Evolution of UCP1

Michael J. Gaudry, Kevin L. Campbell, and Martin Jastroch

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Abstract

Brown adipose tissue (BAT), the specialized heat-producing organ found in many placental mammals including humans, may be accessible for clinical drug intervention to help combat metabolic diseases. Understanding the biology of BAT and its thermogenic uncoupling protein 1 (UCP1) will benefit from an assessment of its evolution, answering where UCP1 originated and how it has been modified and integrated into cellular energy metabolism. Here, we review topical insights regarding the molecular evolution of UCP1—also reconstructing the proximate and ultimate factors selecting for brown fat thermogenesis in placental mammals. This new thinking on "old" events will assist our understanding of how thermogenic mitochondrial uncoupling was integrated into the physiology of the brown adipocyte. Recent comparative studies examining the occurrence of UCP1 in vertebrates not only identified the ancient (pre-mammal) rise of UCP1 but also its repeated downfall during mammalian evolution as

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evidenced by multiple independent gene loss and/or inactivation events. Together with the comparative physiology of various species, we may be able to find conditions that favor UCP1 thermogenesis and, learning from these insights, identify molecular networks that will be useful to pharmacologically stimulate the tissue.

Keywords
Brown adipose tissue · Evolution · Metabolic disease · Thermogenesis · Uncoupling protein

1 Brown Adipose Tissue Thermogenesis

Brown adipose tissue (BAT) is a specialized organ in placental mammals that enables non-shivering thermogenesis (NST) via molecular mechanisms centered in the mitochondria that lower metabolic efficiency. In addition to the dense mitochondrial content of this tissue imparting its brown coloration, BAT is both advantageously situated near vital organs of the body (i.e., interscapular, subscapular, dorso-cervical and axillary regions, as well as near the kidneys) and highly vascularized allowing for effective transfer of heat to the circulatory system (Oelkrug et al. 2015). The rapid energy turnover in brown adipocytes is enabled by high mitochondrial concentrations of uncoupling protein 1 (UCP1). UCP1 short-circuits the proton-motive force that typically drives ATP synthesis, increasing substrate oxidation and, consequently, enhancing cellular heat output (Cannon and Nedergaard 2004). BAT is widely accepted to enable both small-bodied mammals and the neonates of many larger-bodied species to survive acute and chronic cold challenges, as well as to facilitate rewarming in lineages that utilize torpor (Oelkrug et al. 2010, 2013; Cannon and Nedergaard 2004; Nicol et al. 2009). While multilocular lipid droplets of brown adipocytes provide enhanced surface area to facilitate rapid lipolysis and catabolism (Keipert and Jastroch 2014), heat output of BAT can also be maintained via glucose oxidation without the contribution of lipolysis (Shin et al. 2017). This indifference regarding substrate preference further highlights the predominant role of UCP1 for mitochondrial energy turnover. However, a pivotal role for UCP1 in thermogenesis is not universal among mammals (Gaudry et al. 2017; Keipert et al. 2017; Meyer et al. 2012; Golozoubova et al. 2001; Ukropec et al. 2006), as alternative molecular mechanisms to produce heat via ATP-consuming processes or other endogenous uncouplers have been proposed (Ikeda et al. 2017; Kazak et al. 2017b; Long et al. 2016). These thermogenic pathways offer new pharmacological potential, but notably, their measured physiological impacts have so far been exclusively restricted to UCP1 knockout mice (Kazak et al. 2017b; Long et al. 2016). For pigs, which naturally lack UCP1 (Berg et al. 2006), and mice, an ATP-consuming calcium futile cycle through SERCA/ryanodine receptor activity has been proposed as an elegant alternative heat-generating mechanism in beige adipose tissue (Ikeda et al. 2017). While pharmacological as well as gain- and loss-of-function experiments targeting SERCA and ryanodine receptors show the impact on mitochondrial respiration

