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Hughes, S. Miwa, C.E. Cooper, D.A. Svistunenko, R.A. Smith, M.D. Brand, J. Biol. Chem. 278 (2003) 48534–48545.
[3] K.S. Echtay, D. Roussel, J. St-Pierre, M.B. Jekabsons, S. Cadenas, J.A. Stuart, J.A. Harper, S.J. Roebuck, A. Morrison, S. Pickering, J.C. Clapham, M.D. Brand, Nature 415 (2002) 96–99.
[4] K.S. Echtay, T.C. Esteves, J.L. Packay, M.B. Jekabsons, A.J. Lambert, M. Portero-Otín, R. Pamplona, A.J. Vidal-Puig, S. Wang, S.J. Roebuck, M.D. Brand, EMBO J. 22 (2003) 4103–4110.
[5] V. Azzu, M.D. Brand, Trends Biochem. Sci. 35 (2010) 298–307.

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5P3

GMP reductase modulates purine nucleotide concentrations and uncoupling protein 1 (UCP1) activity

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In brown adipose tissue (BAT), uncoupling protein 1 (UCP1) is the central component of non-shivering thermogenesis that provides heat to defend body temperature in the face of low ambient temperatures. UCP1 facilitates a proton flux across the mitochondrial inner membrane thereby uncoupling respiration from ATP synthesis. In the resting state purine di- and triphosphate nucleotides (GDP, GTP, ADP, ATP) repress UCP1 activity, while fatty acids liberated upon adrenergic activation overcome this inhibition and activate UCP1 upon cold exposure. So far, cold induced alterations in cellular nucleotide concentration are not thought to play a role in UCP1 regulation. In BAT, guanosine monophosphate reductase (GMPR) is strongly upregulated upon cold exposure on both the mRNA and the protein level. This enzyme catalyzes the reaction of GMP back to the common presurcor of both guanosine and adenosine nucleotides, inosine monophosphate (IMP). We aimed to unravel a possible role of GMPR and an altered purine nucleotide metabolism in the regulation of UCP1 activity.

Concordant with its molecular function, forced expression of GMPR in a heterologous expression system led to a shift in the ratio between adenosine and guanosine nucleotides without a disturbance in overall cellular energy balance. In the presence of UCP1, however, the GMPR mediated A/G shift was accompanied by a loss in triphosphate nucleotides indicating an impaired energy supply. Furthermore, in the presence of both UCP1 and GMPR cells displayed an increased basal and fatty acid induced proton leak respiration. Both findings imply that the enzymatic action of GMPR leads to an increased UCP1-mediated proton leak by altering purine nucleotide concentrations.

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5P4

Evidence for a brown adipocyte specific enhancer in the first intron of the murine Ucp3 gene

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Uncoupling protein 3 (UCP3) is a mitochondrial carrier with multiple assigned functions including the transport of protons to reduce ROS formation and modulate calcium homeostasis, the export of excess free fatty acids or lipid radicals and the transport of chloride or pyruvate. UCP3 is expressed but differentially regulated in skeletal muscle (SKM) and brown adipose tissue (BAT). Initial characterization of the basal promoter revealed binding sites for PPARγ, MyoD and thyroid receptor but the underlying mechanisms differentiating gene expression between BAT and SKM are not known to date. Our group identified an intronic element required for Ucp3 expression in BAT. Subsequently a PPAR response element (PPRE) in juxtaposition of our intronic element was found.

Using band shift assays, RNA interference and reporter gene assays we demonstrated SP1/SP3 binding to our intronic element in two brown adipocyte cell lines. Inhibition of SP factor DNA binding either by site directed mutagenesis or mithramycine treatment abolished PPAR agonist activation of reporter gene constructs. Selective deletion of the known PPREs revealed that the intronic PPRE next to our SP binding site is sufficient and required for PPAR agonist activation. Transactivation was solely conveyed by the PPARγ agonist rosiglitazone while neither PPARα nor PPARδ agonists were of relevance in our system. Finally stepwise deletions covering the whole first intron assisted by bioinformatics revealed a putative MyoD binding site right next to the dual SP/PPAR element.

Using the very same reporter constructs, PPAR stimulation, regardless of PPAR subtype, was not able to activate our reporter gene constructs in C2C12 cells. This is well in line with data from SKM or C2C12 cells where mutation of the SP1/3 element had no effect on UCP3 expression. As published data demonstrate that endogenous UCP3 mRNA expression can be induced by PPARγ agonists in those cells, we conclude that PPAR action in SKM, in contrast to BAT, is neither mediated by the intronic PPRE nor by the PPRE in the core promoter.

Taken together, we propose a complex enhancer region located within the first intron. This module, consisting of at least SP, PPAR and MyoD binding sites, does not only confer the responsiveness of UCP3 to PPARγ stimulation in BAT but also differentiates regulation of expression between skeletal muscle and brown adipose tissue.

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5P5

Phospholipase iPLA$_2$γ–dependent regulation of uncoupling protein UPC2 in insulinoma INS-1E cells

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We tested a hypothesis that reactive oxygen species (ROS)-dependent activation of mitochondrial phospholipases leads to an increase in respiration and to more intensive attenuation of mitochondrial ROS production due to UCP2-dependent uncoupling. This was tested on the model of pancreatic β-cells, INS-1E cells, by using the loss-of-function approach through UCP2 silencing. Oxygen consumption was assayed in intact INS-1E cells under varying levels of glucose after overnight incubation in low glucose (3 mM). Glucose addition exhibited a steady increase in respiration which was further stimulated by sub-millimolar concentrations of tert-butyl hydroperoxide (TBHP, 0.25 – 1 mM). The effect of TBHP was significantly lower in UCP2-silenced cells than in control cells. The TBHP-induced respiration increase [1] was fully inhibited by (R)-bromoenol lactone ((R)-BEL, 10 – 50 μM), a selective inhibitor of phospholipase iPLA$_2$γ,