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A Futile Approach to Fighting Obesity?

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The current obesity epidemic has focused a great deal of attention on mechanisms controlling energy balance. While diet and nutrient absorption affect energy intake, on the other side of the equation, energy expenditure is determined by basal metabolism, physical activity, and adaptive thermogenesis. Given various challenges in modulating these energy balance mechanisms to combat human obesity, many efforts have concentrated on how it might be possible to achieve weight loss through increased thermogenesis. In this issue of *Cell*, Kazak et al. describe a previously unrecognized molecular pathway for thermogenesis in fat cells.

Non-shivering thermogenesis occurs primarily in brown adipose tissue in rodents, but also has been detected in so called ''beige'' adipocytes, thought to reside mainly in subcutaneous fat tissue interspersed with classic white adipocytes (Wu et al., 2012; Young et al., 1984). Much of human thermogenic fat most closely resembles the rodent beige adipose tissue (Shinoda et al., 2015). Beige and brown adipocytes both express uncoupling protein 1 (Ucp1), which resides on the inner mitochondrial membrane. While the electron transport chain drives protons into the intermembrane space in mitochondria, creating a proton gradient across the inner membrane to drive the synthesis of ATP (Figure 1A), Ucp1 creates a pore through which protons disperse into the mitochondrial matrix, thereby generating heat and uncoupling ATP synthesis (Figure 1B). Cold exposure or increased sympathetic activity stimulated by feeding activates thermogenesis through adrenergic activation of *Ucp1* expression (Ricquier et al., 1986; Scarpace et al., 1997).

Although Ucp1 is well established as an important component of thermogenesis, investigators have long known that the transcriptional regulation of *Ucp1* cannot fully explain thermic responses. For example, the thermic effect of feeding is far too rapid to be explained by a transcriptional effect alone (Scarpace et al., 1997). Furthermore, *Ucp1* knockout mice can adapt to chronic cold exposure when the temperature transition is gradual (Golozoubova et al., 2001). Non-shivering thermogenesis has also been characterized in muscle, where Sarcolipin (*Sln*) uncouples ATP hydrolysis from Ca^{2+} transport, thereby creating a futile cycle that generates heat (Bal et al., 2012). However, *Ucp1/Sln* double-knockout mice still retain the ability to maintain thermal regulation when slowly adapted to the cold (Rowland et al., 2015), leaving a gap in our understanding of how thermogenesis occurs.

To fill in this gap, Bruce Spiegelman and his colleagues, including mass spec expert Steven Gygi, conducted proteomic and genomic studies comparing beige, white, and brown adipocytes (Kazak et al., 2015). KEGG pathway analysis of proteins preferentially expressed in beige versus brown fat revealed several components of the arginine/creatine and proline metabolism pathways. These findings were confirmed when the analysis was limited to proteins specifically enriched in purified mitochondrial fractions. Proteins that promote both creatine synthesis and phosphorylation, including the mitochondrial creatine kinase CMKT2 and the majority of ATP synthase subunits, were elevated in mitochondria from beige fat. Creatine kinase (CK) activity was also specifically induced in beige fat mitochondria derived from mice exposed to cold, suggesting that it is somehow under adrenergic control. Together, these findings hinted that a futile creatine phosphorylation and dephosphorylation cycle might somehow be involved in generating heat specifically in mitochondria from beige adipocytes.

CK catalyzes the phosphorylation of creatine using ATP, generating phosphocreatine and ADP. In tissues with high ATP demands, such as skeletal muscle, the high-energy phosphate bound to creatine can be transferred to ADP to generate cytosolic ATP (Wyss and Kaddurah-Daouk, 2000). If creatine were serving to regenerate mitochondrial ATP through classical CK-mediated phosphotransferase activity, it would be expected to boost respiration as a molar equivalent

