Metabolic remodeling: a pyruvate transport affair

Heike Rampelt¹ & Martin van der Laan^{1,2}

Metabolic remodeling is a major determinant for many cell fate decisions, and a switch from respiration to aerobic glycolysis is generally considered as a hallmark of cancer cell transformation. Pyruvate is a key metabolite at the major junction of carbohydrate metabolism between cytosolic glycolysis and the mitochondrial Krebs cycle. In this issue of *The EMBO Journal*, Bender *et al* show that yeast cells regulate pyruvate uptake into mitochondria, and thus its metabolic fate, by expressing alternative pyruvate carrier complexes with different activities.

See also: T Bender *et al* (April 2015)

The cytosolic reduction of pyruvate to lactate in mammalian cells or fermentation of pyruvate to ethanol in yeast supports rapid cell proliferation and forms the basis for the famous Warburg effect (aerobic glycolysis) exhibited by many cancers. In contrast, pyruvate import into mitochondria and conversion to acetyl-CoA results in its complete oxidation via the Krebs cycle and maximal ATP generation by oxidative phosphorylation, a metabolic state that is adopted by differentiated mammalian cells and glucose-deprived yeast (Fig 1) (Vander Heiden *et al*, 2009). Although it has been long known that a pyruvate carrier exists in the inner mitochondrial membrane, its molecular identity remained elusive until recently (Bricker *et al*, 2012; Herzig *et al*, 2012). The mitochondrial pyruvate carrier (MPC) is a heteromeric complex composed of the MPC1 and MPC2 subunits in mammalian cells and in *Drosophila*, or Mpc1 and either Mpc2 or Mpc3 in yeast. The subunit Mpc1 was discovered as the target of the previously identified MPC inhibitor UK5099.

The yeast orthologs Mpc2 and Mpc3 share 80% identity, but display differential expression depending on the growth media. Ablation of MPC subunits disrupts pyruvate metabolism, as evidenced for instance by impaired synthesis of lipoic acid and concomitant growth defects in yeast, and by lethality of a sucrose diet in *Drosophila* (Bricker *et al*, 2012; Herzig *et al*, 2012). A recent study addressed the expression patterns of the yeast MPC subunits and reported that Mpc2 is synthesized during fermentative growth, whereas Mpc3 is induced by salt stress and upon shift to respiratory growth conditions (Timón-Gómez *et al*, 2013). However, it remained unclear whether indeed different forms of MPC complexes exist in the inner mitochondrial membrane and how subunit composition may affect MPC function, largely because the interpretation of phenotypes was confounded by the metabolic shifts required to induce the synthesis of the different Mpc proteins.

New work from the Martinou laboratory now provides a comprehensive picture of MPC function and regulation in yeast (Bender *et al*, 2015). The authors directly demonstrate the formation of alternative mitochondrial pyruvate carrier complexes under fermentative (MPC_{FERM}) versus respiratory (MPC_{OX}) growth conditions: MPC_{FERM} is composed of Mpc1 and Mpc2 subunits, whereas Mpc1 and Mpc3 constitute MPC_{OX} (Fig 1). Moreover, Bender *et al* resolve the disparate topology predictions regarding Mpc proteins and demonstrate that Mpc1 contains two transmembrane segments with the connecting loop located in the intermembrane space (IMS), whereas Mpc2 and Mpc3 have three transmembrane segments and additionally expose their C-terminal

hydrophilic domains to the IMS. Importantly, the use of a plasmid system with constitutively active promoters allowed them to dissect functional differences between the MPCs. Both complexes were similarly able to rescue the growth defects of a triple MPC deletion under various growth conditions. However, MPC_{OX} displays a higher transport activity than MPC_{FERM}, which depends on the C-terminal region of Mpc3. A hybrid protein composed of the N-terminal transmembrane domain of Mpc2 and the C-terminal IMS domain of Mpc3 formed an active MPC complex together with Mpc1 that exhibited a transport activity similar to MPC_{OX}.

