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Review

Metabo-Devo: A metabolic perspective of development

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ABSTRACT

In the last years, several reports have established the notion that metabolism is not just a housekeeping process, but instead an active effector of physiological changes. The idea that the metabolic status may rule a wide range of phenomena in cell biology is starting to be broadly accepted. Thus, current developmental biology has begun to describe different ways by which the metabolic profile of the cell and developmental programs of the organism can crosstalk. In this review, we discuss mechanisms by which metabolism impacts on processes governing development. We review the growing body of evidence that supports the notion that aerobic glycolysis is required in cells undergoing fast growth and high proliferation, similarly to the Warburg effect described in tumor cells. Glycolytic metabolism explains not only the higher ATP synthesis rate required for cell growth, but also the uncoupling between mitochondrial activity and bioenergetics needed to provide anabolism with sufficient precursors. We also discuss some recent studies, which show that in addition to its role in providing energy and carbon chains, the metabolic status of the cell can also influence epigenetic regulation of developmental processes. Although metabolic aspects of development are just starting to be explored, there is no doubt that ongoing research in this field will shape the future landscape of Developmental Biology.

Contents

Abbreviations: 2-OG, 2 oxoglutarate; 5mC, 5 methylcytosine; 6mdA, N 6 methyldeoxyadenosine; AldoA, aldolase A; CB, chorioallantoic branching; CBC, crypt base columnar cell; D-2-HG, D 2 hydroxyglutarate; dERR, *Drosophila* estrogen-related receptor; DMOG, dimethyloxalylglycine; GAPDH, glyceraldehyde 3 phosphate dehydrogenase; GLUT, glucose transporters; HAT, histone acetyl transferase; HDAC, histone deacetylase; HDM, histone demethylases; HK, hexokinase; HMT, histone methyltransferases; iPSC, induced pluripotent stem cell; L-2-HG, L 2 hydroxyglutarate; LDH, lactate dehydrogenase; MAT, S adenosyl methionine transferase; MPC, mitochondrial pyruvate transporter; msMSC, mouse skin mesenchymal stem cell; NB, neuroblast; NC, neural crest; PDH, pyruvate dehydrogenase; PDHK, pyruvate dehydrogenase kinase; PFKFB4, 6 phosphofructo 2 kinase/fructose 2,6 biphosphatase 4; PGI, phosphoglucose isomerase; PK, pyruvate kinase; PSC, pluripotent stem cell; PSM, presomitic mesoderm; RGC, retinal ganglion cell; SAM, S adenosylmethionine; TPI, triose phosphate isomerase; ZGA, zygotic-genome activation.

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1. Introduction

Many advances in the history of science occurred when two aspects of nature, hitherto considered separate classes of phenomena, were revealed as different aspects of the same process or entity. The American physicist Richard Feynman pointed this out at the beginning of his immortal *Lectures on Physics*, and called these instances *amalgamations* (Feynman et al., 2011). Maybe the most notorious example of amalgamation is the discovery that light, electricity and magnetism are in fact different facets of the electromagnetic field. But more examples can be found elsewhere: Modern thermodynamics only emerged when heat was understood in terms of the laws of mechanics; our current model of the chemical bond was developed only when chemical phenomena like the properties and reactivity of different substances were re-analyzed at the light of quantum mechanics (Allen, 2008); and at the core of current biology, the Modern Synthesis is mainly a Mendelian re-interpretation of Darwin's proposals (Gould, 1982).

The history of embryology, or developmental biology as we call it nowadays, is also full of examples of qualitative advancements triggered by the interactions between scientists, techniques or ideas that arose from diverse scientific fields (Horder, 2001). In fact, the most important advances towards our current conception of development can be interpreted as amalgamations.

The prehistory of embryology lies in the discussion between preformation *vs* epigenesis from the seventeenth century, which can hardly be considered scientific by today standards. It was not until the mid-nineteenth century when Darwinian notions provided the first light needed to start building a scientific understanding of development. Ernst Haeckel, Darwin's main defender at late 1800s in Germany, was one of the first to read the gathered knowledge of comparative embryology through a lens tinted by the then-new Darwinian conception of evolution. This evolutionary perspective allowed Haeckel to convert a huge amount of descriptive data to a coherent theory of development: the *biogenetic law* (Haeckel, 1866). Albeit Haeckel's proposal was abandoned long ago, the fact that embryos reflect the course of evolution still remains as a central premise in developmental biology (Horder, 2001).

