

# Making the most of what you've got: Optimizing residual OXPHOS function in mitochondrial diseases

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Keywords: OXPHOS, cytochrome oxidase, mitochondrial diseases, ROS, PGC-1alpha

**Patients affected by mitochondrial OXPHOS disorders are still faced with a grim lack of therapeutic options. In this Closeup, Carlos Moraes revisits the recent data by Giovanni Manfredi on PKA's functions in the mitochondria and now its modulation can improve respiration and ATP production in COX-defective cells.**

Patients with defects in the oxidative phosphorylation (OXPHOS) system are not as rare as once believed. It is now estimated that approximately 1 in 5000 born children will develop a mitochondrial disorder (Cree et al, 2009). This heterogeneous group of disorders can manifest as child- or adulthood encephalopathies, myopathies or multi-organ syndromes (Cree et al, 2009), and their genetic makeup is complicated by the fact that both nuclear and mitochondrial DNA contribute key factors necessary for OXPHOS function. Strong efforts from government agencies and philanthropic foundations dedicated to funding an expanding number of laboratories researching mitochondrial diseases have increased our understanding of these disorders. However, patients with defects

in OXPHOS function are still faced with a grim lack of therapeutic options.

» . . . 1 in 5000 born children will develop a mitochondrial disorder. «

Giovanni Manfredi at Cornell University and collaborators previously identified a soluble adenylate cyclase (sAC) that is partially localized to mitochondria (mt-sAC) (Acin-Perez et al, 2009a). The mt-sAC produces the cAMP needed for the regulation of a mitochondrial protein kinase A (PKA) pool. In this issue, the same group now shows that by stimulating this pathway, cells with defective oxidative phosphorylation (OXPHOS) function have improved respiration and adenosine triphosphate (ATP) production (Acin-Perez et al, 2009b).

» . . . the impact of various COX defects is reduced when PKA is activated. «

Although the gamut of PKA substrates within mitochondria is not known, subunits I and IV of cytochrome *c* oxidase (COX) have increased phosphorylation when the mitochondrial levels of cAMP are elevated (Acin-Perez et al, 2009a).

This correlates with a higher activity of the enzyme complex, suggesting that this post-translational modification has an important role in regulating COX activity. Acin-Perez et al find that the impact of various COX defects is reduced when PKA is activated.

The authors tested a number of different cell lines with COX defects caused by mutations in either mitochondrial or nuclear genes and consistently saw an improvement in COX activity either by using pharmacological PKA agonists or by promoting higher levels of sAC in mitochondria. Conversely, COX activity in these cells decreased when a PKA inhibitor was used. The magnitude of these effects is greater in COX deficient than in wild-type controls, suggesting that defective cells have a better capacity (and/or an increased need?) to upregulate this pathway. In fact, phosphorylation of COX subunits is lower in COX-deficient cells than in wild-type controls.

Recently, it was shown that an increase in mitochondrial biogenesis could also improve cellular ATP levels in OXPHOS deficient cells and tissues (Bastin et al, 2008; Srivastava et al, 2009; Wenz et al, 2008). Increases in mitochondrial biogenesis are commonly observed in OXPHOS defective cells and they are believed to be a natural compensatory adaptation to the defect. In many cases, such increase in mitochondrial biogenesis does not protect cells from a bioenergetic crisis, as despite the increased numbers, the mitochondria

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DOI 10.1002/emmm.200900049

remain defective. However, when there is residual OXPHOS activity, then mitochondrial proliferation has a beneficial effect. Increases in mitochondrial biogenesis are mediated by PGC-1 $\alpha$ , a transcriptional co-activator that binds and stimulates a group of transcription factors (e.g. NRF-1, NRF-2, ERR $\alpha$ , PPAR $\alpha,\beta,\gamma$ ) associated with the expression of genes coding for mitochondrial proteins (e.g. Cyt *c*, tFAM). Acin-Perez et al also observe an increase in mitochondrial proliferation and PGC-1 $\alpha$  expression in COX deficient cells. Interestingly, activation of the mt-sAC/mitochondrial PKA pathway inhibits this compensatory mechanism. Once COX activity (and by inference OXPHOS function) is above a certain functional threshold, the mitochondrial biogenesis compensatory pathway is not activated.