rates in beige adipocytes, the bioenergetic data of the study surprisingly reveal no impact on ATP-linked respiration, which is in stark contrast to the suggested ATP-dependent model of thermogenesis. Notably, increased ATP consumption would stimulate both glycolytic and oxidative ATP production, at least based upon our current understanding of cellular bioenergetics. Further experiments are thus required to delineate whether calcium futile cycling contributes to thermogenesis in pigs or whether this mechanism remains fishy (in the sense that it was originally proposed for and remains limited to the cranial heater organ of billfishes, swordfish, and the butterfly mackerel; Morrissette et al. 2003). It should be taken into consideration that the disturbance of calcium homeostasis in the Ikeda study may have affected calcium-sensitive dehydrogenases (e.g., pyruvate dehydrogenase), providing a simple explanation for the observed cellular phenomena. Additional research is required to determine whether these and other alternative mechanisms of heat production have any physiological significance in nature, or not. With regard to mitochondrial uncoupling, other UCPs have been suggested to thermogenically compensate if BAT-mediated NST becomes impaired, but this idea has been refuted several times (Golozoubova et al. 2001; Nedergaard and Cannon 2003), mainly due to inconclusive evidence regarding uncoupling activity (Cadenas et al. 2002; Shabalina et al. 2010: Nabben et al. 2011). A potential role for UCP3 in thermogenesis by uncoupled respiration has been recently revived in (some) pig breeds lacking UCP1 (Lin et al. 2017), but the conclusions have to be taken with similar caution as the observed uncoupling cannot be reconciled with our current knowledge on mitochondrial respiratory control (Jastroch et al. 2018). Notably, UCP1-ablated mice further render BAT dysfunctional by inflammation and electron transport deficiencies in the cold (Oelkrug et al. 2010; Kazak et al. 2017a; Keipert et al. 2017), most likely also compromising ATP output. Thus, it remains questionable whether BAT of UCP1 knockout mice represents a good model to investigate some proposed ATP-dependent thermogenic pathways. ATP-dependent thermogenic pathways in beige adipose tissue may be significant if glycolytic and oxidative ATP production can provide sufficient capacities. Furthermore, it should not be forgotten that heat generated by muscle shivering is able to compensate for the lack of brown fat NST (Golozoubova et al. 2001).

2 The Rise of UCP1

Utilizing a comparative approach to investigate the evolution of UCP1 over millions of years could provide valuable insights of both its function and capacity for medical intervention. UCP1, first discovered in the late 1970s (Ricquier and Kader 1976; Heaton et al. 1978), was long believed to have originated with the evolution of eutherian BAT as a unique strategy to defend high body temperatures in the cold (Cannon and Nedergaard 2004). However, tracing orthologous *UCP1* loci by comparative genomics unambiguously revealed the presence of this six-exon gene in teleost fishes, discernible from *UCP2* and *UCP3* paralogs by its conserved syntenic arrangement (i.e., 5′-*TBC1D9-UCP1-ELMOD2*-3′; Fig. 1; Jastroch et al. 2005).

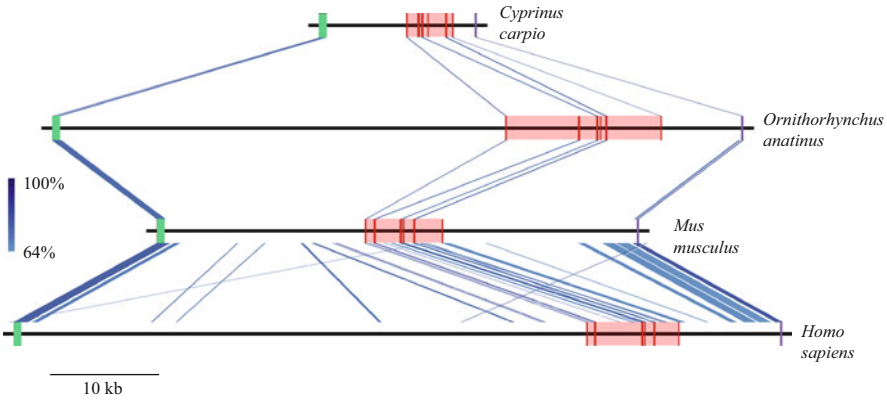

Fig. 1 Sequence identity comparison made in Easyfig 2.2.2 displaying the conserved synteny of the *UCP1* gene among representative vertebrate species (common carp [*Cyprinus carpio*], platypus [*Ornithorhynchus anatinus*], house mouse [*Mus musculus*], and human [*Homo sapiens*]) with respective accession numbers of LHQP01013372.1, NW_001794248.1, AC122890.4, and LOQN01003215.1. *UCP1* exons are symbolized by solid red bars with intervening intron sequences shaded in red. Terminal exons of flanking genes *TBC1D9* and *ELMOD2* are symbolized with green and purple boxes, respectively

