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► **To cite this version:**

David Carling, Benoit Viollet. Beyond energy homeostasis: the expanding role of AMP-activated protein kinase in regulating metabolism. *Cell Metabolism*, Elsevier, 2015, 6 (21), pp.799-804. <inserm-01171523>

HAL Id: inserm-01171523

<http://www.hal.inserm.fr/inserm-01171523>

Submitted on 4 Jul 2015

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Meeting report on AMPK 2014

Beyond energy homeostasis: the expanding role of AMP-activated protein kinase in regulating metabolism

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The recent exciting advances in our understanding of the regulation of the energy sensor AMP-activated protein kinase (AMPK), together with renewed appreciation of its importance in maintaining cellular function, brought together leading scientists at a recent FASEB-sponsored meeting in September 2014. Here, we report some of the highlights of this conference.

Introduction

The AMP-activated protein kinase (AMPK) is a sensor of cellular energy status that directs metabolic adaptation to support cellular growth and survival. AMPK is activated by changes in the cellular AMP:ATP and ADP:ATP ratios, and functions to restore energy homeostasis by switching off biosynthetic pathways consuming ATP while switching on catabolic pathways that produce ATP. Over the last 20 years, the AMPK field has grown enormously from a few dozen papers to over 1000 published per year. The field has grown from a group of ten to twelve investigators interested in the biological actions of AMPK in the 1990s to literally hundreds of investigators worldwide studying this pathway. Indeed, recent research has expanded the paradigm of AMPK as a metabolic sensor and to the more global concept that AMPK has broad effects on cellular function, providing synergy between research in energy metabolism and other areas of science such as cancer and immunology. The downstream actions of AMPK regulate numerous steps in cellular metabolism, cell growth, organelle regulation including mitochondrial biogenesis, autophagy, oxidative stress and cell polarity. As a result, AMPK is an attractive target for the development of drugs aimed at preventing and/or treating metabolic diseases and cancer. Recent breakthroughs in the field of AMPK research were presented on the occasion of a FASEB Scientific Research Conference entitled "AMPK: biological action and therapeutic perspectives". This was the 8th international conference in the series (the original conference was held in Boston in 2000, organized by Dr. Neil Ruderman, Boston Medical Center/Boston University School of Medicine) with the last five meetings organized on the behalf of FASEB Scientific Research Conference. This last conference was held from September 28 to October 3, 2014, in the

picturesque town of Barga, just outside of Lucca, in Italy. In a beautiful location providing an inspiring and intimate setting, scientists from 22 different countries gathered together to discuss basic AMPK biology and provide future direction for AMPK therapy in disease-focused research (**Figure 1**). More than 50 excellent and varied talks, including studies of basic and clinical relevance, addressed recent breakthroughs in the expanding field of AMPK. It would not be possible to provide a comprehensive report of all of the talks, and so in this short report, we have selected some of the highlights that arose during the meeting.

Deconstructing the complexity of AMPK regulation.

AMPK is a heterotrimeric complex composed of one catalytic α -subunit comprising a typical Ser/Thr kinase domain, one scaffold β -subunit containing a carbohydrate binding module (CBM) and one regulatory γ -subunit containing four cystathionine- β -synthase (CBS) domains, that serve to bind adenine nucleotides. The mechanism of AMPK activation involves two steps, a reversible phosphorylation at a conserved residue (Thr172 in the rat catalytic subunit sequence) within the activation loop in the α -subunit, and a stimulatory allosteric effect upon binding of AMP within the CBS domains of the γ -subunit. In addition, AMP and ADP binding regulates AMPK by promoting Thr172 phosphorylation by the upstream kinases, liver kinase B1 (LKB1) and Ca²⁺/calmodulin dependent protein kinase kinase β (CaMKK β), and by protecting Thr172 from dephosphorylation by phosphatases. All these effects of AMP are antagonized by binding of ATP, providing a very sensitive mechanism for the activation of AMPK in conditions of cellular energy stress. Grahame Hardie (University of Dundee) reviewed the current knowledge of the “canonical” mechanisms for the regulation of AMPK by energy stress and presented alternative “non-canonical” mechanisms, including activation by CaMKK β , and repression by Akt and other kinases. Recent studies suggested that phosphorylation of a serine/threonine-rich insert (ST loop) within the α -subunit inhibits subsequent phosphorylation of Thr172 by blocking access of upstream kinases LKB1 or CaMKK β to Thr172 and represents antagonistic mechanisms to downregulate AMPK signaling (Hawley et al., 2014). Yvonne Oligschläger (Maastricht University) provided new insight into the regulation of glycogen binding to AMPK through autophosphorylation at Thr148 within the CBM of the β -subunit. Thr148 phosphorylation was found to inhibit glycogen localization of AMPK thus affecting cellular glycogen turnover (Oligschläger et al., 2015). Shengcai Lin (Xiamen University) presented a novel mechanism by which AMP regulates AMPK activation. In response to starvation or exogenous AMP, AMP binding triggers the interaction between myristoylated AMPK and the scaffold protein AXIN that also binds to LKB1. Of note, ADP has no effect on the AXIN-based complex formation. The formation of the AXIN-AMPK-LKB1 complex facilitates the phosphorylation of AMPK by LKB1 and causes AMPK activation. In follow-up studies, it was demonstrated that this mechanism occurs at the late endosome/lysosome surface and is dependent on the v-ATPase-Ragulator complex (Zhang et al., 2014). Interestingly, v-ATPase and Ragulator proteins are known to be important components for recruiting and activating mechanistic target of rapamycin complex-1 (mTORC1) to the lysosome under nutrient-rich conditions. These results suggest a general role of late endosome/lysosome in sensing of nutrient by both the AMPK and the mTORC1 signaling pathways, thereby providing a switch between catabolism and anabolism. The functional consequence for AMPK recruitment at the late endosomal/lysosomal protein complex v-ATPase/ Ragulator on the inverse relationship

