5.   Doi, R. *et al.* (2010) CLOCK regulates circadian rhythms of hepatic glycogen synthesis through transcriptional activation of Gys2. *J. Biol. Chem.* 285, 22114–22121

6.   Panda, S. *et al.* (2002) Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* 109, 307–320

7.   Terazono, H. *et al.* (2003) Adrenergic regulation of clock gene expression in mouse liver. *Proc. Natl. Acad. Sci. U. S. A.* 100, 6795–6800

8.   Ishida, A. *et al.* (2005) Light activates the adrenal gland: timing of gene expression and glucocorticoid release. *Cell Metab.* 2, 297–307

9.   Kondratov, R.V. *et al.* (2006) Early aging and age-related pathologies in mice deficient in BMAL1, the core component of the circadian clock. *Genes Dev.* 20, 1868–1873

10. McDearmon, E.L. *et al.* (2006) Dissecting the functions of the mammalian clock protein BMAL1 by tissue-specific rescue in mice. *Science* 314, 1304–1308

Spotlight

Antagonism between Antiviral Signaling and Glycolysis

Anoop Singh Chauhan,[1,2,3] Li Zhuang,[1] and Boyi Gan (iD)[1,3,*]

RIG-I-like receptor (RLR)-mediated interferon production is critical for antiviral responses. A recent study (Zhang *et al.*, *Cell*, 2019) uncovered a reciprocal inhibition between RLR signaling and glycolysis: lactate produced by glycolysis inhibits RLR signaling by binding to RLR signaling component mitochondrial antiviral-signaling (MAVS), whereas RLR activation suppresses glycolysis through inhibiting glycolysis enzyme hexokinase.

Type I interferons (IFNs), such as IFN-α and IFN-β, not only are central to host innate and adaptive immunity against viral infection, but also can exert antitumor and immunomodulatory activities [1]. Type I IFNs are induced following the recognition of pathogen-associated molecular patterns by different pattern recognition receptors, such as retinoic-acid-inducible gene I (RIG-I)-like receptors

(RLRs) [2]. RIG-I is an RLR that senses cytosolic viral double-strand RNAs (dsRNAs) to initiate downstream signaling pathways, including the mitochondrial antiviral-signaling (MAVS)-TANK binding kinase 1 (TBK1)-interferon regulatory factor 3 (IRF3) signaling axis, culminating in IRF3-mediated type I IFN transcription. The RIG-I-MAVS interaction occurs on mitochondria, the 'powerhouses of the cell', indicating a potential crosstalk between this antiviral signaling and cellular metabolism; however, whether cellular metabolism plays any role in regulating this crucial host defense signaling pathway has remained elusive.

To investigate potential crosstalk between RLR signaling and cellular metabolism, Zhang *et al.* [3] carried out an untargeted metabolomic analysis to capture alterations in cellular metabolism following RLR activation. This analysis revealed a significant decrease of most metabolites involved in glycolysis and the tricarboxylic acid (TCA) cycle following the initial RLR activation, suggesting that RLR signaling inhibits glycolysis. Once imported into cells, glucose is first converted to glucose-6-phosphate by hexokinase, followed by a series of glycolytic reactions that break down glucose into pyruvate [4]. Since RLR activation increased intracellular glucose levels but decreased most other glycolytic intermediates downstream of hexokinase, the authors reasoned that hexokinase might be the crucial step regulated by RLR signaling. Further analyses indeed revealed a decrease in hexokinase activity during the initial stages of RLR activation. Mechanistically, the authors showed that RLR activation hampers mitochondrial localization of hexokinase (which is required for its activation) [5] by inhibiting hexokinase interaction with MAVS, a mitochondria-localized adaptor protein critical for RLR signaling [2]. Upon recognition of dsRNA by RIG-I, a conformational switch in RIG-I promotes RIG-I interaction with MAVS and causes the

displacement of hexokinase from MAVS and therefore from mitochondria, resulting in impaired hexokinase activation and decreased glycolysis.

The authors further speculated that the altered glucose metabolism upon RLR activation may in turn regulate RLR signaling. In support of this hypothesis, the authors found that, upon RLR activation, cells cultured in low-glucose media or treated with 2-deoxy-glucose (a glycolysis inhibitor) exhibited increased type I IFN production and decreased viral replication compared with cells cultured in high-glucose media or treated with vehicle. Importantly, similar results were confirmed in fasting mice treated with low glucose compared with those treated with high glucose. These results suggest that the downregulation of glucose metabolism promotes RLR signaling.