This unequivocally revealed that *UCP1* predated the evolution of endothermy and was present in a common ancestor of ray- and lobe-finned fishes (~420 million years ago [MYA]), thus spurring a re-examination of its evolutionary origin.

Common carp (*Cyprinus carpio*) *UCP1* mRNA is detected in a wide range of tissues including the liver, brain, intestine, and kidney, but interestingly not adipose tissue (Jastroch et al. 2005). The high expression of the fish *UCP1* orthologue in the liver of the common carp has been independently confirmed by other studies (Bermejo-Nogales et al. 2014; Murakami et al. 2015; Wen et al. 2015). Interestingly, the regulation of fish UCP1 by environmental cues differs vastly between organs as cold-exposed carp downregulate *UCP1* mRNA levels in the liver (Jastroch et al. 2005), but increase expression in brain tissue (Jastroch et al. 2007). In particular, this latter upregulation in certain areas of the brain fostered speculation of local thermogenesis in the neuronal tissues of carp (Jastroch et al. 2007). A similar role for UCP1 has also been suggested in the brain of hibernating thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*; Laursen et al. 2015), but these results await confirmation by other studies. Additionally, one has to take into account that local thermogenesis would not only require UCP1, but also high oxidative capacity and rapid metabolic fluxes. Although liver mitochondrial uncoupling in carp coincides with the presence of *UCP1* mRNA (Jastroch et al. 2007), the involvement of other mitochondrial anion carriers, such as GDP-sensitive uncoupling activity of the adenine nucleotide transporter (Khailova et al. 2006), has not yet been ruled out. In short, future studies are required to fully elucidate the myriad functions of UCP1 in ectothermic vertebrates and nonplacental mammals (monotremes and marsupials), though its physiological roles are likely important as it is highly conserved and

evolving under strong purifying selection within these lineages (Gaudry et al. 2017). Similarly, *UCP2* and especially *UCP3* are remarkably well conserved among vertebrates (Gaudry et al. 2017), and while these paralogous members of the UCP gene family have been proposed to fulfill several functions including catalyzing mild uncoupling to mitigate the production of harmful reactive oxygen species, to date, a consensus has not been reached on their functions, and any suggestions of their thermogenicity remain unsubstantiated (Brand and Esteves 2005; Echtay 2007; Mailloux and Harper 2011; Lin et al. 2017).

When we focus on the most basal branch of the mammalian family tree, monotremes, RNA-seq BLAST searches reveal the presence of *UCP1* translation in a surprisingly wide range of tissue types (Gaudry and Campbell 2017). Indeed, *UCP1* transcripts are found in platypus (*Ornithorhynchus anatinus*) testis, ovary, liver, kidney, heart, and brain tissue, which is reminiscent of the expression pattern seen in ectothermic vertebrates. By contrast, available published evidence suggests marsupial UCP1 may be restricted to adipose depots, which often appear brownish in coloration and are located in the pectoral regions of juvenile gray short-tailed opossums (*Monodelphis domestica*) and the interscapular regions of the fat-tailed dunnart (*Sminthopsis crassicaudata*; Jastroch et al. 2008). Furthermore, this latter species utilizes daily torpor and upregulates UCP1 expression following cold exposure, raising the possibility of a heat-producing role. Yet, so far, evidence for thermogenesis by marsupial UCP1 is lacking, as the fat-tailed dunnart was later shown to be incapable of adaptive NST through classical noradrenaline injection (Polymeropoulos et al. 2012). It remains unknown whether monotreme or marsupial UCP1 permits mitochondrial proton leak as it does in eutherians. Notably, a 5′ enhancer box located ~3–5 kb upstream of the *UCP1* gene, believed to contribute to high UCP1 expression in eutherian BAT, first arose in a stem eutherian ancestor and is absent in non-eutherian mammals (Jastroch et al. 2008; Gaudry and Campbell 2017). This difference may partly underlie comparatively lower *UCP1* transcription in marsupial adipose depots relative to that of comparably sized eutherians (Rousset et al. 2004). While this "brownish" adipose tissue may still contribute to heat production in this lineage, further studies are required to clarify this and other potential physiological roles.