between AMPK and mTORC1 signaling pathways were discussed. To add a further layer of complexity in the regulation of AMPK activation, Bruce Kemp (St Vincent Institute of Medical Research and University of Melbourne) provided evidence for activation of naive AMPK independently of Thr172 phosphorylation (Scott et al., 2014). The small molecule AMPK activator A769662 was found to activate AMPK not phosphorylated on Thr172. In contrast to adenine nucleotides, A-769662 does not bind the γ -subunit but requires the β -subunit and its autophosphorylation at Ser108 within the CBM to allosterically activate AMPK. Interestingly, a synergistic AMPK activation was found with the combination of A-769662 and AMP, independently of both α -subunit Thr172 and β -subunit Ser108 phosphorylation (Scott et al., 2014). These findings raise the exciting possibility that combinatorial strategies based on AMP and small molecule activators may be relevant for AMPK-based therapy in disease-focused research and further studies are eagerly anticipated.

Over the last few years, knowledge gained through the availability of mammalian AMPK crystal structures has fundamentally advanced the understanding of the mechanism by which adenine nucleotides regulate AMPK activity. Structural information on AMPK complexes may also help for the design of specific small-molecule activators for clinical applications. Crystal structures of AMPK bound with AMP revealed that only three of the four potential nucleotide-binding sites within the γ -subunit contribute to nucleotide regulation. Binding studies using AMPK in solution revealed that two sites (sites 1 and 3) are competitively bound by either AMP, ADP, or ATP. In contrast, nucleotide bound as site 4 was not exchangeable. Site 2 is unoccupied, possibly because this site lacks an aspartate residue found in the other sites that interacts with the ribose ring of the bound nucleotide. Binding of AMP at site 1 was assigned to the effect on allosteric activation, whereas binding of AMP or ADP at site 3 to the protection against dephosphorylation. An important breakthrough came from the crystal structure of AMPK regulatory core fragment and the kinase domain of the α -subunit providing insights on the conformational changes induced by binding of adenine nucleotides and the dynamics of communication among the different subunits. Flexible components from the α -subunit (α -RIM/ α -hook) interact with the exchangeable nucleotide-binding site 3 on the γ -subunit and favor a conformation change in which Thr172 interacts with the C-terminal regions of the α - and β -subunits, offering a protection against dephosphorylation. Steve Gamblin (The Francis Crick Institute) presented results based on structural studies for full-length human AMPK $\alpha 2\beta 1\gamma 1$ heterotrimer in complex with the small molecule activators 991 and A-769662 (Xiao et al., 2013). The molecular mechanisms for these two compounds are very similar as they bind to the same site localized at the interface of the kinase domain and the CBM of the β -subunit. Binding of 991 and A-769662 stabilizes the interaction between the CBM and the kinase domain, which in turn has a stabilizing effect on the interaction of the activation loop structure, enhancing the protection against dephosphorylation of T172. In addition to X-ray crystallography data, Matt Sanders (The Francis Crick Institute) presented results from biophysical methods (circular dichroism and biolayer interference) to compare binding of small molecule activators to AMPK complexes, showing that compound 991 binds significantly tighter to $\alpha 2/\alpha 1\beta 1\gamma 1$ and $\alpha 1\beta 2\gamma 1$ heterotrimers than A-769662. Ravi Kurumbail (Pfizer) described similar molecular mechanisms based on the crystal structure of full-length $\alpha 2\beta 1\gamma 1$ heterotrimer bound with a series of ligands related to A-769662 (Calabrese et al., 2014). A-769662 and its analog bind to a novel allosteric pocket between the CBM and the kinase domain, and mediate their effects primarily through decreasing K_m for

the substrate peptide. In contrast, a different molecular mechanism has been reported for AMP, which regulates AMPK by increasing the V_{max} of phosphorylation reaction. Uwe Schlattner (INSERM and University Grenoble Alpes) presented a complementary approach to structural studies to gain insight into the role of CBS domains for allosteric AMPK activation. He developed an AMPK FRET sensor based on the nucleotide-induced conformational switch model. This molecular sensor allows a direct and real-time readout of the AMPK conformational change upon ligand binding and may be exploited for various analytical applications as well as versatile tools for screening and *in vivo* applications.

