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# A Coupled Understanding of UCP2 Function and Metabolic Reprogramming

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**Abstract.** Uncoupling Proteins are a family of integral proteins found on the inner mitochondrial membrane and are thought to uncouple fuel oxidation and ATP synthesis in mitochondria. Uncoupling Protein-2 (UCP2) is a member of this family. Intriguingly, increase in level of UCP2 expression is associated with metabolic reprogramming and the Warburg effect (switch from reliance on citric acid cycle to glycolysis for energy production) in cancer cells. A recent study by Vozza and co-workers (2014) revealed that UCP2 catalyses the export of C4 metabolites from the mitochondrial matrix into the cytosol. This new understanding of the function of UCP2 explains how it contributes to metabolic reprogramming and is of immense value in understanding the progression of cancer and other metabolic disorders, and ultimately in developing drugs to treat them.

Mitochondria are known as the power plants of cells where fuels are oxidised and the vast majority of cellular ATP is produced. It is known that in mitochondria, fuel oxidation and ATP synthesis are highly coupled processes. As proposed in the well-established chemiosmotic theory, mitochondria utilise energy from fuel oxidation to generate a proton gradient across the inner mitochondrial membrane and the energy available in this electrochemical gradient is harnessed to synthesise ATP (Mitchell, 1979).

Uncoupling Proteins (UCPs) are a family of integral proteins found in the inner mitochondrial membrane that are thought to uncouple fuel oxidation and ATP synthesis. Uncoupling Protein 1 (UCP1), discovered in 1984, is the founding member of this family (Nicholls and Locke, 1984) and its function has been well characterised.

UCP1 is found exclusively in mitochondria of brown adipocytes, where it functions to mediate proton leak across the inner mitochondrial membrane, thus dissipating the proton gradient generated by fuel oxidation. As a result, the energy stored by the proton gradient is converted into heat instead of ATP (Garlid and Jaburek, 1998). The UCP1 mediated proton leak is known to be the underlying mechanism of non-shivering heat production in mammals and neonatal humans (Cannon and Nedergaard, 1985).

Uncoupling Protein 2 (UCP2) was discovered in 1997 (Fleury *et al*., 1997) and found to be a mitochondrial protein with broad tissue distribution. This protein was hypothesised to share a similar uncoupling activity as UCP1 on the basis of sequence similarity between the two proteins (Lentes et al., 1999). Experiments conducted to measure the effect of UCP2 expression on uptake of potential-sensitive fluorescent dye in yeast and mammalian cells also showed that UCP2 decreased the mitochondrial membrane potential (Gimeno *et al.*, 1997), which is consistent with the hypothesis that it has uncoupling activity.

However, whether UCP2 indeed functions as an uncoupling protein under physiological conditions is still not clearly established. A study on the levels of UCP2 expression in yeast showed that a decrease in the mitochondrial membrane potential was only observed at levels of UCP2 expression much higher than physiological levels (Stuart *et al*., 2001), suggesting that UCP2 possesses no or only mild uncoupling activity. Furthermore, the possibility remains that the observed effect of UCP2 to decrease the mitochondrial membrane potential may not be due to uncoupling, but inhibition of either fuel oxidation or proton gradient generation.

More intriguingly, changes in the levels of UCP2 expression have been implicated in the metabolic switch from glucose to fatty acid metabolism in mitochondria (Pecqueur *et al*., 2008) and in promoting the Warburg effect (switch in reliance from citric acid cycle to glycolysis for glucose metabolism) in cancer cells (Samudio *et al*., 2009). These observations suggest that UCP2 plays a role in the reprogramming of metabolic pathways.

In a recent paper, Vozza and co-workers revealed compelling evidence that UCP2 is a metabolite transporter which exports C4 metabolites in exchange for uptake of inorganic phosphate ($P_i$) and $H^+$ (Vozza *et al*., 2014). This putative function of UCP2 offers significant new insights into the mechanism underlying the metabolic switches that have been associated with changes in the level of UCP2 expression.

The group began their investigation of UCP2 function by examining the effect of silencing UCP2 gene expression on the metabolism of glucose and glutamine via the mitochondria, using human hepatocarcinoma cells (HepG2) as a model. It was observed that in glucose-containing media, cells with silenced UCP2 expression showed a higher inner mitochondrial membrane potential, and a higher ATP:ADP ratio compared to wild-type cells. In glutamine-containing media, cells with silenced UCP2 expression showed a lower inner mitochondrial membrane potential and a lower ATP: ADP ratio compared to wild-type cells. More importantly, in both glucose and glutamine-containing media, UCP2-silenced cells showed higher concentrations of C4 intermediates of the citric acid cycle in the mitochondria. The group reasoned that UCP2-silenced cells exhibited loss of export of C4 metabolites from the

mitochondria, causing C4 metabolites to accumulate in the matrix. This would increase the rate of oxidative phosphorylation and cause feedback inhibition of anaplerotic reactions such as glutaminolysis. This led the group to the hypothesis that UCP2 functions as a metabolite transporter.

To test their hypothesis, the group characterised the transport activity of UCP2 by reconstituting recombinant UCP2 into liposomes and performing transport assays, as well as performing mitochondrial swelling experiments. They were able to determine that UCP2 transport activity involves an inward $H^+/P_i$ coupled symport, and that UCP2 also catalyses a malate/$H^+$ antiport. The group then characterised the substrate specificity of UCP2 by measuring the uptake of radioactive phosphate into proteoliposomes preloaded with various substrates. Uptake of radioactive phosphate was observed when proteoliposomes were preloaded with malate, oxaloacetate, or aspartate, allowing the group to determine that UCP2 was a specific transporter of these substrates.

From their investigations, the group concluded that UCP2 is a metabolite transporter that catalyses the import of $H^+$ and $P_i$ and export of the C4 metabolites malate, oxaloacetate and aspartate. This new understanding of the function of UCP2 has significant explanatory power to account for why metabolic reprogramming has been associated with changes in UCP2 expression. UCP2 expression is known to be inducible by fasting conditions (Boss *et al*., 1997) and this is perplexing if UCP2 is understood as an uncoupler, which would only exacerbate the short-supply of ATP during fuel shortage. However, if UCP2 exports C4 metabolites from the matrix into the cytosol, then it would increase gluconeogenesis to cope with fuel shortage.

It has already been noted that UCP2 expression is upregulated by oncogenes in cancer cells and associated with their metabolic reprogramming (Samudio *et al*., 2009). The new understanding of UCP2 as a C4 metabolite exporter explains how the mitochondrial carrier protein contributes to the switch from reliance on citric acid cycle to glycolysis for glucose metabolism. It also explains how UCP2 can reduce the production of reactive oxygen species (ROS). The major source of cellular ROS is the mitochondrial electron transport chain. By negatively regulating the citric acid cycle, less reducing equivalents are produced, leading to lower electron transport chain activity. Through this mechanism, UCP2 may play a role to prevent the triggering of apoptosis in cancer cells.

The function of UCP2 that has been elucidated in the study by Vozza *et al*. (2014), coupled with a better understanding of the role in UCP2 in metabolic reprogramming, is of immense value in understanding physiological processes and pathogenesis of diseases, and ultimately in developing drugs to treat them.

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