modulating the mitochondrial biogenesis program.

RESULTS AND DISCUSSION

Complementary RNA and Protein Profiling of Mitochondrial Biogenesis

We sought to better define the mitochondrial biogenesis program in C2C12 mouse myotubes by performing complementary RNA and protein profiling. To maximize the transcriptional activation of mitochondrial genes, we overexpressed PGC-1α—the predominant transcriptional coactivator that drives mitochondrial biogenesis—more than 200-fold using an adenovirus-mediated delivery system. We chose C2C12 cells as a model because overexpression of PGC-1α in this cell line is sufficient to cause an approximate doubling of mitochondrial mass in 3 days (Wu et al., 1999). This approach allows us to assess the relative contribution of posttranscriptional mechanisms in regulating mitochondrial gene expression and provides a more complete assessment of the cell-wide proteomic changes that accompany PGC-1α-induced mitochondrial production.

Following PGC-1α overexpression, we tracked changes in cellular mRNA and protein levels using microarrays and quantitative SILAC proteomics (Ong and Mann, 2006), respectively (Figure 1A). Consistent with previous studies, our microarray analyses showed that PGC-1α causes a robust increase in the transcript abundance of nuclear-encoded mitochondrial genes, especially those involved in oxidative phosphorylation (OxPhos) (Figure 1B). Our SILAC data revealed similar results for protein levels: of 442 mitochondrial proteins quantified using at least two unique peptides, 263 significantly increased in abundance (Figure 1C). As expected, PGC-1α-induced mRNA and protein fold changes were largely consistent in the direction of change (Figure S1A). However, the protein and mRNA abundances differed by as much as 9-fold for nuclear-encoded mitochondrial genes, and for 97 of these genes, mRNA abundance was increased, whereas the corresponding protein abundance was decreased (Table S1; Figure S1B). This mRNA:protein discordance reveals that regulation of protein stability or translation is likely to be important for titrating the expression level of select genes during PGC-1α-induced mitochondrial biogenesis. More broadly, these observations reveal the utility of our resource for spotlighting mitochondrial proteins whose expression may be subject to multiple levels of regulation.

Iron Chelation Causes a Pervasive Dampening of Mitochondrial Protein and Transcript Levels

Our experimental approach also enabled us to investigate peripheral genes and pathways that may be important for the mitochondrial biogenesis program. Interestingly, we noted that proteins involved in regulating cellular iron levels were among the most highly up- and downregulated proteins in our SILAC analyses. The transferrin receptor, which is responsible for transporting transferrin-bound iron into cells, increased more than 4-fold with PGC-1α overexpression (Figure 1C). Reciprocally, the level of ferritin heavy chain, part of the ferritin complex that sequesters cellular iron, was decreased (Figure 1C). These results suggest that iron might be essential for the mitochondrial biogenesis program. Increased cellular iron availability during mitochondrial biogenesis might simply be necessary to accommodate the mitochondrial proteins that contain iron as a cofactor or may occur in anticipation of increased output from the mitochondrial iron-sulfur cluster biogenesis pathway (Lill, 2009; Richardson et al., 2010). However, the magnitude of these changes prompted us to explore whether cellular iron levels might impact the mitochondrial biogenesis program holistically.

To test whether loss of cellular iron is sufficient to induce a restructuring of cellular mitochondrial content, we modified the experimental approach outlined in Figure 1A. Here, in lieu of PGC-1α overexpression, we treated the cells with deferoxamine (DFO), a clinically used cell-permeable iron chelator (Chaston and Richardson, 2003). Strikingly, the microarray results revealed that DFO treatment strongly diminished the abundance of mitochondrial transcripts (Figure 1D). Overall, the magnitude of the DFO effect was as robust as the powerful PGC-1α effect and was also most prominent for genes encoding proteins involved in OxPhos (Figures 1E and S1C). Once again, our mRNA and protein measurements were largely consistent in direction and magnitude (Figure S1D); however, there were notable differences in these measurements among the OxPhos complexes. Complexes I and II, each of which contain multiple iron-sulfur clusters, were most strongly affected, with protein levels more decreased than the corresponding mRNA levels (Figure 1F). Conversely, transcripts encoding complex V subunits were decreased, whereas their corresponding protein levels were increased or unchanged (Figure 1F). A recent, large-scale study of protein dynamics found that mitochondrial proteins, including complex I and complex V, have largely similar turnover rates under normal conditions (Price et al., 2010), suggesting that the differences in complex I and complex V subunit levels following iron deprivation likely involve posttranscriptional regulation.

To assess whether cellular iron levels affect PGC-1α-induced mitochondrial biogenesis, we repeated the experimental approach a third time with PGC-1α adenovirus added to cells simultaneously with DFO. Here, the presence of DFO had a