Peripheral and central regulation of energy balance by AMPK

Although AMPK was originally identified as a sensor of cellular energy status by coordinating anabolic and catabolic pathways to balance nutrient supply with energy demand, it is now clear that it also participates in the control of whole-body energy homeostasis by integrating signals from the cellular environment and whole organism. There are a number of hormones involved in the regulation of whole-body energy balance which alter AMPK activation. Takashi Kadowaki (University of Tokyo) discussed the role of AMPK in mediating the anti-diabetic and anti-atherogenic actions of adiponectin, a hormone that is derived from adipose tissue and is reduced in obesity-linked diseases. Adiponectin exerts its effects by binding to the adiponectin receptors, AdipoR1 and AdipoR2, which mediate increased AMPK and peroxisome proliferator-activated receptor (PPAR)- α activities to regulate normal glucose and lipid metabolism and insulin sensitivity. He reported the identification of an orally active small-molecule AdipoR1/R2 agonist termed AdipoRon with very similar effects to adiponectin in muscle and liver, with activation of AMPK and PPAR- α pathways and ameliorated insulin resistance and glucose intolerance in obese diabetic mice on a high-fat diet (Okada-Iwabu et al., 2013). Another layer of signal integration for the regulation of whole-body energy balance happens at the level of hypothalamic AMPK that acts by controlling both feeding and energy expenditure. Miguel López (Universidade de Santiago de Compostella) further emphasized the role of AMPK in the ventromedial nucleus of the hypothalamus as a central integrator of peripheral signals and mediator of the effects of thyroid hormones, bone morphogenetic protein 8B (BMP8B), nicotine and oestradiol on the regulation of peripheral metabolism and particularly, brown adipose tissue thermogenesis (Martinez de Morentin et al., 2014).

Investigating emerging AMPK signaling pathways

AMPK regulates a wide array of physiological events and, consistently, is expected to have a tremendous impact on survival, growth and development at the organismal level. In addition, impairment of the metabolic control of AMPK has been associated with the pathological context as well as in many prevalent human diseases including type 2 diabetes, insulin resistance, cardiovascular diseases and cancer. In order to explore more deeply the various signaling pathways controlled by AMPK, many groups have generated and analyzed various animal models, ranging from rodents to the worm *C. elegans*. Pascal Froment (INRA) talked about the role of AMPK in male and female reproduction. Male mice deficient in AMPK α 1 show a decrease in fertility due to structural abnormalities and alterations in the motility of spermatozoa. Results from mice deficient in AMPK α 1 in the Sertoli cells established the importance of AMPK on

Sertoli-germ cell interactions and shaping of the sperm head. Deletion of AMPK α 1 in the oocyte also revealed a role for AMPK on oocyte quality and oocyte/somatic cell communication (Bertoldo et al., 2015). Richard Roy (McGill University) used *C. elegans* to demonstrate how AMPK coordinates the initiation of germ line stem cell division with environmental conditions. During periods of nutrient/energy stress, AMPK is required to buffer adverse epigenetic processes in these cells that ultimately cause reproductive defects in subsequent unstressed generations.

Ian Salt (University of Glasgow) focused on the role of AMPK in adipose tissue, influencing not only metabolism in adipocytes but also acting to suppress pro-inflammatory signaling pathways. He also discussed the importance of perivascular adipose tissue (PVAT) in the pathophysiology of cardiovascular disease and how dysregulation of AMPK in PVAT can influence vascular function in obesity. In the same vein, Neil Ruderman (Boston University Medical Center) described diminished AMPK activity in multiple adipose tissue depots from insulin resistant patients. This is associated with oxidative stress, increased expression of inflammatory cytokines and decreased expression of oxidative phosphorylation genes in visceral fat. By contrast, the marked increase in insulin sensitivity observed post bariatric surgery is accompanied by a significant increase in AMPK activity and decreased oxidative stress in subcutaneous fat.

AMPK has emerged as a key mediator of the adaptation to environmental cues. Mark Evans (University of Edinburgh) provided genetic evidence for the contribution of AMPK to ventilatory adjustments during hypoxia in that mice lacking AMPK exhibit hypoventilation and apneas rather than hyperventilation. Thus AMPK signaling links oxygen to energy supply at the whole-body level. Rémi Mounier (CNRS and Université Claude Bernard Lyon1) spoke about the regulation of stem cell fate during post-natal skeletal muscle regeneration. In response to muscle injury, muscle stem cells (also called satellite cells) become activated and trigger cellular reprogramming that results in enhanced proliferation, differentiation, and/or self-renewal. By using mice deleted of AMPK α 1 in satellite cells, he showed that AMPK orchestrates muscle stem cell fate by promoting metabolic reprogramming.

AMPK in cancer: friend or foe?

More than 10 years ago, the discovery of the tumor suppressor LKB1 as an upstream kinase of AMPK established a direct connection between the regulation of energy metabolism and cancer. However, accumulated data indicate that the functional role of AMPK in cancer initiation and progression is much more complex than expected. On one hand, AMPK can mediate the tumor suppressive signaling of LKB1 and is anti-tumorigenic; on the other hand AMPK is required for cancer cell survival under metabolic stress such as hypoxia and glucose deprivation and is pro-tumorigenic. Thus, depending on the context, AMPK has a dual role in carcinogenesis. Almut Schulze (Universität Würzburg) reminded the participants that cancer cells undergo unique metabolic reprogramming during transformation and many oncogenic signaling pathways directly regulate the activity of metabolic processes to support unrestrained cell growth (Lewis et al., 2015). Rosa Señaris (Universidade de Santiago de Compostella) discussed the involvement of AMPK in the initiation and progression of astrocytic tumors. She demonstrated that AMPK is found constitutively activated in astrocytes