» ...increase in mitochondrial biogenesis could also improve cellular ATP levels in OXPHOS deficient cells. . . «

Reactive oxygen species (ROS) have been shown to induce PGC-1 $\alpha$  (Irrcher et al, 2009), and Acin-Perez et al also show that antioxidants inhibit this acti-

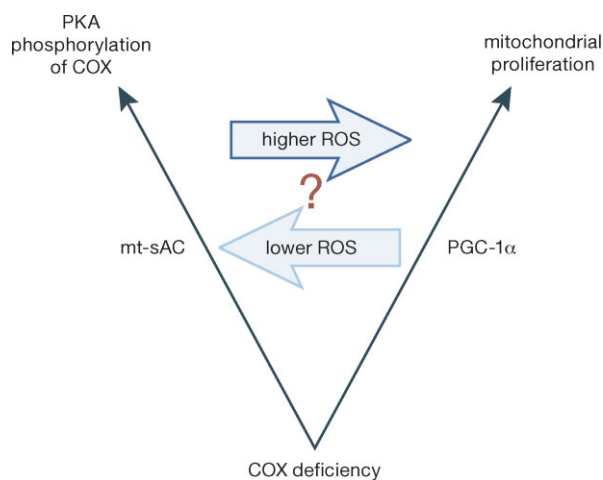
vation, suggesting that mitochondrial proliferation is mediated by ROS. The authors propose that the increase in COX activity mediated by the mt-sAC/PKA prevents the surge in ROS associated with the COX defect and consequently, mitochondrial proliferation would not ensue (Fig 1). This is an attractive model, although the mechanism by which a partial COX deficiency leads to an increase in ROS is not understood. The relationship between inhibition of OXPHOS and increased ROS (either as a signal or a damaging agent) has been a source of much work, and has also provided one of the most enduring models to explain aging and degenerative diseases. Increased ROS damage is consistently observed in aged, degenerating tissues and impaired OXPHOS is commonly the alleged culprit for the ROS damage. However, recent work in worms and mice seem to challenge this concept. Partial inhibition (at different levels) of a large number of OXPHOS-related genes can increase longevity in *Caenorhabditis elegans* and increased ROS is not observed at any level of inhibition (Rea et al, 2007). Mice with a proof-reading deficient polymerase gamma also accumulate mutations and an OXPHOS defect, but ROS is not increased (Kujoth et al, 2005; Trifunovic et al, 2005). ROS is actually decreased in some tissues

of the mutator mouse (Christiaan Leeuwenburgh, personal communication). It may well be that the nature of the OXPHOS inhibition dictates the effects on ROS generation but the stubbornly turbid relationship between OXPHOS impairment and ROS levels is likely to haunt the field for a few more years.

What appears clear though is that residual COX activity can be optimized by at least two pathways: (1) allosterically activating the enzyme, e.g. by promoting phosphorylation and/or isoform switching and (2) increasing mitochondrial biogenesis thereby elevating the levels of competent mitochondria per cell.

» ...COX can be stimulated by pharmacological manipulation. «

Either approach is bound to have limitations when it comes to patient treatment. The work of Acin-Perez et al elegantly demonstrates that COX can be stimulated by pharmacological manipulation. The magnitude of this allosteric activation may be sufficient to overcome a partial biochemical defect, but not in cases where the residual amount of enzyme is too low. In addition, many mitochondrial disorders present with multiple OXPHOS complex defects. This is particularly true for diseases caused by mutations in mitochondrial DNA that affect global mitochondrial protein synthesis. On the other hand, increasing mitochondrial biogenesis has its own set of problems too. Large increases in mitochondrial mass in the mouse heart was associated with cardiomyopathy (Russell et al, 2004). Similarly to the activation pathway, increase of mitochondrial biogenesis is likely to be effective only when there are sufficient functional mitochondria to proliferate and supply the cell with adequate levels of ATP. In addition, activating PGC-1 $\alpha$  to promote mitochondrial proliferation can activate a large number of genes, which may not be desirable in certain conditions.



**Figure 1. Compensatory mechanisms for COX deficiencies.** Acin Perez et al (2009) proposed that COX deficiencies can lead to a mitochondrial proliferation, possibly because of ROS signalling increasing PGC-1 $\alpha$  expression. However, if increased activation by mt-sAC/PKA phosphorylation ensues, the levels of ROS are kept low and mitochondrial proliferation does not take place. The precise role of ROS in these pathways is still unclear.