In sharp contrast to ectothermic vertebrates and the platypus, eutherian UCP1 is predominantly expressed in brown adipocytes and, under certain physiological conditions (e.g., cold stress), within white adipose tissue depots. These latter UCP1-positive cells are referred to as "beige" or "brite" (brown-in-white) adipocytes, as they take on further brown adipocyte-like characteristics such as increased mitochondrial density and multilocularity (Harms and Seale 2013). Apart from some reports on UCP1 expression in neurons and thymocytes (Carroll et al. 2005; Adams et al. 2008; Laursen et al. 2015), eutherian UCP1 expression appears highly tissue-specific. Moreover, UCP1 in BAT mitochondria is exceedingly concentrated and can represent as much as 8% of mitochondrial membrane proteins in brown adipocytes (Rousset et al. 2004), some 100- to 1000×-fold higher than expression levels of UCP2 and UCP3 in other tissues (Brand and Esteves 2005).

Among a wide range of eutherian mammals, the role of UCP1 has been well-documented to be thermogenic and, for some species, contribute a crucial survival advantage in cold environments, including mouse knockout models that confirm an essential role of UCP1 to acute cold challenges. For instance, UCP1 is implicated in BAT-mediated NST of the rock elephant shrew (*Elephantulus myurus*; Mzilikazi et al. 2007) and lesser hedgehog tenrec (*Echinops telfairi*; Oelkrug et al. 2013), two affiliates of the Afrotherian superorder. Some features of BAT in Afrotherian species may be considered archetypal, such as no regulation of BAT mass and UCP1 content in the elephant shrews, or the unusual abdominal location of BAT in the tenrec. This finding is in line with recent work that reveals marked differences in putative promoter and enhancer elements in non-model versus murid rodent model systems (Gaudry and Campbell 2017). Indeed, some promoter elements (e.g., CRE-4 and CCAAT box) postulated to contribute to *UCP1* transcription in the mouse and rat are absent in non-murid species. Also, while the enhancer is generally conserved, relatively few motifs (CRE-3, PPRE, and RARE-3) thought to be key for *UCP1* expression in rodents display high identity to homologous sequences in other lineages, highlighting the likely evolution of differential transcriptional control mechanisms within the eutherian radiation. In members of both the Laurasiatheria and Euarchontoglires superorders, BAT-mediated NST has been detailed in a wide range of studies as reviewed by Oelkrug et al. (2015). Notably, the Euarchontoglires superorder also includes humans, who are known to express BAT as newborns and even into adulthood (Nedergaard et al. 2007; Cypress et al. 2009; van Marken Lichtenbelt et al. 2009; Virtanen et al. 2009). Thus, due to its widespread occurrence across the eutherian radiation, the rise of adaptive UCP1 thermogenesis presumably originated following the marsupial/placental divergence. Recent fossil-calibrated timetrees (Springer et al. 2017; Liu et al. 2017) accordingly place this event within a ~100 million year window between the Middle Jurassic and Late Cretaceous (~180 and 80 MYA). The global climate over much of this period is generally characterized as a warm-to-hot "greenhouse" with no polar glaciations and only small latitudinal temperature gradients, though the early Middle Jurassic may have been cooler with latitudinal gradients more in line with the present (O'Brien et al. 2017; Alberti et al. 2017). Ancestral reconstructions also infer that the Late Cretaceous crown eutherian ancestor was a small-bodied (<245 g) scansorial insectivore with an altricial reproductive strategy (O'Leary et al. 2013) and was probably heterothermic (Lovegrove 2012). Therefore, despite likely having evolved in a much warmer world, BAT may have arisen as a mechanism to maintain elevated body temperatures in newborns (Rowlatt et al. 1971), for offspring thermoincubation (Oelkrug et al. 2013), and/or as a more efficient method of rewarming from bouts of torpor (Oelkrug et al. 2011). Although UCP1-mediated NST has even been proposed to have facilitated the expansion of modern eutherian groups to cold ecological niches (and temperature seasonality) that emerged in the early Oligocene (~34 MYA), it should be noted that the advent of thermogenic BAT predates these events by 50–150 million years.