expressing oncogenic HRasV12 and help to coordinately support high cell division rates by increasing extracellular lipid internalisation and reducing energy expenditure through the inhibition of *de novo* fatty acid synthesis (Rios et al., 2014). Conversely, Tracey Rouault (NIH) provided an example where AMPK acts as a tumor suppressor in hereditary renal cancer caused by fumarate hydratase (FH) deficiency. Reduced AMPK activity contributes to the oncogenic growth of FH-deficient cells by promoting lipid synthesis and anabolic reactions (Tong et al., 2011). Along the same line, Russell Jones (McGill university) demonstrated that AMPK can function as a tumor suppressor in Myc-dependent lymphoma. Disruption of AMPK signaling promotes metabolic reprogramming of cancer cells with stabilization of the hypoxia-inducible factor-1 α (HIF-1 α) to drive the Warburg effect. In this context, he presented evidence supporting a role for AMPK in the control of mitochondrial OXPHOS and ROS production. Dave Carling (MRC Clinical Sciences Centre) discussed the role of AMPK cascade in prostate cancer progression, and presented evidence that activation of AMPK in prostate cancer cells increases cell migration.

Annapoorni Rangarajan (Indian Institute of Science) presented a novel role for AMPK in the acquisition of anoikis-resistance, a hallmark of solid tumors. By phosphorylating the anti-apoptotic protein PEA15, AMPK contribute to the anchorage-independent growth of breast cancer cells (Hindupur et al., 2014). She evoked the exciting possibility that targeting the AMPK-PEA15 axis might prevent breast cancer dissemination and metastasis. Masahisa Jinushi (Hokkaido University) is interested in molecular mechanisms by which macrophage-mediated phagocytosis of tumor cells and regulates tumor immunosurveillance. He described the participation of AMPK α 1 with T cell immunoglobulin- and mucin domain-containing molecule-4 (TIM-4) in the activation of autophagic processes, which are critical for degrading ingested tumors and their associated antigens (Baghdadi et al., 2013). These results indicate that targeting the TIM-4-AMPK α 1 interaction may constitute a unique strategy for augmenting antitumor immunity and improving cancer chemotherapy.

Novel regulatory mechanisms in cardiac and skeletal muscle metabolism

Over the past decade, AMPK has emerged as a key player in the regulation of myocardial metabolism in response to ischemia, pressure overload and heart failure. Lawrence Young (Yale) discussed AMPK regulation of mitochondrial function after ischemia/reperfusion. He also spoke about the therapeutic potential of D-dopachrome tautomerase (DDT), a novel secreted protein from cardiomyocytes with important autocrine/paracrine actions, for preventing cardiac injury during ischemia (Qi et al., 2014). Interestingly, the cardioprotective effect of DDT requires downstream AMPK activation via a calcium-dependent, energy-independent mechanism implicating CaMKK β as the primary upstream kinase. Beside this protective action during myocardial ischemia, AMPK activation also limits the development of cardiac myocyte hypertrophy and increases cardiomyocyte response to insulin. Luc Bertrand (Université catholique de Louvain) described new molecular mechanisms and AMPK downstream targets involved in its insulin-sensitizing and anti-hypertrophic actions. Sandrine Horman (Université catholique de Louvain) presented evidence that AMPK α 1 plays a critical role in cardiac fibroblasts/ myofibroblast biology, providing new perspectives and potential therapeutic approaches that could counter the adverse left ventricular remodeling of infarcted hearts (Noppe et al., 2014).

Erik Richter and Jorgen Wojtaszewski (both University of Copenhagen) reminded us of the important role of AMPK in skeletal muscle metabolism in response to exercise, both in terms of glucose uptake and fatty acid oxidation. Erik Richter explored the relative role of AMPK and Ca^{2+} in increasing glucose uptake during muscle contraction and demonstrated that both metabolic activation of AMPK and Rac1-dependent rearrangement of the actin cytoskeleton, but not Ca^{2+} released from the sarcoplasmic reticulum, fully account for contraction-induced muscle glucose transport (Jensen et al., 2014). Jorgen Wojtaszewski highlighted the differences in the fiber type-specific expression and regulation of AMPK during exercise in humans (Kristensen et al., 2015). He also discussed the importance of AMPK in fatty acid oxidation during exercise (Fentz et al., 2015) and provided evidence to support that AMPK activation is sufficient to increase skeletal muscle insulin sensitivity (Kjobsted et al., 2014).