Simultaneously controlling these two mechanisms may ultimately benefit patients and we look forward to seeing the application of these approaches. In cases where residual COX is too low, and neither allosteric activation, increased mitochondrial biogenesis, nor combination therapies can bring ATP production to acceptable levels, the use of alternative oxidases from non-vertebrates (AOX), as recently reported (Dassa et al, 2009, Matsukawa et al, 2009, Perales-Clemente et al, 2008), may have a complementary role in future therapeutic approaches for patients with COX-deficient mitochondrial disorders.

### Acknowledgements

This work was supported by NINDS and the Muscular Dystrophy Association.

### References

- Acin-Perez *et al* (2009a) Modulation of mitochondrial protein phosphorylation by soluble acyl-CoA oxidase ameliorates cytochrome oxidase defects. *EMBO Mol Med* 1: 392–406
- Acin-Perez R, Salazar E, Kamenetsky M, Buck J, Levin LR, Manfredi G (2009b) Cyclic AMP produced inside mitochondria regulates oxidative phosphorylation. *Cell Metab* 9: 265–276
- Bastin J, Aubey F, Rotig A, Munnich A, Djouadi F (2008) Activation of peroxisome proliferator-activated receptor pathway stimulates the mitochondrial respiratory chain and can correct deficiencies in patients' cells lacking its components. *J Clin Endocrinol Metab* 93: 1433–1441
- Cree LM, Samuels DC, Chinnery PF (2009) The inheritance of pathogenic mitochondrial DNA mutations. *Biochim Biophys Acta* in press.
- Dassa EP, Dufour E, Goncalves S, Paupé V, Hakkaart GAJ, Jacobs HT, Rustin P (2009) Expression of the alternative oxidase complements cytochrome c oxidase deficiency in human cells. *EMBO Mol Med* 1: 30–36
- Irrcher I, Ljubicic V, Hood DA (2009) Interactions between ROS and AMP kinase activity in the regulation of PGC-1 $\alpha$  transcription in skeletal muscle cells. *Am J Physiol Cell Physiol* 296: C116–C123
- Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, Hofer T, Seo AY, Sullivan R, Jobling WA, *et al* (2005) Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science* 309: 481–484
- Matsukawa K, Kamata T, Ito K (2009) Functional expression of plant alternative oxidase decreases antimycin A-induced reactive oxygen species production in human cells. *FEBS Lett* 583: 148–152
- Perales-Clemente E, Bayona-Bafaluy MP, Perez-Martos A, Barrientos A, Fernandez-Silva P, Enriquez JA (2008) Restoration of electron transport without proton pumping in mammalian mitochondria. *Proc Natl Acad Sci USA* 105: 18735–18739
- Rea SL, Ventura N, Johnson TE (2007) Relationship between mitochondrial electron transport chain dysfunction, development, and life extension in *Caenorhabditis elegans*. *PLoS Biol* 5: e259
- Russell LK, Mansfield CM, Lehman JJ, Kovacs A, Courtois M, Saffitz JE, Medeiros DM, Valencik ML, McDonald JA, Kelly DP (2004) Cardiac-specific induction of the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator-1 $\alpha$  promotes mitochondrial biogenesis and reversible cardiomyopathy in a developmental stage-dependent manner. *Circ Res* 94: 525–533
- Srivastava S, Diaz F, Iommarini L, Aure K, Lombes A, Moraes CT (2009) PGC-1 $\alpha$ /beta induced expression partially compensates for respiratory chain defects in cells from patients with mitochondrial disorders. *Hum Mol Genet* 18: 1805–1812
- Trifunovic A, Hansson A, Wredenberg A, Rovio AT, Dufour E, Khvorostov I, Spelbrink JN, Wibom R, Jacobs HT, Larsson NG (2005) Somatic mtDNA mutations cause aging phenotypes without affecting reactive oxygen species production. *Proc Natl Acad Sci USA* 102: 17993–17998
- Wenz T, Diaz F, Spiegelman BM, Moraes CT (2008) Activation of the PPAR/PGC-1 $\alpha$  pathway prevents a bioenergetic deficit and effectively improves a mitochondrial myopathy phenotype. *Cell Metab* 8: 249–256