Mechanisms of mitochondrial response to variations in energy demand in eukaryotic cells

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Devin A, Rigoulet M. Mechanisms of mitochondrial response to variations in energy demand in eukaryotic cells. *Am J Physiol Cell Physiol* 292; C52–C58, 2007. doi:10.1152/ajpcell.00208.2006.—This review focuses on the different mechanisms involved in the adjustment of mitochondrial ATP production to cellular energy demand. The oxidative phosphorylation steady state at constant mitochondrial enzyme content can vary in response to energy demand. However, such an adaptation is tightly linked to a modification in both oxidative phosphorylation yield and phosphate potential and is obviously very limited in eukaryotic cells. We describe the three main mechanisms involved in mitochondrial response to energy demand. In heart cells, a short-term adjustment can be reached mainly through metabolic signaling via phosphotransfer networks by the compartmentalized energy transfer and signal transmission. In such a complex regulatory mechanism, Ca2+ signaling participates in activation of matrial dehydrogenases as well as mitochondrial ATP synthase. These processes allow a large increase in ATP production rate without an important modification in thermodynamic forces. For a long-term adaptation, two main mechanisms are involved: modulation of the mitochondrial enzyme content as a function of energy demand and/or kinetic regulation by covalent modifications (phosphorylations) of some respiratory chain complex subunits. Regardless of the mechanism involved (kinetic regulation by covalent modification or adjustment of mitochondrial enzyme content), the cAMP signaling pathway plays a major role in molecular signaling, leading to the mitochondrial response. We discuss the energetic advantages of these mechanisms.

yeast; C6 glioma cells; muscle; kinetic regulation

MITOCHONDRIA ARE INTRACELLULAR organelles that play an important role in eukaryotic cells. They are known to intervene not only in energy transduction and some intermediary metabolism pathways, but also in Ca2+ homeostasis, cell signaling, and apoptosis. One important feature of these organelles is that they harbor their own circular genome. In mammalian cells, the mitochondrial DNA contributes 13 mRNA, 22 tRNA, and 2 rRNA molecules, which are essential to mitochondrial function. All 13 mRNA molecules encode components of the oxidative phosphorylation apparatus. These 13 components are combined with nuclear encoded proteins to form multisubunit holoenzymes: cytochrome oxidase, respiratory chain complexes 1 and 3, and ATP synthase. The function of these holoenzymes is clearly impaired if the contribution of the mitochondrial or nuclear genome is absent (20). Indeed, most of the proteins belonging to mitochondria are encoded by the nuclear genome. Thus mitochondrial biogenesis involves the coordinated expression of hundreds of genes at the nuclear and the mitochondrial level. Over the past few years, there has been a better understanding of the interaction between these two genomes leading to functional mitochondria. Depending on the physiological stimulus, several transcription factors are known to be activated, and their recognition sequence on various mitochondrial protein-encoding genes has been identified. Moreover, mitochondrial composition can change. Indeed, mitochondrial content is commonly estimated by 1) the maximal respiratory capacity, 2) the activity of a typical marker enzyme, e.g., citrate synthase, or 3) the content of a single mitochondrial protein. This assumption is valid if one considers the mitochondrial content to be regulated as a whole; i.e., expression of every single mitochondrial protein is regulated in the same way. This assumption should be seriously reconsidered, since mitochondrial protein composition has been shown to vary, e.g., in response to chronic exercise (19, 21, 31, 43). Such a peculiar organelle biogenesis might be linked to the fact that mitochondrial biogenesis requires the cooperation of the nuclear and mitochondrial genomes. Thus, depending on the signaling pathway involved and the way it regulates the expression of genes encoding mitochondrial proteins, mitochondrial compositions might be different.