In the aftermath of World War II, the successes and new techniques of cell biology brought fresh air into the then-stagnated field of embryology. Thus, the focus was shifted from whole embryo studies to cell-based analysis. A paradigmatic research of that time was the discovery by John Gurdon that a full new frog can develop from an enucleated frog's egg in which a nucleus from a gut cell had been transplanted (Gurdon, 1962). This research, based on the previously established knowledge from cell biology that most genetic information resides in the nucleus, proved that every nucleus contains the required information to execute the developmental program.

The molecular biology revolution of the late twentieth century has deeply influenced developmental biology. Maybe the most successful example of a gene-based approach to embryology was the identification of single genes related to the control of the *Drosophila* segmented body form, and the hierarchical relation between them (Nüsslein-Volhard and Wieschaus, 1980). From this point onwards, the role in development of dozens of morphogens, transcription factors, growth factors and signaling molecules has been identified.

The interest on the metabolic aspects of development has existed since the early twentieth century, when Joseph Needham published a detailed study called *Chemical Embryology* (Needham, 1931). However, due to technical limitations, as well as to the lack of a proper theoretical background on this matter, research did not progress much further. In the last five years, a new interest in metabolism has started to emerge among developmental biologists. Gradually, the idea that metabolism and signaling are deeply intertwined, with one affecting the other and *vice versa*, is forging its way as a widely accepted biological premise (Teleman, 2016). However, the true extent of the developmental control elicited by metabolism has just started to be tested out. Ongoing research is set to clarify if we are truly living a new amalgamation in developmental biology, in which the metabolic point of view is incorporated to this ever-changing field.

In this review we will discuss different ways by which the metabolic status of the cell and developmental programs of the organism can talk with each other. First, we will examine current bibliography that supports the notion that metabolic shifts observed during development reflect changes in cellular energy requirements, as well as in the requirement of anabolic precursors. Reciprocally, the capability of the cell to re-wire its entire metabolism seems to be an unavoidable requirement for cell differentiation. A more complex perspective will emerge when we will discuss recent reports that link metabolic-driven epigenetic regulation with cell differentiation and some milestones of embryogenesis. Finally, we will analyze some recent studies that show that metabolism not only can adapt in response to signaling pathways, but also modulates them.

2. Bioenergetics of development

2.1. The Warburg effect

Otto Warburg was the first to study, in the 1920's, the metabolism of tumors. He reported that tumor cells undergo a metabolic reprogramming that turns them unable to fully oxidize carbon chains through mitochondrial catabolism, forcing those cells to rely on glycolysis as their main ATP source (Warburg, 1956). This finding led Warburg to build a metabolic-centered theory of the origin of cancer. He proposed that an impaired respiratory capacity of cancer cells, mainly due to mitochondrial dysfunction, was the driving force behind its malignant transformation (Warburg, 1956). By the same time, Herbert Crabtree noticed the high dependence of tumor cells on glucose availability, and suggested that glucose uptake has a depressing effect over oxygen consumption (Crabtree, 1929). Warburg hypothesis failed to convince the scientific community, and in the 1950's Sidney Weinhouse found that some tumor cells are still able to fully oxidize lipids and carbohydrates to carbon dioxide, retaining their oncogenic properties (Weinhouse et al., 1951). Thus, Weinhouse proposed that impaired mitochondrial activity of cancer cells reflect the Crabtree effect: It was a consequence, and not a cause, of the increased glycolytic flux. Today it is widely accepted that most tumor cells use this strictly glycolytic pathway for ATP synthesis, even in conditions of oxygen availability (Gatenby and Gillies, 2004).

Many metabolic genes (Table 1) are known to be dysregulated in different tumor cells, leading to cancer-characteristic metabolic profiles. Fig. 1 shows the metabolic pathways in which these proteins are involved.

