The electrochemical gradient over the inner mitochondrial membrane is the driving force for cellular ATP production. For this, electrons, which are derived from succinate and NADH, are passed along the mitochondrial electron transfer chain, and the resulting energy is used to pump protons. The transport of the positively charged protons into the mitochondrial inner membrane space builds up an electric gradient mitochondrial membrane potential ($\Delta \Psi$) and a chemical gradient $\Delta $pH. The effective proton motive force is $\Delta \Psi \approx 2.3\text{RT} \Delta $pH. Mitochondria are not only the central cellular producers of ATP but also a significant source of reactive oxygen species (ROS), which are formed as a consequence of electron leak from the respiratory chain. This relatively frequent event occurs when an electron, particularly at complex I, complex III, or ubiquinone, escapes from the transfer chain and reacts with oxygen. The resulting superoxide anions ($O_2^-$) are to a great extent detoxified by manganese superoxide dismutase (MnSOD), and homozgyous deletion of this important antioxidative enzyme is lethal. A low transfer rate of electrons through the respiratory chain increases the ratio of reduced to oxidized compounds and therefore favors electron escape onto oxygen and thus ROS production. Because a high $\Delta \Psi$ hinders proton pumping over the inner mitochondrial membrane, high $\Delta \Psi$ is thought to decrease the electron transport rate through the respiratory chain, and thus to increase ROS formation. Conversely, a reduction of $\Delta \Psi$ reduces mitochondrial ROS formation.1 Besides increasing the ADP-to-ATP ratio and thus the turnover of complex V, respiratory chain uncoupling compounds have been shown to lower $\Delta \Psi$. Chemical uncouplers like dinitrophenol not only massively reduce $\Delta \Psi$ but also lower mitochondrial ROS formation to an extent that ROS, in the presence of an intact antioxidant defense, become virtually undetectable. Mechanistically, uncouplers, which are also abused for fat loss therapy, are lipophilic redox-cyclers that move protons over the inner mitochondrial membrane and bypass the ATP synthase. Importantly, with the protein family of uncoupling proteins (UCP1 to UCP5), such a function is also achieved physiologically.1 UCP1 is highly expressed in brown adipose tissue, and its uncoupling function facilitates thermogenesis. UCP4 and UCP5 are expressed tissue-specifically, whereas UCP2 and UCP3 show a different and broader expression. Because UCP2 to UCP5 uncouple less extensively than UCP1, they do not contribute to thermogenesis, and their negative impact on cellular ATP production is small.2 Rather, these UCPs mediate a low degree of mitochondrial uncoupling that mainly keeps $\Delta \Psi$ below a certain threshold and therefore limits mitochondrial ROS production. Despite this theoretical basis for a potent antioxidative function of UCP2 and UCP3, the physiological significance of these proteins is just emerging. With respect to endothelial cells, overexpression of UCP2 promotes endothelial function,3 but the role of endogenous UCP2 for vessel formation has not been studied.

In this issue of Circulation Research, Shimasaki et al4 report the fundamental importance of UCP2 for angiogenesis by using UCP2−/− and MnSOD−/− mice.4 Genetic deletion of UCP2 reduced endothelial cell proliferation and migration in culture, attenuated endothelial cell outgrowth from the mouse aorta, and, most importantly, greatly attenuated the restoration of perfusion in the murine femoral artery ligation model. In keeping with the theoretical concept of UCP2 being an antioxidative enzyme, Shimasaki et al4 observed similar functional deficits in the angiogenic response of MnSOD−/− mice. Downregulation or knockdown of either UCP2 or MnSOD increased mitochondrial $O_2^-$ level. Conversely, overexpression of UCP2 in cells with decreased MnSOD expression as well as MnSOD overexpression in cells with decreased UCP2 expression both normalized ROS level. A similar redundancy was operative functionally as well; overexpression of UCP2 in MnSOD−/− endothelial cells or mice improved endothelial cell function and restored normal angiogenesis and overexpression of MnSOD increased proliferation in UCP2−/− cells. With these data, a functionally relevant antioxidative function of UCP2 in endothelial cells has been established (Figure).