Mitochondria are responsible for countless functions, including the ATP generation from metabolic fuels through oxidative phosphorylation. Strong reducing agents, such as NADH and FADH2, donate electrons to the respiratory chain, resulting in the establishment of an electrochemical potential difference in protons across the mitochondrial inner membrane. This proton electrochemical potential difference is, in
turn, used by the mitochondrial $F_0F_1$-ATP synthase, which couples the proton input to ATP synthesis. Mitochondria have to make energy conversion meet energy demand, which can vary to a consequential extent. To do so, one can expect mitochondria to use at least three means, which are not exclusive. The first is a change in the oxidative phosphorylation rate. Inasmuch as it is well established that oxidative phosphorylation in the living cell is not functioning to its capacity (i.e., the maximal respiratory capacity is usually higher than the spontaneous respiratory rate), an increase or a decrease in oxidative phosphorylation rate would allow an increase or a decrease in energy conversion flux (i.e., the rate of ATP synthesis). Such an adaptation involves a modification in associated forces and in oxidative phosphorylation yield (15, 32). The second is a change in the oxidative phosphorylation steady state by kinetic regulation of one or more controlling steps, such as complex I and cytochrome $c$ oxidase. Finally, energy demand could also be met by a change in the level of mitochondrial enzymes per cell, with a constant steady state in the activity of each compound. In this last case, associated forces and oxidative phosphorylation yield may not be affected.

In this short review, we evaluate what is known about the mechanisms of mitochondrial adaptation to changes in cellular energy demand.

**MITOCHONDRIAL RESPONSE THROUGH VARIATIONS IN CELLULAR MITOCHONDRIAL ENZYME CONTENT**

Strict adjustment between growth and mitochondrial enzyme content in yeast. Microbial growth is a striking adaptation of microorganisms to environmental changes, with the goal to reach a compromise among growth rate, growth yield, and thermodynamic growth efficiency. The growth yield is the part of the energy input (substrate consumption) that is converted to biomass during growth. Over the last two decades, a complete thermodynamic description of growth processes has been obtained by establishment of the balanced chemical reactions for anabolism and catabolism. Thus, by application of nonequilibrium thermodynamics, two parameters have been defined to assess growth efficiency: thermodynamic Gibbs energy efficiency and thermodynamic enthalpy efficiency. The estimation of the former parameter for microbial growth remains difficult because of the lack of knowledge about the Gibbs energy change of substrates, products, and biomass. In contrast, the quantification of enthalpy efficiency has been successfully achieved for microorganisms as well as cultured mammalian cells. This approach is based on the continuous measurement of heat production and the exhaustive determination of substrate and by-product concentrations, thus allowing the construction of enthalpy balances (see Refs. 17, 50, and 51 for reviews). In microorganisms, the strategy of optimizing growth rate and growth yield has mainly been studied under conditions of active exponential growth (17, 26, 42). We analyzed the variation in enthalpy growth yield under conditions of long-term adaptation of yeast cells in response to carbon source depletion in the culture medium. The enthalpy growth yield is defined as the enthalpy variation corresponding to the complete combustion of the formed biomass divided by the enthalpy variation corresponding to the complete combustion of the consumed substrates. We particularly focused on the role of the mitochondrial compartment during the transition from exponential growth to stationary phase during aerobic metabolism.

As stated above, growth is the result of the coupling between substrate catabolism and various anabolic reactions during net biomass synthesis. Under respiratory metabolism, energy transduction is mainly achieved through mitochondrial oxidative phosphorylation. In this process, ATP cycling, which depends on the phosphate potential (ATP/ADP-Pi), plays a central role. Energy delivered by substrate catabolism (energy input) is partly used for biomass synthesis and dissipated as heat. Energy output necessary for cell homeostasis and life is referred to as maintenance.

