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Review

The molecular features of uncoupling protein 1 support a conventional mitochondrial carrier-like mechanism

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ABSTRACT

Uncoupling protein 1 (UCP1) is an integral membrane protein found in the mitochondrial inner membrane of brown adipose tissue, and facilitates the process of non-shivering thermogenesis in mammals. Its activation by fatty acids, which overcomes its inhibition by purine nucleotides, leads to an increase in the proton conductance of the inner mitochondrial membrane, short-circuiting the mitochondrion to produce heat rather than ATP. Despite 40 years of intense research, the underlying molecular mechanism of UCP1 is still under debate. The protein belongs to the mitochondrial carrier family of transporters, which have recently been shown to utilise a domain-based alternating-access mechanism, cycling between a cytoplasmic and matrix state to transport metabolites across the inner membrane. Here, we review the protein properties of UCP1 and compare them to those of mitochondrial carriers. UCP1 has the same structural fold as other mitochondrial carriers and, in contrast to past claims, is a monomer, binding one purine nucleotide and three cardiolipin molecules tightly. The protein has a single substrate binding site, which is similar to those of the dicarboxylate and oxoglutarate carriers, but also contains a proton binding site and several hydrophobic residues. As found in other mitochondrial carriers, UCP1 has two conserved salt bridge networks on either side of the central cavity, which regulate access to the substrate binding site in an alternating way. The conserved domain structures and mobile inter-domain interfaces are consistent with an alternating access mechanism too. In conclusion, UCP1 has retained all of the key features of mitochondrial carriers, indicating that it operates by a conventional carrier-like mechanism.

Contents

Abbreviations: UCP, uncoupling protein; AAC, mitochondrial ADP/ADP carrier; DPC, dodecylphosphocholine (Fos-Choline-12); 10MNG, decyl maltose neopentyl glycol.

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1. Introduction

Uncoupling protein 1 (UCP1) is the defining feature of brown fat and is responsible for the unique thermogenic properties of the tissue [1–3]. The protein catalyses the leak of protons across the mitochondrial inner membrane, dissipating the electrochemical proton gradient that would otherwise drive ATP production by ATP synthase. As a result, the energy from the oxidation of respiratory substrates is instead released as heat. Hence, substrate oxidation is 'uncoupled' from ADP phosphorylation. UCP1 is the original uncoupling protein that defined the name and, unlike its homologues within the mitochondrial carrier family of metabolite transporters (e.g. UCP2 and 3), is the only carrier that is undisputed in having a proton leak function (see Refs. [4,5]). Brown fat that is rich in UCP1 has long been known to facilitate non-shivering thermogenesis in newborn mammals to help defend body temperature against the cold. However, over recent years it has come to prominence that the tissue is present in adult humans too [6,7]. Humans exhibit both 'classical' brown fat, with characteristics similar to the developmental tissue studied in newborns, and 'beige' brown fat, a recruitable form in white adipose tissue (see Ref. [8] for an overview); the major depots are found in the supraclavicular region and the neck [6,9]. These tissues utilise UCP1 and, when activated (e.g. by cold exposure), have the potential to contribute significantly to whole-body energy expenditure [7,10–12]. Notably, the occurrence of brown fat in humans correlates with leanness [7,9,13]. In mice, thermogenesis by brown fat has been shown to clear lipids and dispose of glucose from the blood, reducing metabolic disease [14], while the genetic ablation of brown fat [15], or UCP1 specifically [16], induces obesity. Methods to encourage brown adipose tissue and, importantly, activate thermogenesis via UCP1 in the absence of physiological stimuli [11,17,18] could provide therapies to combat obesity and related diseases [19].

UCP1 activity is well regulated in brown adipocytes, where it is induced in response to cold exposure or over-feeding through the sympathetic nervous system, facilitating adaptive thermogenesis (see Ref. [20] for review). Signals from the brain stimulate the release of catecholamines, such as noradrenaline, in innervated brown adipose tissue, which activate adrenergic receptors (most significantly β_3) in the plasma membrane of brown adipocytes to initiate intracellular cAMP-dependent signaling (Fig. 1). $G_{\alpha s}$ protein, dissociated from stimulated receptors, activates adenylate cyclase, increasing cellular cAMP, which leads to changes in UCP1 activity both acutely and through transcriptional regulation of the UCP1 gene [21–23]. In the acute response, cAMP activates protein kinase A, which, among several targets, is believed to phosphorylate hormone sensitive lipase and the perilipin protein that protects stored lipid droplets from lipase breakdown, activating and deactivating them, respectively [20,24,25]. The resulting lipolysis of triglyceride stores releases free fatty acids into the cytosol, which act as a fuel for mitochondrial oxidation, and, importantly, as the direct activator of UCP1 [26], switching on thermogenesis (Fig. 1). UCP1 binds and is inhibited by cytosolic (Mg^{2+}-free) purine nucleotide (see Ref. [27]), ATP and ADP being the most relevant. The mechanism of how fatty acids activate UCP1 and overcome nucleotide inhibition is debated (see below).