Pyruvate, the end product of glycolysis, has two major fates. Under aerobic conditions, pyruvate is transported into mitochondria and converted to acetyl-CoA, which then enters the TCA cycle, followed by electron transport and oxidative phosphorylation to completely oxidize glucose for ATP production. Under anaerobic conditions, pyruvate is converted to lactate by lactate dehydrogenase (LDHA), and lactate is subsequently exported out of cells, a process called anaerobic glycolysis [4]. (Although it is much less efficient in producing ATP, anaerobic glycolysis allows cells to regenerate NAD^+ needed for the early steps in glycolysis in the absence of oxygen, whereas under aerobic conditions NAD^+ can be regenerated through the electron transport chain in which oxygen serves as the final electron acceptor.) Using a series of approaches to switch between these two metabolic routes downstream of pyruvate, the authors elegantly demonstrated that it is the lactate generated by the LDHA reaction that negatively regulates RLR signaling. Further analyses using mice treated with an LDHA inhibitor

or *Ldha* knockout mice confirmed this finding *in vivo*.

These observations raised the question of how lactate inhibits RLR signaling. Intriguingly, biotin-labeled lactate pull-down experiments coupled with mass spectrometry analysis revealed a specific binding between lactate and MAVS (but not other proteins involved in RLR signaling). Similarly, immunoprecipitation of MAVS revealed its specific binding to lactate but not pyruvate. Domain mapping experiments revealed that MAVS binds to lactate via the transmembrane (TM) domain in MAVS that is responsible for its

mitochondrial localization. In addition, an *in vitro* synthesized peptide representing the TM domain inhibited the MAVS–lactate interaction and largely abolished the inhibitory effects of lactate on RLR signaling. Finally, the authors showed that lactate inhibits MAVS mitochondrial localization, RIG-I-MAVS interaction, and MAVS aggregation (which are critical for mediating RLR signaling), likely through the direct sensing of lactate by MAVS.

Together, the results of this study reveal previously unrecognized reciprocal inhibitory effects between RLR-mediated

antiviral signaling and glycolysis. On one hand, lactate produced by glycolysis inhibits RLR signaling through direct binding to MAVS (Figure 1A); on the other hand, in response to viral infection, RLR activation suppresses glycolysis and lactate production through inhibiting hexokinase mitochondrial localization (Figure 1B). Like other important discoveries, these remarkable findings also raise several outstanding questions for future investigation. Living organisms have evolved elegant mechanisms, mainly through nutrient sensors, to sense and respond to nutrient availability [6]. Notable examples include AMPK-mediated energy sensing and amino acid
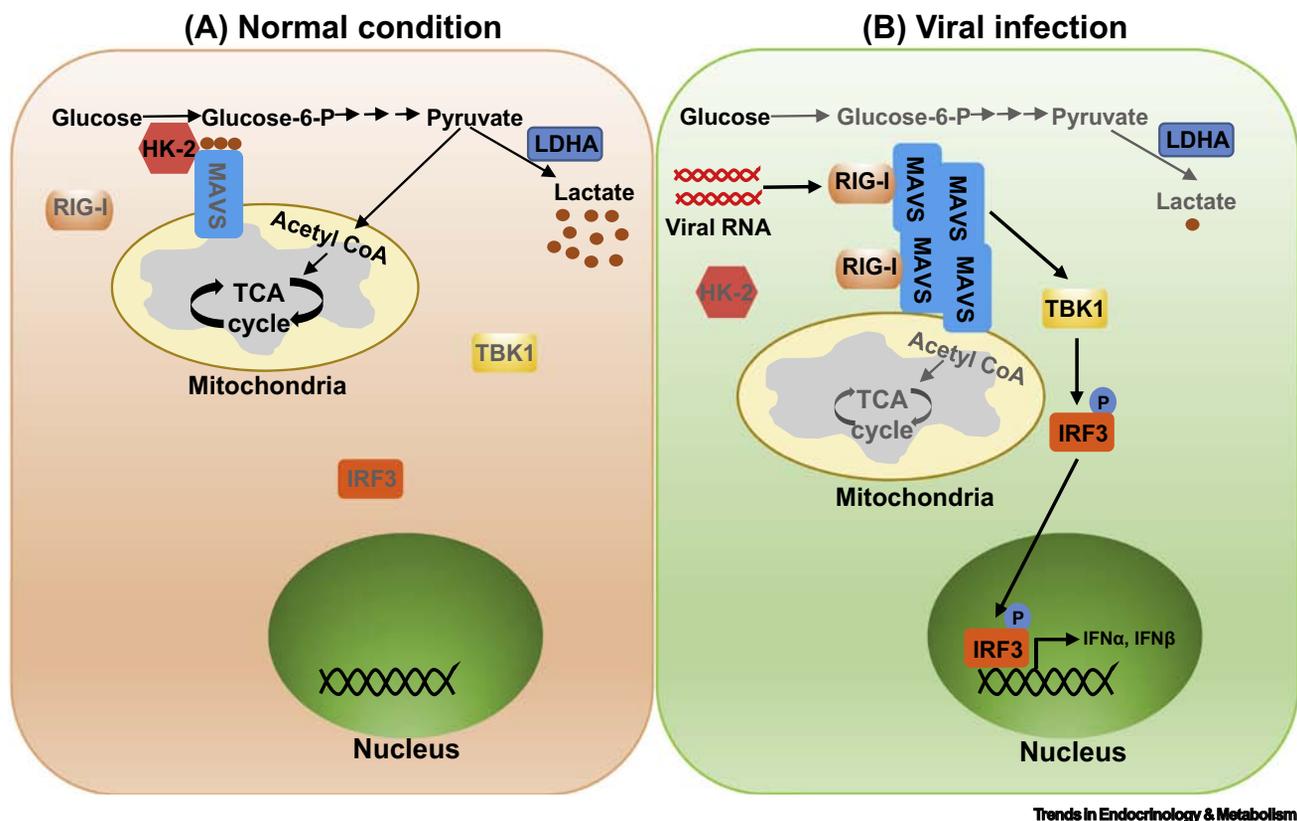