During the growth of yeast in a medium containing a limiting amount of a nonfermentable substrate such as lactate, we estimated the growth yield from measurement of four parameters: the net substrate (lactate) consumption, the by-product formation, the biomass formed, and the heat production, which is the part of the energy input lost for maintenance and energy transduction processes. Three distinct growth phases can be defined: 1) in the early exponential phase, the heat production increased in parallel with biomass production; 2) in the transition (or late exponential) phase, when about one-third of the lactate had been consumed, the heat dissipation pattern no longer correlated with the biomass increase; and 3) in the stationary phase, lactate was exhausted and the heat production rate eventually reached a constant and low value, consistent with the growth arrest (10). Energy balance in each phase may be calculated with integrated values of net substrate consumption (energy input) and net product formation, including biomass and heat dissipation (energy output), transformed into energy units. The part of the energy input converted to biomass was nearly the same during the early exponential and
Fig. 2. Mitochondrial oxidative phosphorylation. Oxidative phosphorylation results in a chemiosmotic coupling between $F_0F_1$-ATP synthase and the proton pumps of the respiratory chain. At any steady state (e.g., constant proton electrochemical potential difference across the inner membrane, constant ATP synthesis, and respiratory rates), the proton efflux by the respiratory chain compensates the proton leak and the proton influx through the ATP synthase. Inset: ATP-to-O ratio as a function of respiratory rate during Pi titration of the OL556 strain (6), in which the high-affinity phosphodiesterase (PDE2) and CDC25 were inactivated, rendering this strain sensitive to exogenous cAMP. Consequently, in the presence of cAMP in the culture medium, OL556 cells possess all the characteristics of Ras-cAMP pathway-overactivated mutant strains (13). OL556 cells were aerobically grown with 0.2% lactate, addition of 3 mM cAMP increased the growth rate from 0.15 to 0.2 per hour, which is close to the growth rate measured in the presence of 2% lactate (Table 1). In this case, the growth rate remained unchanged after cAMP addition. Moreover, for cells grown with 0.2% lactate, cAMP addition did not significantly change the cell protein content expressed as dry mass. In contrast, cAMP treatment led to a markedly high protein accumulation in cells grown with 2% lactate. By multiplying the cell protein content by the respective growth rate, we observed that cAMP addition increased the protein synthesis rate to about the same extent regardless of the concentration of lactate in the medium (45% and 55% increase transition phases, indicating that the enthalpy growth yield remained constant (10). However, during the transition phase, the ATP demand for growth decreased and the nature of the mechanism(s) responsible for the maintenance of the enthalpy growth yield was in question.

We observed a parallel decrease in the activities of all the measured mitochondrial enzymes (i.e., D,L-lactate dehydrogenase and citrate synthase) as well as a decrease in the amount of cytochromes, indicating that the adaptive process involves a decrease in the amount of mitochondria per cell, but not a change in the oxidative phosphorylation steady state (10). If the part of energy used for maintenance is taken into account, it can be concluded that mitochondria themselves are the major heat dissipative system in a fully aerobic metabolism (11) and that the parallel decrease in the ATP demand and in the amount of mitochondria when growth rate decreases leads to a constant enthalpy growth yield.