Up-regulation of the UCP1 gene occurs as part of adaptive thermogenesis following adrenergic stimulation. The signaling process and regulation of UCP1 transcription is multifaceted (see [21,23] for a review), involving the activation of the p38 MAP kinase pathway downstream of protein kinase A stimulation by cAMP. The UCP1 gene has a complex enhancer region upstream of the promoter, where cAMP response elements facilitate transcriptional control [28]. The region contains binding sites for several nuclear receptor transcription factors, including the peroxisome proliferator-activated receptor γ (PPARγ), retinoic X receptor (RXR), 9-cis retinoic acid receptor (RAR) and thyroid hormone receptor (TR), consistent with the transactivation of the UCP1 gene in response to their respective ligands [23]. The active control of expression, tied to physiological cues, means that UCP1 protein concentrations in brown adipose tissue mitochondria can vary significantly. The protein is a relatively abundant mitochondrial carrier in conventional brown fat mitochondria, even in warm adapted animals. In cold adapted animals, however, UCP1 levels can increase to represent as much as ~10% of the mitochondrial inner membrane protein [29].

The features of the UCP1 protein first started to emerge from early studies with isolated mitochondria (see Ref. [30]). The 'recoupling' effect of nucleotides on mitochondrial respiration and the demonstration of an externally-exposed GDP-binding site led to the proposal that a 'nucleotide binding protein' was responsible for the unusual ion transport properties of brown fat mitochondria [31]. Ricquier and Kader [32] were first to highlight a 32 kDa mitochondrial protein that increases in concentration in association with cold acclimation, which corresponded to the same protein in later 8-azido-ATP labeling studies identified to be responsible for controlling energy dissipation [29]. The purification of the protein, first by using immobilised nucleotide [33] and later by 'negative chromatography' methods developed for the ADP/ATP carrier [34,35], led to investigations into the properties of what was then first termed "uncoupling protein". These studies revealed UCP1 as an integral membrane protein like the ADP/ATP carrier but with distinct nucleotide binding properties. Purine nucleotides (ATP, ADP, GTP and GDP) specifically bind with high affinity at low pH (<6), stabilising the detergent-solubilised protein [35]. The stoichiometry of nucleotide binding and oligomeric state of UCP1 was also addressed, but has since been revised in our recent work (see below and [36]). The identification of the UCP1 gene and amino acid sequence [37,38] confirmed that UCP1 is related to the ADP/ATP carrier, and is a member of the mitochondrial carrier family of metabolite exchangers, which all share the same basic structure and membrane topology [39–41]. They are composed of three ~100-amino acid homologous domains, each comprising two transmembrane α-helices separated by a loop and small α-helix on the matrix side [41]. The first helix of each domain contains the signature motif PX[DE]XX[RK], which is well conserved across the protein family. Following the reconstitution of isolated UCP1 into liposomes, key characteristics associated with the uncoupling activity of isolated brown adipose tissue mitochondria could be demonstrated [42–44]. Namely, proton conductance activated by fatty acids, which can be inhibited by nucleotides. However, despite many studies over the years, the molecular mechanism of UCP1 has not been resolved.

2. Proposed mechanisms of UCP1 activation

There have been many claims for various cellular metabolites interacting with and regulating UCP1, however, most are controversial and are likely to relate to the many technical difficulties in studying this type of protein (see "Unsubstantiated mechanistic and structural claims" below). What is clear is that proton conductance by UCP1 is activated by free fatty acids and inhibited by purine nucleotides. Several models have been proposed to explain the interplay that occurs with these effectors, each derived from observations largely based on different methodological approaches. Studies with isolated mitochondria have suggested that fatty acids and nucleotides influence uncoupling activity with simple competitive kinetics [45,46], supporting a 'functional competition' model (Fig. 2A). In this proposal, fatty acids act on