The low yield glycolytic metabolism of proliferating cells may seem paradoxical at a first glance. However, additional aspects need to be considered when studying metabolism at a whole-cell level. Based on empirical evidence from cancer biology, there are two main non mutually-exclusive interpretations of the Warburg effect (DeBerardinis et al., 2008; Schuster et al., 2015):

1. The *kinetic interpretation* contemplates the fact that the ATP production rate is considerably higher in glycolysis as compared to mitochondrial oxidative phosphorylation (Pfeiffer et al., 2001). Thus, considering a condition in which glucose availability is non-restrictive, the Warburg metabolic profile would lead to a higher rate of ATP synthesis, supporting the augmented proliferation rate of cancer cells. This interpretation would also explain the glucose-dependency of most tumors.
2. The *anabolic interpretation* highlights the need for metabolic intermediates in biosynthetic pathways. The core of the altered metabolic status lies in the inhibition of pyruvate mitochondrial conversion into acetyl-CoA (Fig. 1). This blockade is achieved mainly by decreased pyruvate dehydrogenase (PDH) activity (Hitosugi et al.,

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Table 1
List of the Warburg-related genes reported in the literature. Physiological roles of the proteins, as well as the kind of deregulation observed in cancer cells, are also depicted in the table.

Gene	Metabolic function	Dysregulation in transformed cells	References
GLUTs transporters	Glucose transporters in plasma membrane	Upregulation of mainly isoforms 1 and 3	(Medina and Owen, 2002; Szablewski, 2013)
Hexokinase (HK)	Phosphorylates glucose in glycolysis	Isoform II becomes more abundant	(Mathupala et al., 2006)
Phosphoglucose Isomerase (PGI)	In glycolysis, it interconverts glucose-6-phosphate and fructose-6-phosphate	Tumors secrete PGI to blood circulation, where it acts as cytokine	(Baumann et al., 1990; Filella et al., 1991)
6 Phosphofructo 2 kinase/fructose 2,6 bisphosphatase	Branching point in glycolysis towards pentose phosphate pathway	Isoform 3 becomes more abundant, shifting the flux towards glycolysis	(Kessler et al., 2008; Minchenko et al., 2005)
Phosphoglycerate mutase	Conversion of 3 phosphoglycerate to 2 phosphoglycerate in glycolysis	Upregulation of isoform 1	(Ren et al., 2010)
Pyruvate kinase (PK)	Conversion of phosphoenolpyruvate to pyruvate	PKM2 isoform becomes more abundant, slowing down glycolytic flux (bottleneck at the end of the pathway)	(Christofk et al., 2008; Desai et al., 2014)
Lactate Dehydrogenase (LDH)	Reduction of pyruvate to lactate	Upregulation of LDHA isoform	(Miao et al., 2013; Semenza, 2000; Shim et al., 1997)
Monocarboxylate Transporters	Lactate transporters in plasma membrane	Elevated expression of MCT4	(Baek et al., 2014)
MPCs transporters	Mitochondrial carriers of pyruvate	Downregulation of both MPC1 and MPC2 isoforms	(Bricker et al., 2012; Schell et al., 2014)
Pyruvate Dehydrogenase (PDH)	Synthesis of Acetyl-CoA from pyruvate	Inhibition through phosphorylation mediated by augmented Pyruvate Dehydrogenase Kinase activity	(Hitosugi et al., 2011; Holness and Sugden, 2003)
Pyruvate Dehydrogenase Kinase (PDHK)	Inhibition of PDH	Overexpression of PDHK 1 isoform	(Hitosugi et al., 2011; Holness and Sugden, 2003; Kaplon et al., 2013)
Glutaminase	Generates glutamate from glutamine	Increased expression mediated by Myc	(Wise et al., 2008)
ASC amino acid transporter 2	Glutamine transporter	Increased expression mediated by Myc	(Wise et al., 2008)
Isocitrate Dehydrogenase	Isocitrate decarboxylation, producing 2 oxoglutarate	Mutations leading to altered enzymatic activity: 2 hydroxyglutarate synthesis	(Dang et al., 2009; Ward et al., 2010)