Involvement of the cAMP-protein kinase A signaling pathway in the yeast mitochondrial adaptive response. In mammalian cells, several cAMP targets and transcription factors seem to be involved in the upmodulation of mitochondrial biogenesis when energy demand increases (see below). In the yeast Saccharomyces cerevisiae, the Ras-cAMP-protein kinase pathway is involved in many physiological adaptations of cells when the environmental changes. This includes the diauxic shift, responses to nutrient starvation, oxidative stress, and heat shock (6, 23, 40, 44, 46, 53). We analyzed the ability of various mutants of the Ras-cAMP-protein kinase pathway to develop their mitochondrial compartment when grown on lactate. In the yeast Ras cascade (Fig. 3), CDC25 catalyzes the conversion of GDP-Ras1 and GDP-Ras2 to GTP-Ras1 and GTP-Ras2, which are the activators of CYR1, the adenylate cyclase (47) catalyzing cAMP synthesis. The cAMP intracellular concentration thus depends on the respective activities of CYR1 and the phosphodiesterases PDE1 and PDE2. High cAMP concentrations promote the dissociation of the regulatory subunit (BCY1 (49)) from the catalytic subunits [TPK1, TPK2, and TPK3 (48)], thus activating the catalytic subunits of protein kinase A (PKA), which phosphorylates a variety of substrates. The mutants used in our study (12) were 1) a mutant disrupted for Ras2, which leads to underactivation of the Ras-cAMP signaling pathway and 2) three mutants in which the Ras-cAMP-protein kinase signaling pathway is overactivated: IRA1 and IRA2 gene disruptants, an RAS2val19 point mutant, and a BCY1 inactivation mutant. The mutant disrupted for the Ras2 gene is characterized by a slight decrease in the content of all the respiratory chain cytochromes and in maximal respiratory capacity. By contrast, regardless of the mutation, overactivation of this signaling pathway induced a twofold increase in the cellular content of all the cytochromes, except cytochrome oxidase in the RAS2val19 mutant. These changes in cytochrome content correlated with the variation in respiratory capacity.

A direct involvement of cAMP in the regulation of cellular mitochondrial content has been further underscored by studying the OL556 strain (6), in which the high-affinity phosphodiesterase (PDE2) and CDC25 were inactivated, rendering this strain sensitive to exogenous cAMP. Consequently, in the presence of cAMP in the culture medium, OL556 cells possess all the characteristics of Ras-cAMP pathway-overactivated mutant strains (13). OL556 cells were aerobically grown with 0.2% or 2% lactate as the carbon source. In the presence of 0.2% lactate, addition of 3 mM cAMP increased the growth rate from 0.15 to 0.2 per hour, which is close to the growth rate measured in the presence of 2% lactate (Table 1). In this case, the growth rate remained unchanged after cAMP addition. Moreover, for cells grown with 0.2% lactate, cAMP addition did not significantly change the cell protein content expressed as dry mass. In contrast, cAMP treatment led to a markedly high protein accumulation in cells grown with 2% lactate. By multiplying the cell protein content by the respective growth rate, we observed that cAMP addition increased the protein synthesis rate to about the same extent regardless of the concentration of lactate in the medium (45% and 55% increase concentration-to-oxygen consumption flux ratio).
in the presence of 0.2% and 2% lactate, respectively). At the same time, cAMP addition significantly changed the respiratory activity of cells grown in the presence of 2% lactate and, to a lesser extent, in the presence of 0.2% lactate. This suggested a global activation of the metabolism of these cAMP-treated cells. Moreover, the effect of 3 mM cAMP on the enthalpy growth yield was dependent on the carbon source limitation; i.e., in 0.2% lactate, the decrease in enthalpy growth yield was 20%, whereas it reached 40% in the presence of 2% lactate (Table 1). This was further confirmed by the assessed enthalpy balance. The part of the energy input lost as heat or stored as biomass was not affected by variations in lactate concentration. cAMP treatment in the presence of 0.2% lactate increased the heat production and decreased the biomass yield. Finally, a more pronounced effect of cAMP was observed in the presence of 2% lactate: an increase in by-product formation (pyruvate + acetate) and heat production and a large decrease in biomass formation (13).