Figure 1. Model Depicting Lactate-Mediated Regulation of Type I Interferon (IFN) Production. (A) Under normal physiological conditions, hexokinase 2 (HK-2) is localized on mitochondria partly through interacting with mitochondrial antiviral-signaling (MAVS) and catalyzes the conversion of glucose to glucose-6-P, which is subsequently converted to pyruvate through a series of glycolytic reactions. Pyruvate can either be shunted into the tricarboxylic acid (TCA) cycle in mitochondria or converted to lactate by lactate dehydrogenase (LDHA). Lactate binds to MAVS and inhibits downstream RIG-I-like receptors (RLR) signaling. (B) Upon viral infection, viral RNAs promote the retinoic-acid-inducible gene I (RIG-I)–MAVS interaction and displace HK-2 from mitochondria, resulting in decreased glycolytic flux and reduced lactate production. This subsequently relieves MAVS from its lactate-mediated inhibition, leading to the activation of downstream RLR signaling and interferon regulatory factor 3 (IRF3)-mediated type I IFN production. The grey color in gene names or arrows indicates inactivation of corresponding proteins or suppression of corresponding pathways. Abbreviations: glucose-6-P, glucose-6-phosphate; TBK1, TANK binding kinase 1;

sensors to couple amino acid availability with mTORC1-mediated cell growth. This study is the first to identify a direct sensor for lactate. Although lactate has traditionally been viewed as a waste product of glycolysis, it is now increasingly appreciated that lactate can serve as a critical energy source, signaling molecule, and biosynthetic precursor in cells [7]. The study by Zhang and colleagues, therefore, will have a far-reaching impact on our understanding of lactate in physiology and disease and will surely motivate future studies to identify additional nutrient sensors in cellular metabolism. Moreover, elevated levels of LDHA or lactate have been reported in patients with viral infections [8,9], suggesting that the virus might hijack the glycolysis pathway to promote lactate production, thereby escaping from the host defense. Finally, cancer cells often exhibit increased lactate production, even under aerobic conditions (the Warburg effect) [4]. Considering the important roles of type I IFNs in cancer immunosurveillance [1], the Warburg effect might be used by cancer cells to dampen this immunosurveillance. Further studies will be directed to further testing these hypotheses and to translating these intriguing findings into novel treatments for cancer and viral infections.