An increased level of endothelial $O_2^-$ has been traditionally associated with decreased nitric oxide availability, endothelial nitric oxide synthase uncoupling, and impaired endothelium-dependent relaxation. Interestingly, although impaired endothelium-dependent relaxation has previously been noted in UCP2−/− mice,5 the authors of the present study could not reproduce this finding for unknown reasons.6 The functional attenuation of UCP2−/− endothelial cells in the present study was rather mediated by an induction of a premature senescent phenotype, which resulted from a ROS-mediated phosphorylation and thus activation of the transcription p53 and the subsequent induction of the cell cycle inhibitors p16ipkα and p21cip/waf. Interestingly, p53 activation and p21cip/waf

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Editorial

Do Not Overcharge the System or It Will Explode!
How Mitochondrial Uncoupling Protein 2 Maintains Endothelial Function

Ralf P. Brandes
accumulation also had an impact on mitochondrial morphology because they induced their fragmentation, probably as a consequence of attenuated mitofusin 1 and 2 expressions. Small interfering RNA against mitofusins decreased endothelial proliferation. However, given the direct inhibitory effects of p16<sup>ink4a</sup> and p21<sup>cip1/waf1</sup> on cell cycle progress, it is unlikely that the UCP2<sup>−/−</sup>-induced attenuation of proliferation is exclusively mediated by loss of mitofusin expression. Collectively, these observations by Shimasaki et al<sup>4</sup> demonstrate that by limiting mitochondrial O$_2$<sup>−</sup> formation, UCP2 maintains normal mitochondrial dynamics, normal endothelial function, and angiogenesis, and prevents the development of a ROS-mediated premature senescence phenotype.

Another interesting aspect of the work by Shimasaki et al<sup>4</sup> was the observation that expression of UCP3 in endothelial cells is somewhat constitutive, whereas UCP2 is massively induced in proliferating cells by a pathway partially sensitive to c-Jun-N-terminal kinase (JNK) inhibitors. Multiple signaling pathways regulate UCP2 mRNA, involving elements like the transcription factor SP1, p53, forkhead box protein A1 and Sirtuin 1,2,6 and the transcription factor SP1, peroxisome proliferator-activated receptor and its coactivator (PGC1α, AMPK), forkhead box protein A1 and Sirtuin 1,2,6 and therefore the role of JNK in proliferation-induced UCP2 induction could be direct but could also be a consequence of some interactions of JNK with these pathways. Given the impact of PGC1α and AMPK on mitochondrial function and biogenesis, it is tempting to speculate that the increase in UCP2 expression during proliferation was just a consequence of mitochondrial biogenesis. Proliferation, however, did not affect the expression of UCP3 or of the cytochrome oxidase of complex IV rendering this alternative less likely. UCP2, however, has a low half-life of only 30 minutes, whereas the half-life of UCP1 and complexes of the respiratory chain is ≥30 hours. This indicates that UCP2 will acutely show much more dramatic changes in expression than other mitochondrial components.

Mitogen-activated protein kinase (MAP) kinases are closely involved in ROS signaling, which is the consequence of the high redox sensitivity of MAP kinase phosphatases. This aspect might be relevant to the present study because JNK phosphorylation is increased by ROS and because the authors report that JNK mediated UCP2 expression. The fact that UCP2 limits ROS level suggests a negative feedback loop. Such a mechanism also seems to contribute to the anti-inflammatory activity of UCPs,<sup>7</sup> an aspect also of relevance to the vascular system; transplantation of UCP2<sup>−/−</sup> bone marrow into LDLR<sup>−/−</sup> mice increased atherosclerosis development,<sup>8</sup> and global UCP2<sup>−/−</sup> mice exhibited increased inflammatory activity and enhanced aortic macrophage deposition in response to atherogenic diet.<sup>9</sup>

In many cell types, including smooth muscle cells and fibroblasts, ROS-stimulated MAP kinase activation promotes proliferation, and therefore it is unlikely that the antiproliferative effect of UCP2 knockout observed in endothelial cells in the study by Shimasaki et al<sup>4</sup> is universal; for example pulmonary arterial smooth muscle cells harvested from UCP2<sup>−/−</sup> mice exhibited accelerated proliferation<sup>10</sup> and increased apoptosis resistance.<sup>11</sup>

The impact of proliferation and UCP2 on cellular ATP production is controversial.<sup>2</sup> JC-1 measurements in the study by Shimasaki et al<sup>4</sup> suggested that proliferating cells have a lower ΔΨ but higher ATP content. Although the latter value has to be interpreted with caution because other metabolic markers and the ADP content were not provided, it illustrates that accumulation of UCP2 does not necessarily attenuate the cellular ATP supply but rather acts to limit cellular ROS levels. A mechanistic explanation is that the activities of UCP2 and UCP3 are ROS-dependent because lipid peroxidation products and potentially ROS-stimulated signaling cascades increase their uncoupling activity.<sup>1</sup> By such a mechanism, UCP2 and UCP3 would keep ΔΨ at an optimal level, sufficient to drive ATP synthesis but low enough to limit ROS formation. Thus, in analogy to a generator, UCPs are the pressure relief valves that prevent overcharge of the system. The present work by Shimasaki et al<sup>4</sup> impressively documents how central this function is to endothelial cell biology.

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None.

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