Molecular phylogenetic studies reveal long stem eutherian branch lengths in both UCP1 nucleotide and amino acid trees relative to those of UCP2 and UCP3 (Fig. 2), demonstrating an elevated substitution rate that may have led to the hypothesized

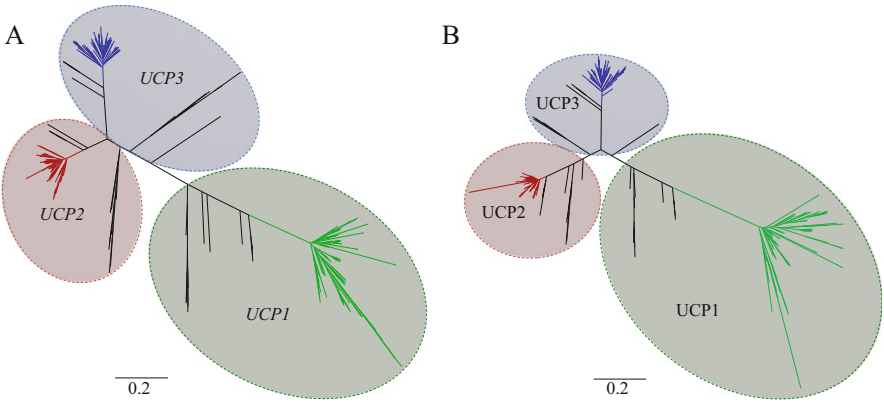

Fig. 2 UCP gene trees of nucleotide coding sequences (**a**) and virtually translated amino acid sequences (**b**) from the data set of Gaudry and Campbell (2017; $n = 448$). Approximately-maximum-likelihood phylogenetic trees were built using FastTree 2.1.5 with default settings. Branch lengths denote number of substitutions per site. Branches of non-eutherians are displayed in black, while eutherian branches are colored in green (UCP1), red (UCP2), and blue (UCP3). Note the lengths of the stem eutherian branches are substantially longer for UCP1 than both UCP2 and UCP3 indicating an increased rate of nucleotide and amino acid substitution

novel function of UCP1 that permitted physiologically significant proton translocation across the mitochondrial inner membrane (Saito et al. 2008; Hughes et al. 2009; Gaudry and Campbell 2017). Saito et al. (2008) attributed this apparent gain of thermogenic function to directional/positive selection, although follow-up studies with improved (larger and more diverse) data sets indicate non-synonymous (dn) to synonymous (ds) nucleotide substitution ratios along the stem eutherian branch of 0.5–0.6, which is more in line with relaxed evolutionary constraints rather than positive selection (defined as dn/ds > 1; Hughes et al. 2009; Gaudry et al. 2017). Additionally, while eutherian UCP1s exhibit numerous unique amino acid residues relative to non-eutherian mammals, to date, no evidence demonstrates that these substitutions can be accredited to positive selection (Fig. 3; Hughes et al. 2009; Gaudry et al. 2017). However, future studies may be able to target these residues to determine if they do in fact alter proton translocation across the mitochondrial inner membrane and can serve as potential sites of medical intervention. Comparative cryo-EM or crystal structures will also be key in identifying important amino acid interactions and structure-function relationships underlying the variability in uncoupling activity among vertebrate UCP1s. Unfortunately, attempts to resolve the physical structure of UCP1 have failed so far, and uncertainty remains with many mutational loss-of-function studies, as protein integrity cannot be confirmed. However, it may be possible to scale UCP1 functional features by naturally diversified sequences, which circumvent mutational integrity problems as nature's blueprint has purified functional sequences for the in vivo condition.