[1]Department of Experimental Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
[2]Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham B17 2TT, UK
[3]https://www.mdanderson.org/research/departments-labs-institutes/labs/gan-laboratory.html

*Correspondence:
bgan@mdanderson.org (B. Gan).

https://doi.org/10.1016/j.tem.2019.07.010

References

1. Musella, M. *et al.* (2017) Type-I-interferons in infection and cancer: unanticipated dynamics with therapeutic implications. *Oncoimmunology* 6, e1314424
2. Wu, J. and Chen, Z.J. (2014) Innate immune sensing and signaling of cytosolic nucleic acids. *Annu. Rev. Immunol.* 32, 461–488
3. Zhang, W. *et al.* (2019) Lactate is a natural suppressor of RLR signaling by targeting MAVS. *Cell* 178, 176–189
4. Vander Heiden, M.G. *et al.* (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324, 1029–1033
5. Roberts, D.J. and Miyamoto, S. (2015) Hexokinase II integrates energy metabolism and cellular protection: Akting on mitochondria and TORCing to autophagy. *Cell Death Differ.* 22, 248–257
6. Efeyan, A. *et al.* (2015) Nutrient-sensing mechanisms and pathways. *Nature* 517, 302–310
7. Brooks, G.A. (2018) The science and translation of lactate shuttle theory. *Cell Metab.* 27, 757–785
8. Chen, Y. *et al.* (2013) Human infections with the emerging avian influenza A H7N9 virus from wet market poultry: clinical analysis and characterisation of viral genome. *Lancet* 381, 1916–1925
9. Hunt, L. *et al.* (2015) Clinical presentation, biochemical, and haematological parameters and their association with outcome in patients with Ebola virus disease: an observational cohort study. *Lancet Infect. Dis.* 15, 1292–1299

Spotlight

Neuron–Astrocyte Liaison to Maintain Lipid Metabolism of Brain

Soham Mukherjee[1] and S.N. Suresh[2,*]

Neuron–astrocyte crosstalk is a tightly regulated process that is essential for overall proper functioning of the brain. Maria S. Ioannou *et al.* (*Cell*, 2019) revealed a novel and compelling function of neuron–astrocyte crosstalk that explains how neurons cope with their accumulating toxic lipid particles under various scenarios.

Neurons and astrocytes coordinate a plethora of metabolic processes to ensure the homeostasis of the brain. In the 1980s, a seminal study showing trophic support by astrocytes to neurons indicated their probable crosstalk [1]. Unsurprisingly

thereafter, the literature skyrocketed, demonstrating the synchronized operation of neurons and astrocytes as being quintessential for the well-being of organisms. Although the traditional belief is that astrocytes support the well-being of neurons, growing evidence pinpoints numerous in-built mechanisms helping neurons to cope with stress scenarios. Lipid droplets (LDs) are energy rich and fuel mitochondria-mediated β-oxidation followed by oxidative phosphorylation [2]. Hyperactive neurons generate peroxidated lipids and also have very low mitochondrial fatty acid consumption [3]. This poses the question: how do neurons tackle the accumulating lipids and their adducts? This led the authors to investigate whether astrocytes have any role to play at all in maintaining the lipid homeostasis of neurons, as the preceding literature indicates a bidirectional interaction of neurons and astrocytes. Such a possibility of astrocyte–neuron interaction in the context of lipid metabolism is not yet well characterized. Do neurons transfer lipids to astrocytes? If so, what are the mechanistic underpinnings facilitating such transfer? The current study by Maria S. Ioannou *et al.* in *Cell* sheds light on these questions that are important to perpetuate neuronal and, eventually, organismal homeostasis [4].

N-Methyl-D-aspartate (NMDA)-mediated excitotoxicity in neurons stimulates the formation of LDs. Increased neuronal autophagic flux liberates free fatty acids from lysosomes that amalgamate to form cytosolic LDs. Thus, autophagy assists LD biogenesis to evade lipotoxicity. This corroborates with a previous observation that diacylglycerol acyltransferase 1 (DGAT-1) and elevated autophagic flux mediate LD formation by channeling free fatty acids from lysosomes [5]. Despite autophagy inhibition, there is no exponential accumulation of LDs beyond a certain threshold, probably indicating the existence of an alternative pathway. Under