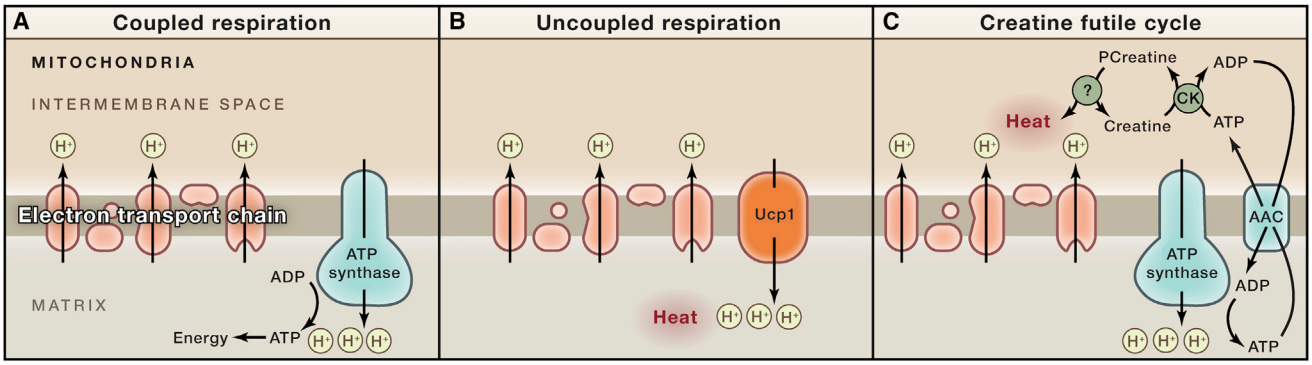

Figure 1. Different Modes of Mitochondrial Respiration

(A) Coupled respiration, which generates ATP.

(B) Thermogenesis through uncoupled respiration by Ucp1, which does not involve ATP synthase.

(C) Thermogenesis by creatine futile cycle, which requires ATP synthase activity, although no net ATP is generated.

to ADP, when ADP concentrations are limiting. Consistent with this classical function of creatine, addition of 0.01 mM creatine in the presence of 1 mM ADP to mitochondria isolated from classic brown fat and muscle had no detectable effect on respiration. However, in beige fat mitochondria, this small amount of creatine produced a large effect on respiratory rate, far exceeding that expected from 1:1 stoichiometry with ADP, suggesting that creatine is regenerated from phosphocreatine via a futile cycle that dissipates the energy as heat (Figure 1C). This idea was supported by direct calorimetry, which demonstrated that addition of small amounts of creatine increased heat production in beige, but not brown, mitochondria. In contrast to Ucp1-mediated thermogenesis, this futile creatine cycle requires coupled ATP synthesis, although no net ATP is generated.

The identification of this futile cycle may advance our understanding of beige-fat-specific thermogenesis in adult humans who possess little if any BAT. However, numerous questions remain about the molecular mechanics of this futile creatine cycle. Notable among these is the mechanism of dephosphorylation. While Kazak et al. note that the mitochondrial phosphatase Phospho1 exhibits an expression pattern that suggests its participation in this cycle, this phosphatase did not catalyze dephosphorylation of phospho-creatine in vitro. The authors propose that Phospho1 may play a unique role at the end of a phosphotransfer chain, but other players are probably involved, and it will be important to identify the relevant phosphatase or transferase(s) that complete this futile cycle.

An equally important question is how the transport and flux of creatine in mitochondria affects the activity of this futile cycle. In principle, even diminishingly small quantities of creatine could continually undergo phosphorylation and dephosphorylation, obviating the need for significant creatine synthesis in beige adipose tissue. Indeed, creatine levels are an order of magnitude higher in brown fat, where this futile cycle does not appear to be active. Along the same lines, it is not clear why the creatine transport inhibitor, β-GPA, which reduces creatine levels by less than 50%, would have such a profound effect on beige fat thermogenesis, as it reduced oxygen consumption in response to β-adrenergic stimulation in beige fat, as well as core body temperature of cold-adapted *Ucp1* knockout mice. Finally, a key question concerns how the cycle may be regulated, particularly in response to adrenergic activation of the beige fat cell.

Additional investigations into this futile cycle by genetic and pharmacological manipulation of its activity will hopefully reveal its relative contribution to energy expenditure in humans and whether or not it is modulated in obesity. If it does prove to be an important component of adaptive thermogenesis, therapeutic or even dietary agents might be employed to activate the process and perhaps achieve weight loss in obese individuals. Let's hope that efforts to decipher the mechanisms of this cycle are not futile.

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