2011; Holness and Sugden, 2003), but also through transcriptional repression of mitochondrial pyruvate transporters (MPC1 and MPC2) (Bricker et al., 2012; Schell et al., 2014), as well as by a switch of pyruvate kinase isoforms towards one with decreased substrate affinity (Fig. 1) (Christofk et al., 2008; Desai et al., 2014). The reduction of pyruvate conversion into acetyl-CoA elicits a double effect. First, it stalls the glycolytic flux, leading to an increase in steady-state concentrations of key glycolytic intermediates, which can then be diverted into anabolic processes (DeBerardinis et al., 2008; Palsson-McDermott and O'neill, 2013): Glyceraldehyde 3 phosphate can be used in glycerol synthesis, fructose 1,6 bisphosphate is required for ribose production in the nucleotide synthesis pathway, and high glucose-6-phosphate levels are necessary to produce, *via* the pentose phosphate pathway, sufficient amounts of NADPH to sustain the biomass accumulation during tumor progression (Fig. 1). Secondly, pyruvate blockade uncouples glycolysis from the Krebs Cycle. Hence, the main carbon source of the Krebs Cycle ends up being 2 oxoglutarate (2-OG), derived from glutamate, which is largely available due to a high glutaminase activity (Fig. 1) (Hensley et al., 2013; Wise et al., 2008). Once the TCA cycle reactions are uncoupled from energetic metabolism, its metabolites can be employed as anabolic precursors: Oxaloacetate can be used for amino acid biosynthesis, succinyl CoA for porphyrin synthesis, and the citrate generated by reductive carboxylation of 2-OG (Mullen et al., 2014) as a precursor for lipid synthesis.

2.2. A physiological role for the Warburg effect

Regardless of the kinetic or anabolic interpretation of the Warburg effect, this phenomenon has ignited a novel interest in metabolism among developmental biologists. Considering that the metabolic reprogramming observed in cancer is actually needed to fuel synthesis of biomass, the idea that this switch could be a physiological feature of cell proliferation (Fig. 2) rather than a malignant characteristic of tumor cells is starting to be discussed and tested out (Agathocleous and Harris, 2013; DeBerardinis et al., 2008).

In fact, this idea was addressed briefly in the 1950s. While studying metabolic requirements for cell division, R. J. O'Connor found a strong correlation between cell proliferation and glycolysis in chick embryo midbrain: Decreased cell proliferation rate observed during developmental progression in this tissue occurs concomitantly with a decrease in glycolytic activity, whilst the respiration rate remains essentially unaltered (O'connor, 1950). Furthermore, two years later, O'Connor found a similar correlation between tissue growth and glycolytic activity at early stages of retina development in chick embryos, whilst at later stages (from day 8 onwards) an unexplained increase in glycolysis, unrelated to tissue growth, was observed (O'connor, 1952).

In the twenty first century, one of the earliest works that explored this concept was published in 2012 by Redel et al. (2012). In this work, the authors reported that *in vitro*-produced pig blastocysts expressed cancer-related isoforms of two key glycolytic enzymes: Hexokinase 2 (HK2) and pyruvate kinase M2 (PKM2), although the role these enzymes play in pig early development has not been further analyzed.

Another study from 2012 showed that dividing progenitor cells of the *Xenopus* retina rely on a highly glycolytic metabolic profile sustained mainly by glycogen, whilst upon final differentiation retinal neurons experience a metabolic switch towards oxidative phosphorylation (Agathocleous et al., 2012). Albeit in previous works proliferating lymphocytes (Wang et al., 1976) or thymocytes (Brand and Hermfisse, 1997) were reported to be glycolytic, this was the first report showing a metabolic shift in an *in vivo* model, overcoming a major limitation of earlier works: That metabolism in cultured cells may depend on experimentally defined nutrient or oxygen conditions. Remarkably, Agathocleous et al. not only characterized the differences between the metabolic status of proliferating progenitors *versus* differentiated non-proliferating neurons, but also showed that after a complete glycolytic