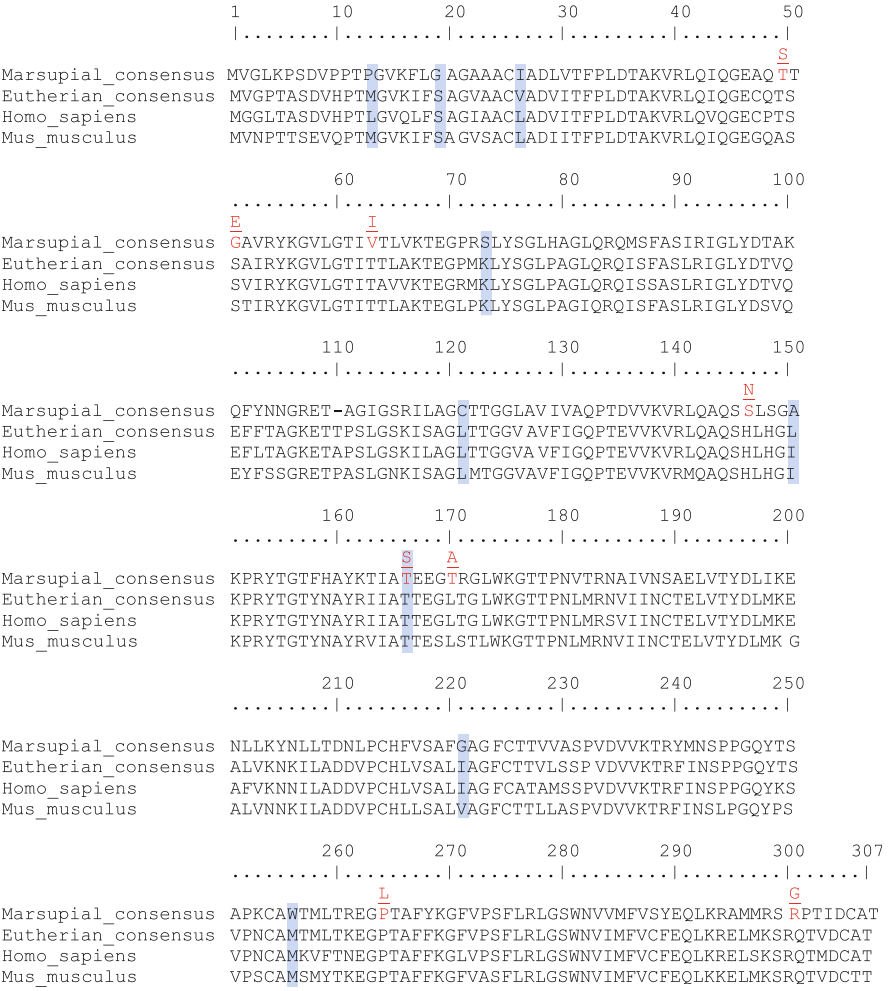

Fig. 3 Deduced UCP1 amino acid alignment of the marsupial consensus sequence, eutherian consensus sequence, human (*Homo sapiens*), and mouse (*Mus musculus*). Consensus sequences were generated from the Gaudry and Campbell (2017) data set by a simple majority and excluding any eutherian *UCP1* pseudogenes. Equally represented amino acids between the four marsupial species (*Monodelphis domestica*, *Macropus eugenii*, *Sminthopsis crassicaudata*, *Sarcophilus harrisii*) are shown in red. Amino acids highlighted in blue have been tested as candidates for positive selection, but these hypotheses were statistically rejected using likelihood ratio tests (Hughes et al. 2009; Gaudry et al. 2017)

3 The Fall of UCP1

Despite the documented benefits of UCP1-mediated NST in several (small) eutherian species, members of a variety of lineages are accomplished endotherms despite lacking a functional UCP1. For instance, birds possess even higher body

temperatures than mammals and defend their body temperature by shivering and possibly other poorly characterized mechanisms. The detection of a *UCP* mRNA, termed avian UCP, fostered early speculation that it may catalyze thermogenesis similar to eutherian UCP1. However, the molecular and physiological evidence remains correlative (Raimbault et al. 2001; Vianna et al. 2001), and functional data in bird mitochondria do not support the typical mode of UCP1 action and may instead be related to reactive oxygen species (Talbot et al. 2004; Criscuolo et al. 2005). Conserved synteny revealed that avian *UCP* is an orthologue of *UCP3* and suggested that *UCP1* and *UCP2* are absent in birds (Emre et al. 2007). In fact, *UCP1* appears to have been excised from the genome in a common ancestor of the Sauropsida lineage (reptiles and birds; Mezentseva et al. 2008; McGaugh and Schwartz 2017).