The decrease in the enthalpy growth yield is not due to an alteration in mitochondrial oxidative phosphorylation leading to a higher respiration rate. Indeed, in permeabilized spheroplasts (1), the (nonphosphorylating) state 4 and the ADP-induced state 3 respiration were about twice as high as when cells were grown with cAMP than without cAMP. Moreover, the respiratory control ratio was slightly higher for cells grown in the presence of cAMP. Additional data (electron microscopy, citrate synthase and D + l-lactate dehydrogenase activities, and cytochrome content) confirmed that the number of mitochondria is almost twice as high in cells grown in the presence of cAMP. However, the isolated mitochondria prepared from cAMP-treated cells are not significantly different from those prepared from untreated cells (13). In conclusion, when conditions where the growth rate was already optimal (high lactate concentration), addition of exogenous cAMP led to the proliferation of well-coupled mitochondria within the cells. This phenomenon led to uncoupling between biomass synthesis and catabolism and, consequently, a decrease in the enthalpy growth yield. This confirms that mitochondria by themselves are a major heat dissipative system in a fully aerobic metabolism and that a subtle adaptation of the amount of mitochondria to the growth rate is necessary to maintain the enthalpy growth yield. Taken together, our results show that the Ras-cAMP-protein kinase cascade plays an important role in this adaptation but do not exclude the participation of other signaling pathways.

Yeast harbors three cAMP protein kinase (PKA) catalytic subunits, which have >75% identity and are encoded by the TPK (TPK1, TPK2, and TPK3) genes (48). Although they are redundant for viability and functions such as glycogen storage regulation, the three kinases are not redundant for other functions such as pseudohypal growth and regulation of genes involved in trehalose degradation and water homeostasis, as well as iron uptake, which are regulated by Tpk2p (33, 34, 38). Tpk1p is required for the derepression of branched-chain amino acid biosynthesis genes, which seem to have another role in the maintenance of iron levels and DNA stability within mitochondria (39). These data provide evidence for a specificity of signaling through the three PKA catalytic subunits. To elucidate a potential role of one or more of these subunits in the regulation of mitochondrial biogenesis in response to energy demand during growth, we investigated the role of each of the TPKs in this process. We showed that the yeast protein kinase Tpk3p is specifically involved in the regulation of mitochondrial enzyme content at the transition phase when cells are grown in 2% lactate (8). Indeed, compared with the wild-type strain, the Δtpk3, but not Δtpk1 or Δtpk2, strain showed a decrease in the spontaneous respiration rate as well as in the growth rate during the transition phase. Thus this PKA is involved in the cellular response, leading to a decrease in the growth rate when the stationary (i.e., transition) phase is reached. To further characterize the mitochondrial compartment in these cells, we isolated mitochondria from the wild-type and mutant strains. Phosphorylating and uncoupled respiratory rates and enzyme activities (i.e., citrate synthase, cytochrome c oxidase, and oligomycin-sensitive ATPase) were decreased (~40%) in the mutant compared with the wild-type strain. However, when cytochrome content was measured in both strains, the respiratory chain composition was clearly modified. Indeed, whereas cytochromes a + a3, b, and c1 were not significantly affected in the mutant strain, cytochrome c content was largely decreased (40%). The decrease in phosphorylating and uncoupled respiratory rates, as well as cytochrome c oxidase activity, originated in this decrease in cytochrome c content. Under nonphosphorylating conditions, the mitochondria isolated from the mutant exhibited a lower respiratory rate than the wild-type mitochondria for a comparable protonmotive force, indicating that the energy waste was decreased in these mitochondria. This is due to a decrease in the switching process at the level of cytochrome c oxidase (8) originating in an enhancement of the kinetic constraints on the electron flux at the level of cytochrome c, leading to an increase in reactive oxygen species production (unpublished results). This raises the question of an involvement of these species in the adjustment of mitochondrial enzyme content in response to energy demand.

Table 1. Effect of cAMP on growth characteristics of OL556 cells in lactate-supplemented medium