These findings give precedent to the concept that switching between different metabolic states can be accomplished through regulation of pyruvate import into mitochondria. This notion is particularly exciting in light of recent studies that implicate defective pyruvate import via the MPC in cancer cell metabolism (Schell *et al*, 2014; Vacanti *et al*, 2014; Yang *et al*, 2014). A survey across a wide range of cancers found that the *MPC1* locus is lost with a high frequency and that low MPC1 expression is associated with poor survival (Schell *et al*, 2014). When MPC function was compromised, cells relied on glutamine oxidation to maintain a functional Krebs cycle and to support cell growth (Vacanti *et al*, 2014; Yang *et al*, 2014) (Fig 1). Consequently, simultaneous inhibition of MPC and glutamate dehydrogenase abrogated tumor growth (Yang *et al*, 2014). Despite redundant metabolic adaptations of cancer cells to limit pyruvate oxidation, forced expression of MPC resulted in a loss of cancer stemness markers and reverted cells to a low oncogenic potential (Schell *et al*, 2014). Thus, a

¹ Institut für Biochemie und Molekularbiologie, Universität Freiburg, Freiburg, Germany

² BIOS Centre for Biological Signalling Studies, Universität Freiburg, Freiburg, Germany, E-mail: martin.van.der.laan@biochemie.uni-freiburg.de

DOI 10.15252/emj.201591228 | Published online 27 February 2015

Figure 1. The mitochondrial pyruvate carrier (MPC) at the junction between glycolytic and oxidative metabolism.

In respiring yeast, pyruvate produced from the non-fermentable carbon source glycerol is readily imported into the mitochondrial matrix by MPC_{ox}. Conversion of pyruvate to acetyl-CoA and its complete oxidation in the Krebs cycle drive high rates of oxidative phosphorylation (OXPHOS). In fermenting yeast, pyruvate transport into the matrix via the MPC_{FERM} complex is less efficient, resulting in the formation of ethanol from pyruvate in the cytosol. Glycolytic metabolites are used for the synthesis of biomolecules to support rapid cell proliferation. Aerobic glycolysis in cancer cells may be supported by down-regulation or post-translational modification of MPC subunits leading to reduced net uptake of pyruvate into mitochondria and formation of lactate in the cytosol. Cancer cells rely on glutamine oxidation to sustain the Krebs cycle in the mitochondrial matrix, which is a major source of material for anabolic metabolism and cell proliferation.

metabolic switch centered around the MPC determines oncogenicity and the ability to grow in a non-adhesive fashion. This reprogramming mirrors stem cell differentiation into somatic cells, which involves a similar metabolic transition from glycolytic to oxidative metabolism at the expense of proliferative potential (Ito & Suda, 2014). Accordingly, the view is emerging that metabolic reprogramming directly influences cell fate, and it appears that MPC function is a critical variable in this equation.

In contrast to yeast MPC, the activity of the mammalian complex is not regulated via changes in subunit composition. However, the observation that murine Mpc2 is a target of the SIRT3 deacetylase (Hebert *et al*, 2013), a known regulator of mitochondrial metabolism, indicates that mammalian cells may modulate MPC activity in a post-translational manner. Another feasible way to control metabolite uptake into mitochondria is the regulation of carrier protein biogenesis. Mitochondrial metabolite carriers are

encoded by nuclear genes and synthesized by cytosolic ribosomes. These precursor proteins initially enter mitochondria via the general translocase of the outer membrane (TOM complex) (Harbauer *et al*, 2014). Growth of yeast cells in glucose medium induces the phosphorylation of Tom70, the receptor for carrier precursors on the mitochondrial surface, by protein kinase A (Schmidt *et al*, 2011). Phosphorylation of Tom70 impairs the delivery of chaperone-bound carrier precursors to the TOM complex, leading to reduced levels of carrier protein complexes in the inner mitochondrial membrane. A similar regulatory loop may contribute to the control of metabolite uptake into mitochondria in mammalian cells.