Toward new AMPK activators and therapeutic applications

Numerous investigators and major pharmaceutical companies have identified AMPK as a potential target for pharmacologic development in the fields of diabetes, obesity, ischemic heart disease, atherosclerosis, and cancer. In parallel with rapid scientific discovery, pharmaceutical companies are developing specific, direct-acting AMPK agonists that are critically important for pre-clinical translational studies and early stage clinical investigation to progress. Mark Rider (de Duve Institute and Université catholique de Louvain) focused on the effect of the small molecule AMPK activator 991 on glucose uptake in skeletal muscle (Beiroa et al., 2014). Incubation of rat epitrochlearis muscle dose-dependently increases activity of AMPK complexes containing various combination of $\alpha 1$ -, $\alpha 2$ -, $\beta 1$ - or $\beta 2$ -subunits. Importantly, this work revealed that compound 991 increases glucose uptake in an AMPK-dependent manner. Jérôme Tamburini (Inserm, CNRS, Université Paris Descartes) employed a novel AMPK activator GSK621 as a therapeutic agent in acute myeloid leukemia (AML). Rather unexpectedly, mTORC1 activity correlated with the degree of GSK621-induced cytotoxicity and defines a mechanism of synthetic lethality with AMPK activation (Sujobert et al., 2015). It is noteworthy that GSK621 has no effect on normal CD34+ hematopoietic progenitor cells, adding support for the development of AMPK activators as promising therapeutic molecules in AML. Benoit Viollet (Inserm, CNRS and Université Paris Descartes) defined the AMPK-dependent and -independent metabolic actions of indirect and direct AMPK activators on hepatic metabolism. Using mice lacking expression of both AMPK $\alpha 1/\alpha 2$ catalytic subunits in the liver, it was established that AMPK is dispensable for metformin- and AICAR-mediated suppression of glucose production in primary hepatocytes and the AICAR-induced decrease in glucose levels seen *in vivo* (Foretz et al., 2010; Hasenour et al., 2014). In contrast, these drugs, as well as the small molecule AMPK activators A-769662 and compound-13 are ineffective in inhibiting lipogenesis in the absence of hepatic AMPK (Hunter et al., 2014). Angela Woods (MRC Clinical Sciences Centre) presented data using a new genetic model for determining the effect of activation of AMPK *in vivo*. In the liver, expression of a mutant $\gamma 1$ subunit lead to increased basal activity of AMPK, but surprisingly did not lower lipid levels on mice fed either a normal or high fat diet. Greg Steinberg (McMaster University) looked at the functional effects of metformin in combination with salicylate on hepatic metabolism. The two drugs synergistically activate AMPK and greatly enhanced the inhibition of lipogenesis in murine and human hepatocytes (Ford et al., 2015). In addition, low dose metformin and salicylate co-

treatment synergistically reduced fatty liver and enhanced insulin sensitivity in mice fed a high fat diet. These results reinforce the view that combinatorial treatments would be of value to enhance AMPK activation and help to reduce the concentration of individual drugs administered to patients.

Integrating LKB1 and AMPK-related kinases in the regulation of metabolism

LKB1 is a master kinase that phosphorylates and activates AMPK and also a family of 12 closely related protein kinases, including SIK (salt-induced kinase), MARK (microtubule-affinity-regulating kinase) and BRSK (brain-specific kinase). A currently debated issue is the precise role of the AMPK and AMPK-related protein kinases in mediating the downstream effects of LKB1 on metabolism. In this context, Kei Sakamoto (Nestlé Institute of Health Sciences SA) talked about the critical contribution of SIK family members in the LKB1-mediated suppression of hepatic gluconeogenesis gene transcription by regulating downstream transcription factors/co-activators (Patel et al., 2014). These findings support a model where the LKB1-SIKs but not LKB1-AMPK pathway functions as a key gluconeogenic gatekeeper in the liver. Robert Sreton (Children's Hospital of Eastern Ontario Research Institute) looked at the function of SIK2 in pancreatic β -cells and the control of glucose homeostasis. By the use of mice deficient in SIK2 in adult β -cells, it was found that SIK2 is required for glucose-induced insulin secretion and is essential for adaptive β -cell functional compensation in models of hyperglycaemia (Sakamaki et al., 2014). SIK2 phosphorylates the cyclin-dependent kinase 5 activator 1 CDK5R1/ p35 to trigger its ubiquitylation by the E3 ligase PJA2, resulting in activation of calcium signaling and insulin secretion. Of note, SIK2 has a specific role in regulating insulin secretion as silencing SIK1 or SIK3 had no effect in this regard. Olga Göransson (Lund University) also highlighted the importance of SIK2 in the regulation of metabolic processes. She focused on the molecular targets and functions of SIK2 in adipocytes. Based on recent work, she showed data from primary adipocytes demonstrating the role of SIK2 in the regulation of GLUT4 levels and glucose uptake, potentially through its action on CREB regulated transcriptional coactivators (CRTCs) and histone deacetylase (HDAC) 4 (Henriksson et al., 2015). Kristopher Clark (University of Dundee) discussed the therapeutic potential of targeting the SIKs for the treatment of chronic inflammatory diseases. Previous work has revealed that inhibition of the SIKs with the pan-SIK inhibitor HG-9-91-01 elevates the production of anti-inflammatory cytokines, while suppressing the production of pro-inflammatory cytokines in macrophages. He described the SIKs as major targets of the anti-cancer drugs bosutinib and dasatinib that mediate the effects of these drugs on the innate immune system (Ozanne et al., 2015). He proposed to repurpose these therapeutics for the treatment of chronic inflammatory diseases.

Guy Rutter (Imperial College London) investigated the respective roles for LKB1 and AMPK in the control of β -cell identity. In this context, although LKB1 and AMPK signaling only partly overlap to maintain β -cell identity, they act by suppressing the expression of different subsets of hepatic and neuronal genes (Kone et al., 2014). Bipal Dasgupta (Cincinnati Children's Hospital Medical Center) presented an example for the differential role of LKB1 and AMPK in the regulation of cell metabolism. He found that LKB1 is a key player in the metabolic reprogramming (from a glycolytic to oxidative metabolism) during differentiation of Schwann cells, whereas AMPK is largely dispensable. Schwann cells deficient in LKB1 failed to produce the mitochondrial Krebs

cycle substrate citrate, a precursor to cellular lipids, resulting in impaired developmental myelination and axonal integrity (Pooya et al., 2014). In order to identify additional novel targets for AMPK and its related family members, Reuben Shaw (The Salk Institute for Biological Studies) presented a strategy employing *in vivo* quantitative proteomic analysis through metabolic labeling of proteins with a heavy stable isotope of nitrogen. On-going work concerns the identification and characterization of phosphoproteins in metformin-treated wild type versus mice lacking LKB1.