<table>
<thead>
<tr>
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<th>0.2% Lactate</th>
<th>2% Lactate</th>
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<tbody>
<tr>
<td></td>
<td>−cAMP</td>
<td>+cAMP</td>
</tr>
<tr>
<td>Growth rate, h⁻¹</td>
<td>0.15±0.02</td>
<td>0.20±0.01</td>
</tr>
<tr>
<td>Protein content, mg/mg dry wt</td>
<td>0.61±0.02</td>
<td>0.65±0.03</td>
</tr>
<tr>
<td>Protein synthesis rate, μg·min⁻¹·mg dry wt⁻¹</td>
<td>1.5±0.2</td>
<td>2.2±0.2</td>
</tr>
<tr>
<td>ΔJo, growth, O·min⁻¹·mg dry wt⁻¹</td>
<td>210±20</td>
<td>300±20</td>
</tr>
<tr>
<td>Enthalpy growth yield, %</td>
<td>55±3</td>
<td>44±5</td>
</tr>
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Values are means ± SE. OL556 cells were grown aerobically in a minimal medium supplemented with 0.2% or 2% D,L-lactate as carbon source. When added in the culture medium, cAMP was 3 mM. Heat production rate, respiratory rate, biomass, and metabolites in culture medium were measured throughout exponential phase. Data are from Dejean et al. (13). ΔJo, growth corresponds to the oxygen consumption of growing cells in the culture media.
In conclusion, from studies of various strains in different growth phases, it is clear that, in yeast, there is a homeostasis of growth yield due to a tight adjustment of mitochondrial enzyme content to the growth rate. The signaling pathway responsible for such an adjustment is the Ras-cAMP pathway. However, upstream of the Ras proteins and downstream of the PKA, the molecular mechanisms remain to be elucidated, even though the reactive oxygen species seem to act as signaling molecules in this process downstream of the PKA.

Increase in mitochondrial enzyme content in muscle in response to energy demand at the onset of exercise. Muscle mitochondrial content can be increased by 50–100% within 6 wk of endurance training. Chronic contractile activity produced by electrical stimulation of the motor nerve can mimic this mitochondrial biogenesis. Williams et al. (52) were the first to show that chronic contractile activity led to increases in mRNA levels encoding nuclear and mitochondrial gene products.

Numerous rapid events occur at the onset of contractile activity. Two have been shown to be involved in mitochondrial biogenesis: Ca²⁺ signaling and ATP turnover. When released from the sarcoplasmic reticulum and in addition to its role in the actin-myosin interaction, Ca²⁺ can also activate a number of kinases (Ca²⁺-calmodulin kinase II and PKC) and phosphatases, which translocate their signals to the nucleus and alter the rate of gene transcription. Moreover, increases in cytosolic Ca²⁺ levels are known to be matched within the mitochondria downstream of the PKA. This occurs via the activation of dehydrogenases, which require Ca²⁺ for full activity (30). It has also been shown that the mitochondrial ATP synthase was activated by Ca²⁺ (18). Thus Ca²⁺ itself is a signaling molecule allowing an integrated activation of the overall oxidative phosphorylation process. This could lead to an increase in mitochondrial ATP synthesis without a change in mitochondrial and cytosolic phosphate potentials (24). However, quantitative estimates of Ca²⁺ effects in mitochondria are in conflict with the magnitude of changes in the respiratory rate in vivo at the onset of contractile activity (9, 45). More likely, the regulation of oxidative phosphorylation in these cells involves a high level of organization of the metabolic signaling network. In this regard, a new role for spatially arranged intracellular enzyme networks, catalyzed by creatine kinase, adenylate kinase, and glyceraldehyde-3-phosphate dehydrogenase, could participate in the regulation of oxidative phosphorylation in mitochondria during exercise. This dynamic concept points out that metabolic signaling through near-equilibrium enzyme networks, along with other homeostatic mechanisms (3), contributes to efficient intracellular energetic communication in maintaining the balance between cellular ATP consumption and production. As much as this research area is currently very productive and in progress and the present review is mainly focused on the adaptation of respiratory chain enzymes to long-term variations in cellular energy demand, the reader is referred to recent more specialized reviews (4, 5, 14, 41).