Among mammals, suids (pigs and kin) were the first described lineage to lack a functional copy of the *UCP1* gene (Berg et al. 2006). Exons 3–5 of this pseudogene have been deleted, and the remaining exons (1, 2, and 6) are plagued by nonsense and frameshift mutations (Berg et al. 2006). A recent attempt to override genomic evidence with immunological detection (Mostyn et al. 2014) has been criticized for methodological shortcomings (Jastroch and Andersson 2015). Delineating experiments using specific pig UCP1 antibodies further confirmed that this inactivated gene does not result in a translated protein (Hou et al. 2017a). Taken together, this work provides a molecular explanation of why piglets lack BAT (Herpin et al. 2002, Hou et al. 2017b), are vulnerable to cold temperatures, and rely upon shivering thermogenesis and behavioral adaptations, such as maternal nest building, to defend against hypothermia. While Berg et al. (2006) estimated this pseudogene to have arisen ~20 MYA, newborn peccaries also reportedly lack BAT (Rowlatt et al. 1971); thus it seems plausible that a shared inactivation event may have occurred prior to the radiation of Suoidea ~37 MYA. More recent work by Lin et al. (2017) suggested that certain cold-adapted pig breeds compensate for the lack of UCP1 and BAT by upregulating UCP3 expression in thermogenic white/beige adipocytes in response to cold. However, the interpretation that UCP3 contributes to an increased rate of proton leak may be a misconception stemming from a lack of proton leak kinetics data that require simultaneous measurement of mitochondrial membrane potential and oxygen consumption (Jastroch et al. 2018). Thus, UCP3 has not actually been shown to contribute to proton conductance in pigs.

The progressive downfall of *UCP1* throughout the course of eutherian evolution was further traced in two studies by McGaugh and Schwartz (2017) and Gaudry et al. (2017). The latter study employed a comparative phylogenetic approach to reveal that members within nearly half of all traditional eutherian orders (8 of 18) lack functional *UCP1*. These findings corroborate reports that failed to detect discernable BAT depots in neonates of each of these groups (Rowlatt et al. 1971). Notably, as these ancient inactivations were accompanied by dn/ds ratios indicative of neutral evolution, Gaudry et al. (2017) were further able to estimate pseudogenization dates using nucleotide substitution models and phylogenetic bracketing techniques, thereby correlating these independent inactivations to sharp reductions in metabolic intensity or rapid evolutionary increases in body size (and hence species