The Herbert Young Investigator Award and poster prizes

John Kyriakis (Associate Editor at the Journal of Biological Chemistry) presented the Herbert Tabor Young Investigator Award (http://www.jbc.org/site/home/tabor_award/2014/izreig.xhtml) to Dr. Said Izreig (McGill University) for his work on metabolic regulation in lymphoma.

The organizers David Carling and Benoit Viollet awarded Claire Speirs (University of Glasgow) and Pablo Hollstein (The Salk Institute for Biological Studies) prizes for the best poster presentations illustrating the crosstalk between AMPK and JAK-STAT pathway and the control of cell motility through LKB1-MARK signaling, respectively.

Concluding remarks

This conference showcased the advances that have been made in the understanding of the mechanisms of AMPK regulation and our current knowledge of new downstream targets and signaling pathways controlled by AMPK. Given the significance of these findings and the new questions that they raise, we anticipate that the next meetings on AMPK and AMPK-related protein kinases will be equally exciting. Remaining challenges in the field include the translation of basic research into therapeutic approaches and the development of therapeutics, such as isoform-specific small molecule AMPK activators.

Acknowledgements

Picture courtesy of Foto Pastrengo/FASEB.

The organizers of the AMPK 2014 meeting would like to thank FASEB SRC, Pfizer, Beryllium, Rigel, Poxel, Ozgene, Cell Signaling Technology, Sanofi-Aventis, University Paris Descartes, Debiopharm Group, Mercury Therapeutics and Cell Press for sponsoring this meeting. We are grateful to the Biochemical Journal and Journal of Biological Chemistry for providing poster and Herb Tabor awards, respectively.

We are indebted to all the speakers for giving excellent talks and for sharing unpublished data. We apologize to those speakers whose work could not be mentioned owing to space constraints.

References

Baghdadi, M., Yoneda, A., Yamashina, T., Nagao, H., Komohara, Y., Nagai, S., Akiba, H., Foretz, M., Yoshiyama, H., Kinoshita, I., Dosaka-Akita, H., Takeya, M., Viollet, B., Yagita, H., and Jinushi, M. (2013). TIM-4 glycoprotein-mediated degradation of dying tumor

- cells by autophagy leads to reduced antigen presentation and increased immune tolerance. *Immunity* 39, 1070-1081.
- Beiroa, D., Imbernon, M., Gallego, R., Senra, A., Herranz, D., Villarroya, F., Serrano, M., Ferno, J., Salvador, J., Escalada, J., Dieguez, C., Lopez, M., Fruhbeck, G., and Nogueiras, R. (2014). GLP-1 agonism stimulates brown adipose tissue thermogenesis and browning through hypothalamic AMPK. *Diabetes* 63, 3346-3358.
- Bertoldo, M.J., Guibert, E., Faure, M., Ramé, C., Foretz, M., Viollet, B., Dupont, J., and Froment, P. (2015). Specific deletion of AMP-activated protein kinase (α 1AMPK) in murine oocytes alters junctional protein expression and mitochondrial physiology. *PLoS ONE in press*.
- Calabrese, M.F., Rajamohan, F., Harris, M.S., Caspers, N.L., Magyar, R., Withka, J.M., Wang, H., Borzilleri, K.A., Sahasrabudhe, P.V., Hoth, L.R., Geoghegan, K.F., Han, S., Brown, J., Subashi, T.A., Reyes, A.R., Frisbie, R.K., Ward, J., Miller, R.A., Landro, J.A., Londregan, A.T., Carpino, P.A., Cabral, S., Smith, A.C., Conn, E.L., Cameron, K.O., Qiu, X., and Kurumbail, R.G. (2014). Structural basis for AMPK activation: natural and synthetic ligands regulate kinase activity from opposite poles by different molecular mechanisms. *Structure* 22, 1161-1172.
- Fentz, J., Kjobsted, R., Birk, J.B., Jordy, A.B., Jeppesen, J., Thorsen, K., Schjerling, P., Kiens, B., Jessen, N., Viollet, B., and Wojtaszewski, J.F. (2015). AMPK α is critical for enhancing skeletal muscle fatty acid utilization during in vivo exercise in mice. *Faseb J in press*.
- Ford, R.J., Fullerton, M.D., Pinkosky, S.L., Day, E.A., Scott, J.W., Oakhill, J.S., Bujak, A.L., Smith, B.K., Crane, J.D., Blupsilonmer, R.M., Marcinko, K., Kemp, B.E., Gerstein, H.C., and Steinberg, G.R. (2015). Metformin and salicylate synergistically activate liver AMPK, inhibit lipogenesis and improve insulin sensitivity. *Biochem J in press*.
- Foretz, M., Hebrard, S., Leclerc, J., Zarrinpashneh, E., Soty, M., Mithieux, G., Sakamoto, K., Andreelli, F., and Viollet, B. (2010). Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. *J Clin Invest* 120, 2355-2369.
- Hasenour, C.M., Ridley, D.E., Hughey, C.C., James, F.D., Donahue, E.P., Shearer, J., Viollet, B., Foretz, M., and Wasserman, D.H. (2014). 5-Aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR) effect on glucose production, but not energy metabolism, is independent of hepatic AMPK in vivo. *J Biol Chem* 289, 5950-5959.
- Hawley, S.A., Ross, F.A., Gowans, G.J., Tibarewal, P., Leslie, N.R., and Hardie, D.G. (2014). Phosphorylation by Akt within the ST loop of AMPK- α 1 down-regulates its activation in tumour cells. *Biochem J* 459, 275-287.
- Henriksson, E., Sall, J., Gormand, A., Wasserstrom, S., Morrice, N.A., Fritzen, A.M., Foretz, M., Campbell, D.G., Sakamoto, K., Ekelund, M., Degerman, E., Stenkula, K.G., and Goransson, O. (2015). Salt-inducible kinase 2 regulates CRTCs, HDAC4 and glucose uptake in adipocytes. *J Cell Sci in press*.
- Hindupur, S.K., Balaji, S.A., Saxena, M., Pandey, S., Sravan, G.S., Heda, N., Kumar, M.V., Mukherjee, G., Dey, D., and Rangarajan, A. (2014). Identification of a novel AMPK-PEA15 axis in the anoikis-resistant growth of mammary cells. *Breast cancer research* 16, 420.
- Hunter, R.W., Foretz, M., Bultot, L., Fullerton, M.D., Deak, M., Ross, F.A., Hawley, S.A., Shpiro, N., Viollet, B., Barron, D., Kemp, B.E., Steinberg, G.R., Hardie, D.G., and Sakamoto, K. (2014). Mechanism of action of compound-13: an α 1-selective small molecule activator of AMPK. *Chem Biol* 21, 866-879.