In muscle cells, since ATP production is able to match ATP consumption in a wide range and without a variation in phosphate potential, the hypothesis that disturbance in energy metabolism, leading to ATP depletion or a change in the phosphorylation potential, could initiate a compensatory response, ultimately leading to an increase in mitochondrial content (37), seems unlikely. However, an increase in ATP turnover without a variation in cellular ATP levels can also lead to mitochondrial biogenesis. Ca²⁺ induces an increase in cytochrome c mRNA levels mediated by the activation of PKC isoforms (16). However, it also appears that an increase in Ca²⁺ cannot, by itself, lead to an increase in overall mitochondrial content. Indeed, subsequent studies have shown that whereas a number of nuclear genes encoding mitochondrial subunit expression are increased along with cytochrome c, a number of others that would be critical for mitochondrial biogenesis, such as COX subunits IV, Vb, and VIc, are not. Two interpretations arise: 1) Ca²⁺ forms only part of a broader series of signals that mediate modifications in the synthesis of mitochondrial components or 2) the overall stoichiometry of the respiratory chain is modified.

Under conditions of partial mitochondrial uncoupling, which mimics the intense exercise, ATP production matches ATP consumption at lower phosphate potential, and an induction of the nuclear respiratory factor 1 (NRF-1) is observed. Subsequent to this induction, an increase in the expression of its target genes has been observed. It appears that the increase in the mitochondrial respiration or the imbalance between ATP demand and ATP supply provides a stimulus for the sequential induction of a variety of genes involved in the biogenesis of the organelle.

A number of transcription factors have been implicated in mitochondrial biogenesis. They include NRF-1 and NRF-2, peroxisome proliferator-activated receptors-α and -γ, and Sp1 (27). PGC-1 is a transcriptional coactivator that binds peroxisome proliferator-activated receptor-γ and, thus, regulates its activity. It has recently been shown that PGC-1α mRNA increases between 1.5- and 7- to 10-fold after a single bout of exercise (2, 36). Contractile activity has been shown to induce an increase in the mRNA and/or protein levels of several of these transcription factors, consistent with their roles in mediating phenotypic changes as a result of exercise (22). The upstream regulatory regions of genes encoding mitochondrial proteins are highly variable in their composition (27, 29). This variability suggests that a coordinated upregulation of gene transcription in response to contractile activity would be difficult to achieve, unless the multiple transcription factors mentioned above were effective in uniformly upregulating the transcriptional activity of numerous genes. A complete coordination among the responses is not achieved at the protein level (19) and does not seem to be required for an increase in mitochondrial content and activity, i.e., physiological function.

These results tend to show that, similar observations in yeast, energy production meets energy demand mainly through an adjustment in mitochondrial enzyme content. It is obvious that, in muscle cells, this adjustment in mitochondrial enzyme content cannot be continuously correlated to energy demand. Indeed, in these peculiar cells, energy demand varies rapidly (with a time span very different from that of mitochondrial turnover) and to a great extent. Thus the fact that, at the onset of exercise, a muscle harbors more mitochondria implies an increase in energy waste (futile cycles, mitochondrial activity) at rest.

In muscle, mitochondrial response to energy demand involves at least two distinct mechanisms. 1) A short-term event can consist of a fast exchange of metabolic signaling via phosphoryltransfer...
networks by the compartmentalized energy transfer and signal transmission. In such a complex regulatory mechanism, Ca^{2+} signaling participates in an activation of matricial dehydrogenases, as well as the mitochondrial ATP synthase. 2) A long-term adaptation involves an enhancement of mitochondrial biogenesis.