In conclusion, several lines of evidence support the idea that the mitochondrial pyruvate carrier critically shapes cellular metabolism by directly influencing the switch between respiratory and glycolytic growth. Mechanistic insights into MPC

function and regulation, as provided by Bender *et al* (2015), are therefore of paramount importance to our understanding of metabolic reprogramming in health and disease.

Acknowledgements

The authors are supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 746, Excellence Initiative of the German Federal and State Governments (EXC 294 BIOS), and a postdoctoral fellowship of the Peter und Traudl Engelhorn Stiftung (to H.R.).

References

- Bender T, Pena G, Martinou JC (2015) Regulation of mitochondrial pyruvate uptake by alternative pyruvate carrier complexes. *EMBO J* 34: 911–924
- Bricker DK, Taylor EB, Schell JC, Orsak T, Boutron A, Chen YC, Cox JE, Cardon CM, Van Vranken JG, Dephousse N, Redin C, Boudina S, Gygi SP, Brivet M, Thummel CS, Rutter J (2012) A mitochondrial pyruvate carrier required for

- pyruvate uptake in yeast, *Drosophila*, and humans. *Science* 337: 96–100
- Harbauer AB, Zahedi RP, Sickmann A, Pfanner N, Meisinger C (2014) The protein import machinery of mitochondria – a regulatory hub in metabolism, stress, and disease. *Cell Metab* 19: 357–372
- Hebert AS, Dittenhafer-Reed KE, Yu W, Bailey DJ, Selen ES, Boersma MD, Carson JJ, Tonelli M, Balloon AJ, Higbee AJ, Westphall MS, Pagliarini DJ, Prolla TA, Assadi-Porter F, Roy S, Denu JM, Coon JJ (2013) Calorie restriction and SIRT3 trigger global reprogramming of the mitochondrial acetylome. *Mol Cell* 49: 186–199
- Herzig S, Raemy E, Montessuit S, Veuthey JL, Zamboni N, Westermann B, Kunji ER, Martinou JC (2012) Identification and functional expression of the mitochondrial pyruvate carrier. *Science* 337: 93–96
- Ito K, Suda T (2014) Metabolic requirements for the maintenance of self-renewing stem cells. *Nat Rev Mol Cell Biol* 15: 243–256
- Schell JC, Olson KA, Jiang L, Hawkins AJ, Van Vranken JG, Xie J, Egnatchik RA, Earl EG, DeBerardinis RJ, Rutter J (2014) A role for the mitochondrial pyruvate carrier as a repressor of the Warburg effect and colon cancer cell growth. *Mol Cell* 56: 400–413
- Schmidt O, Harbauer AB, Rao S, Eyrich B, Zahedi RP, Stojanovski D, Schönfisch B, Guiard B, Sickmann A, Pfanner N, Meisinger C (2011) Regulation of mitochondrial protein import by cytosolic kinases. *Cell* 144: 227–239
- Timón-Gómez A, Proft M, Pascual-Ahuir A (2013) Differential regulation of mitochondrial pyruvate carrier genes modulates respiratory capacity and stress tolerance in yeast. *PLoS One* 8: e79405
- Vacanti NM, Divakaruni AS, Green CR, Parker SJ, Henry RR, Ciaraldi TP, Murphy AN, Metallo CM (2014) Regulation of substrate utilization by the mitochondrial pyruvate carrier. *Mol Cell* 56: 425–435
- Vander Heiden MG, Cantley LC, Thompson CB (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324: 1029–1033
- Yang C, Ko B, Hensley CT, Jiang L, Wasti AT, Kim J, Sudderth J, Calvaruso MA, Lumata L, Mitsche M, Rutter J, Merritt ME, DeBerardinis RJ (2014) Glutamine oxidation maintains the TCA cycle and cell survival during impaired mitochondrial pyruvate transport. *Mol Cell* 56: 414–424