- Jensen, T.E., Sylow, L., Rose, A.J., Madsen, A.B., Angin, Y., Maarbjerg, S.J., and Richter, E.A. (2014). Contraction-stimulated glucose transport in muscle is controlled by AMPK and mechanical stress but not sarcoplasmic reticulum Ca(2+) release. *Mol Metab* 3, 742-753.
- Kjobsted, R., Treebak, J.T., Fentz, J., Lantier, L., Viollet, B., Birk, J.B., Schjerling, P., Bjornholm, M., Zierath, J.R., and Wojtaszewski, J.F. (2014). Prior AICAR stimulation increases insulin sensitivity in mouse skeletal muscle in an AMPK-dependent manner. *Diabetes in press*.
- Kone, M., Pullen, T.J., Sun, G., Ibberson, M., Martinez-Sanchez, A., Sayers, S., Nguyen-Tu, M.S., Kantor, C., Swisa, A., Dor, Y., Gorman, T., Ferrer, J., Thorens, B., Reimann, F., Gribble, F., McGinty, J.A., Chen, L., French, P.M., Birzele, F., Hildebrandt, T., Uphues, I., and Rutter, G.A. (2014). LKB1 and AMPK differentially regulate pancreatic beta-cell identity. *Faseb J* 28, 4972-4985.
- Kristensen, D.E., Albers, P.H., Prats, C., Baba, O., Birk, J.B., and Wojtaszewski, J.F. (2015). Human muscle fibre type-specific regulation of AMPK and downstream targets by exercise. *J Physiol in press*.
- Lewis, C.A., Brault, C., Peck, B., Bensaad, K., Griffiths, B., Mitter, R., Chakravarty, P., East, P., Dankworth, B., Alibhai, D., Harris, A.L., and Schulze, A. (2015). SREBP maintains lipid biosynthesis and viability of cancer cells under lipid- and oxygen-deprived conditions and defines a gene signature associated with poor survival in glioblastoma multiforme. *Oncogene in press*.
- Martinez de Morentin, P.B., Gonzalez-Garcia, I., Martins, L., Lage, R., Fernandez-Mallo, D., Martinez-Sanchez, N., Ruiz-Pino, F., Liu, J., Morgan, D.A., Pinilla, L., Gallego, R., Saha, A.K., Kalsbeek, A., Fliers, E., Bisschop, P.H., Dieguez, C., Nogueiras, R., Rahmouni, K., Tena-Sempere, M., and Lopez, M. (2014). Estradiol regulates brown adipose tissue thermogenesis via hypothalamic AMPK. *Cell Metab* 20, 41-53.
- Noppe, G., Dufeys, C., Buchlin, P., Marquet, N., Castanares-Zapatero, D., Balteau, M., Hermida, N., Bouzin, C., Esfahani, H., Viollet, B., Bertrand, L., Balligand, J.L., Vanoverschelde, J.L., Beauloye, C., and Horman, S. (2014). Reduced scar maturation and contractility lead to exaggerated left ventricular dilation after myocardial infarction in mice lacking AMPKalpha1. *J Mol Cell Cardiol* 74, 32-43.
- Okada-Iwabu, M., Yamauchi, T., Iwabu, M., Honma, T., Hamagami, K., Matsuda, K., Yamaguchi, M., Tanabe, H., Kimura-Someya, T., Shirouzu, M., Ogata, H., Tokuyama, K., Ueki, K., Nagano, T., Tanaka, A., Yokoyama, S., and Kadowaki, T. (2013). A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity. *Nature* 503, 493-499.
- Oligschlaeger, Y., Miglianico, M., Chanda, D., Scholz, R., Thali, R.F., Tuerk, R., Stapleton, D.I., Gooley, P.R., and Neumann, D. (2015). The recruitment of AMP-activated protein kinase to glycogen is regulated by autophosphorylation. *The Journal of biological chemistry in press*.
- Ozanne, J., Prescott, A.R., and Clark, K. (2015). The clinically approved drugs dasatinib and bosutinib induce anti-inflammatory macrophages by inhibiting the salt-inducible kinases. *Biochem J* 465, 271-279.
- Patel, K., Foretz, M., Marion, A., Campbell, D.G., Gourlay, R., Boudaba, N., Tournier, E., Titchenell, P., Pegg, M., Deak, M., Wan, M., Kaestner, K.H., Goransson, O., Viollet, B., Gray, N.S., Birnbaum, M.J., Sutherland, C., and Sakamoto, K. (2014). The LKB1-salt-inducible kinase pathway functions as a key gluconeogenic suppressor in the liver. *Nat Commun* 5, 4535.