MITOCHONDRIAL RESPONSE THROUGH CHANGES IN KINETIC REGULATION OF MITOCHONDRIAL ENZYMES

C6 glioma cells are cancer cells with a high proliferation rate. Mitochondrial response to energy demand has been studied in C6 glioma cells during growth. When grown on a three-dimensional support, these cells, similar to yeast cells (see above), exhibit different growth phases. In contrast to some kinds of cancer cells, these cells are highly dependent on their respiratory metabolism. We estimated the relative contribution of mitochondrial oxidative phosphorylation to ATP synthesis to be 70–85% (28). Throughout growth, the ATP-to-ADP ratio remained constant; when the growth rate decreased, glycolysis and oxidative phosphorylation decreased similarly. Between the beginning of the exponential phase and the confluence, the cellular respiratory rate and the maximal respiratory capacity were decreased by a factor 5. This is not due to a decrease in mitochondrial enzyme content, since the activities of citrate synthase and complexes III and IV were maintained almost constant (28, 35). In contrast, the specific activity of complex I decreased continuously as the cell density increased. Moreover, there was a good positive correlation between the respiratory rate and the activity of complex I, indicating that the cellular respiratory rate is mainly controlled by the activity of this complex (35). Western blot analysis showed that the amount of complex I was maintained constant throughout growth. Immunodetection of the phosphoserine-containing proteins of immunoprecipitated complex I revealed, among several bands, a major protein band with an apparent molecular mass of ~16–18 kDa. This phosphorylated protein is likely the subunit ESSS, which has been identified in bovine heart mitochondria as one of two complex I phosphorylation sites; the other is MWFE, a 10-kDa protein with expression that is supposed to be linked to cAMP signaling (7). The phosphorylation level of this 18-kDa subunit decreased during growth of C6 glioma cells, indicating that complex I activity and, thus, respiratory rate are kinetically regulated by phosphorylation during growth. Further experiments demonstrated that the 18-kDa subunit phosphorylation is at least under the control of the cAMP signaling pathway (35).

Even though we were not able to decipher the exact molecular mechanism modulating the phosphorylation state of the 18-kDa subunit, it is clear that, in C6 glioma cells, the adjustment of oxidative phosphorylation to energy demand through the growth rate is mainly due to the regulation of the phosphorylation level of a subunit of complex I.

In conclusion, from the studies reported here, it seems that the mitochondrial adaptation to variation in cellular energy demand through modifications of oxidative phosphorylation steady state without kinetic regulation or modification in mitochondrial enzyme content is observed only as a short-term adjustment. For instance, when yeast cells reach the stationary growth phase or when C6 glioma cells are grown in a medium deprived of glutamine, one can observe a growth arrest associated with a decrease in respiratory rate that approaches a nonphosphorylating respiratory rate (10, 28). In heart cells, the short-term adjustment can be reached mainly through metabolic signaling via phosphotransfer networks by the compartmentalized energy transfer and signal transmission. In such a complex regulatory mechanism, Ca^{2+} signaling participates in an activation of matricial dehydrogenases as well as of the mitochondrial ATP synthase. This concerted response leads to an increase in mitochondrial ATP synthesis at nearly constant forces.

For a long-term adaptation, two main mechanisms are involved: the modulation of mitochondrial enzyme content as a function of energy demand and/or the kinetic regulation by covalent modifications of some respiratory chain complex subunits. In yeast, the main process is a tight adjustment of mitochondrial enzyme content to the growth rate, such that the oxidative phosphorylation steady state and, consequently, the growth yield remain constant. In contrast, in C6 glioma cells, the main process is a modulation of the phosphorylation status of at least the ESSS subunit of complex I, leading to a change in the activity of this controlling step at constant mitochondrial enzyme content. Regardless of the mechanism involved (kinetic regulation by covalent modification or adjustment of mitochondrial enzyme content), the cAMP pathway plays a major role in the molecular signaling leading to the mitochondrial response.

From an energetic standpoint, with these long-term adaptation mechanisms, the cell favors the ability to change ATP turnover to a large extent without significant modifications in the forces associated with oxidative phosphorylation and ATP consumption (mitochondrial electrochemical potential difference in protons, intramitochondrial and cytosolic phosphate potentials, and mitochondrial and cytosolic redox potentials). This implies that the maintenance of thermodynamic force homeostasis is vital.

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