diversity). For instance, ancient (likely Cretaceous to early Paleocene) independent *UCP1* inactivations in both Xenarthrans (anteaters, sloths, and armadillos) and pangolins are presumably linked to the adoption of energetically diffuse diets that favored energy-conserving reductions in body temperature and metabolic turnover. By contrast, independent *UCP1* inactivations in the ancestors of proboscideans (elephants and mammoths), hyraxes, sirenians (sea cows), equids (horses and zebras), and cetaceans (whales and dolphins) all temporally coincide with magnitude-scale increases in body size of each lineage. For the terrestrial clades, these shifts in body size were likely driven in part by progressive global cooling in the ~30 million years following the Paleocene-Eocene thermal maximum some 55 MYA (Gaudry et al. 2017); i.e., *UCP1* was primarily inactivated in response to planetary *cooling* as opposed to a relaxation of thermogenic needs in tropic environments as was previously proposed for pigs (Berg et al. 2006). The evolution of larger body size evident from the fossil record of these lineages reduced surface area to volume ratios, allowing for more efficient retention of body heat and, theoretically, reduced their need for BAT-mediated NST. This interpretation is consistent with a strong negative correlation between body size and NST capacity that has been observed for eutherians by Oelkrug et al. (2015). Heldmaier (1971) similarly predicted no thermal benefit of BAT in species above 10 kg. It is thus perhaps unsurprising that a number of large-bodied species that lack UCP1 (mammoths, Steller's sea cows, horses, cetaceans, and even extinct ground sloths) were able to expand their ranges into Arctic and sub-Arctic environments. It should be stressed, however, that *UCP1* pseudogenization did not come without evolutionary costs as it presumably hindered the re-evolution of small body size, thereby limiting current species diversity in UCP1 lacking clades (Gaudry et al. 2017). It is also of note that some large-bodied eutherian species (e.g., rhinoceroses, hippos, giraffes, camels) possess *UCP1* genes with putatively translatable open reading frames, though whether or not the protein is expressed in BAT or beige adipocytes remains unknown. Future functional assays of UCP1 from these species may reveal amino acid substitutions accumulated through neutral evolution or relaxed selection pressures that suppress maximal proton leak or other uncoupling attributes relative to species that are known to rely heavily upon BAT-mediated thermogenesis. For example, UCP1 of extant camels contains a four-residue deletion bordering the putative GDP-binding domain that may compromise functional control of thermogenesis (Gaudry et al. 2017). Overall, the fall of *UCP1* highlights the need to further investigate alternate heat-producing mechanisms or greater heat retention capacities (e.g., arteriovenous rete) in these lineages that may compensate for the lack of BAT-mediated NST, as well as the importance of BAT in eutherians that have retained it throughout evolution, such as humans.

4 Translational Value

The most relevant mammals to the medical community are humans, *Homo sapiens*. Over the last decades, and presumably as a consequence of the modern lifestyle in our society, there has been an increasing prevalence of metabolic diseases such as

obesity and type 2 diabetes. The metabolic syndrome of positive energy balance that leads to corpulence, inflammation, and insulin resistance is usually mimicked in mice to gain further insights into this condition. To overcome disease-promoting aspects, the scientific community concurs that increasing energy expenditure would benefit humans by adjusting energy balance and improving systemic lipid and glucose metabolism. The discovery of BAT in adult humans (Nedergaard et al. 2007; Cypress et al. 2009; van Marken Lichtenbelt et al. 2009; Virtanen et al. 2009) fostered major efforts to harness adipose tissue thermogenesis to this end, and current efforts are geared toward the recruitment and activation of brown and beige cells. In addition to cold exposure, many promising "browning" agents have been discovered (e.g., butyrate metabolite, lactate, BMPs, adenosine) that may have potential to be targeted in humans (Roberts et al. 2014; Carrière et al. 2014; Xue et al. 2014; Okla et al. 2015; Gnad et al. 2014). These avenues await further consolidation, and future research of signaling pathways controlling the differentiation of beige adipocytes may yield additional sites of possible therapeutic/pharmacological intervention.

However, the dominant experimental model organism is the house mouse (*Mus musculus*), and our knowledge from this species is translated to the human condition. Restricting our research to laboratory mice, however, may bare some caveats that can only be rectified by expanding the range of experimental species. The mouse reflects very different thermoregulatory demands compared to humans, which can be up to three orders heavier than mice. Furthermore, the relative amount of brown fat in adult humans is minor, decreasing in mass after neonatal life (Cannon and Nedergaard 2004; Nedergaard et al. 2007). Thus, the amount of UCP1 in adult humans is rather small, with further decreases in obese subjects (Wang et al. 2015). Furthermore, from the viewpoint of a comparative physiologist, medical research performs a 1-vs-1 species comparison, and we cannot be sure which signaling pathways in mice are specialized for this diminutive life-form and which signaling pathways apply to the energy-wasting function that can be applied to humans. Indeed, *UCP1* transcriptional control mechanisms may vary substantially between humans and mice (Gaudry and Campbell 2017), highlighting the need for broad comparative studies that provide translatable insights to human medicine. For all these caveats, nature's diversity provides blueprints for the understanding of BAT, in particular in humans. Embracing Krogh's principle "for such a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied" could be very true to solve the metabolic pandemic with mechanisms of brown adipose tissue.

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