- Pooya, S., Liu, X., Kumar, V.B., Anderson, J., Imai, F., Zhang, W., Ciruolo, G., Ratner, N., Setchell, K.D., Yutaka, Y., Jankowski, M.P., and Dasgupta, B. (2014). The tumour suppressor LKB1 regulates myelination through mitochondrial metabolism. *Nat Commun* 5, 4993.
- Qi, D., Atsina, K., Qu, L., Hu, X., Wu, X., Xu, B., Piecychna, M., Leng, L., Fingerle-Rowson, G., Zhang, J., Bucala, R., and Young, L.H. (2014). The vestigial enzyme D-dopachrome tautomerase protects the heart against ischemic injury. *J Clin Invest* 124, 3540-3550.
- Rios, M., Foretz, M., Viollet, B., Prieto, A., Fraga, M., Garcia-Caballero, T., Costoya, J.A., and Senaris, R. (2014). Lipoprotein internalisation induced by oncogenic AMPK activation is essential to maintain glioblastoma cell growth. *Eur J Cancer* 50, 3187-3197.
- Sakamaki, J., Fu, A., Reeks, C., Baird, S., Depatie, C., Al Azzabi, M., Bardeesy, N., Gingras, A.C., Yee, S.P., and Screaton, R.A. (2014). Role of the SIK2-p35-PJA2 complex in pancreatic beta-cell functional compensation. *Nat Cell Biol* 16, 234-244.
- Scott, J.W., Ling, N., Issa, S.M., Dite, T.A., O'Brien, M.T., Chen, Z.P., Galic, S., Langendorf, C.G., Steinberg, G.R., Kemp, B.E., and Oakhill, J.S. (2014). Small molecule drug A-769662 and AMP synergistically activate naive AMPK independent of upstream kinase signaling. *Chem Biol* 21, 619-627.
- Sujobert, P., Paubelle, E., Grenier, A., Zylbersztejn, F., Lambert, M., Poulain, L., Townsend, E., Brusq, J.M., Nicodeme, E., Decroocq, J., Nepstad, I., Green, A., Hospital, M.A., Jacque, N., Hermine, O., Foretz, M., Viollet, B., Lacombe, C., Mayeux, P., Weinstock, D.M., Moura, I.C., Bouscary, D., and Tamburini, J. (2015). Co-activation of AMPK and mTORC1 is synthetically lethal in acute myeloid leukemia. *Cell Reports* *in press*.
- Tong, W.H., Sourbier, C., Kovtunovych, G., Jeong, S.Y., Vira, M., Ghosh, M., Romero, V.V., Sougrat, R., Vaulont, S., Viollet, B., Kim, Y.S., Lee, S., Trepel, J., Srinivasan, R., Bratslavsky, G., Yang, Y., Linehan, W.M., and Rouault, T.A. (2011). The glycolytic shift in fumarate-hydratase-deficient kidney cancer lowers AMPK levels, increases anabolic propensities and lowers cellular iron levels. *Cancer Cell* 20, 315-327.
- Xiao, B., Sanders, M.J., Carmena, D., Bright, N.J., Haire, L.F., Underwood, E., Patel, B.R., Heath, R.B., Walker, P.A., Hallen, S., Giordanetto, F., Martin, S.R., Carling, D., and Gamblin, S.J. (2013). Structural basis of AMPK regulation by small molecule activators. *Nat Commun* 4, 3017.
- Zhang, C.S., Jiang, B., Li, M., Zhu, M., Peng, Y., Zhang, Y.L., Wu, Y.Q., Li, T.Y., Liang, Y., Lu, Z., Lian, G., Liu, Q., Guo, H., Yin, Z., Ye, Z., Han, J., Wu, J.W., Yin, H., Lin, S.Y., and Lin, S.C. (2014). The lysosomal v-ATPase-Ragulator complex is a common activator for AMPK and mTORC1, acting as a switch between catabolism and anabolism. *Cell Metab* 20, 526-540.

Legend Figure

Figure 1: Attendees of the FASEB Science Research Conference, AMPK: biological action and therapeutic perspectives in Lucca, Italy (September 28 - October 3, 2014).