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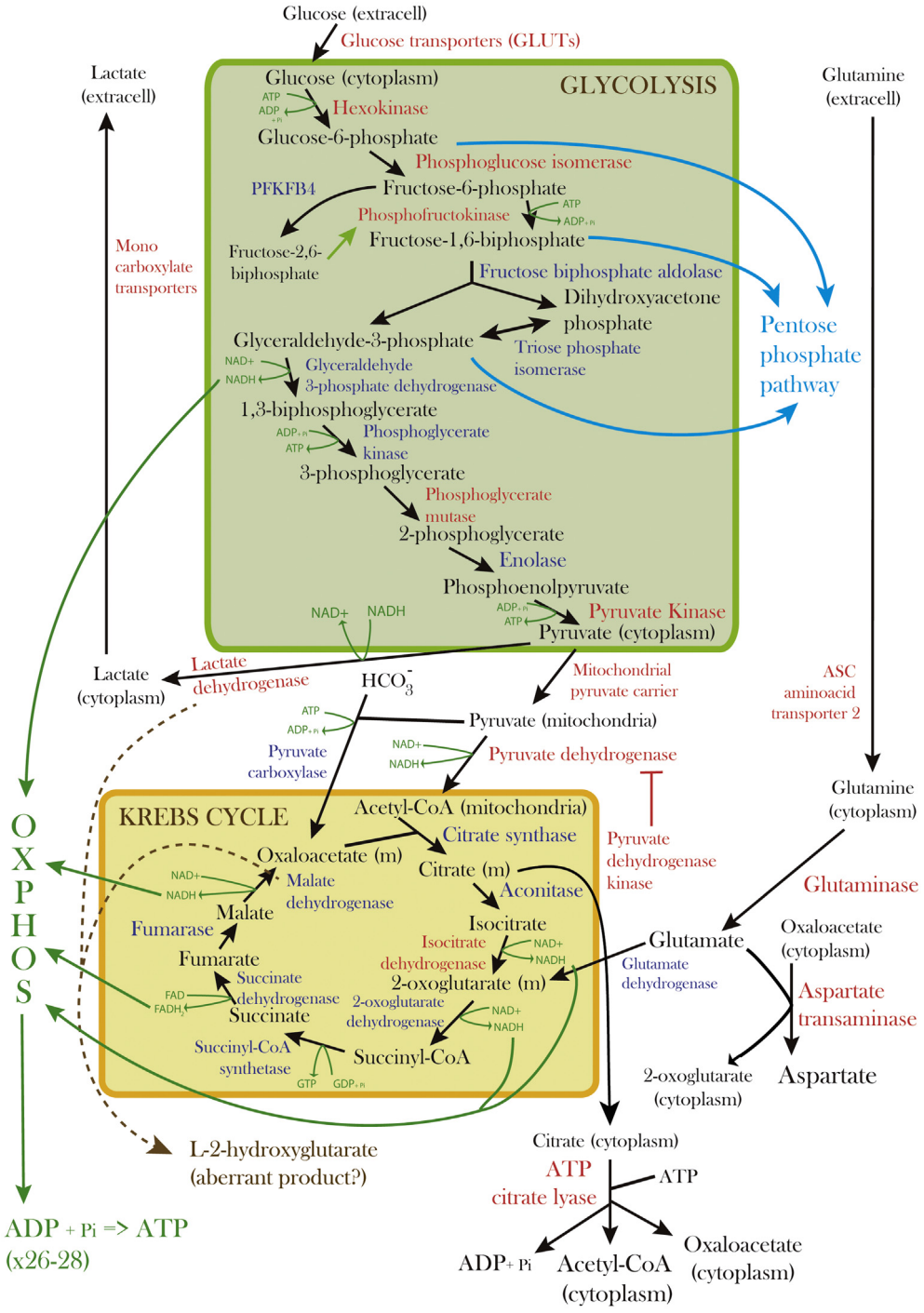

Fig. 1. Metabolic map of the pathways discussed in this review. Enzymes and transporters in red are those for which deregulation in cancer cells has been reported (see Table 1); the rest of them are shown in blue. Molecules directly related to bioenergetics are in green. The **(m)** next to certain metabolites of the Krebs Cycle indicates their mitochondrial sub-cellular localization.

block, progenitors can maintain their energy levels (ATP concentration does not seem to be altered by the treatment) whilst both proliferation and differentiation are seriously compromised. These results indicate that glycolysis and oxidative phosphorylation are actually uncoupled, as expected from the *anabolic interpretation* of the Warburg-effect, and whilst in this case the energetic metabolism relies on mitochondrial activity, a high glycolytic flux seems to be required to sustain the anabolic requirements of proliferating cells.

Aerobic glycolysis was also reported to be necessary for proliferation in mammalian hematopoiesis (Wang et al., 2014). In this research, the authors observed that normal hematopoietic progenitors from murine bone marrow expressed PKM2 and LDHA, cancer-related isoforms of Pyruvate Kinase (PK) and Lactate Dehydrogenase (LDH), respectively (Fig. 1). PKM2 displays lower affinity for its substrate phosphoenolpyruvate, so that its high prevalence in tumors, as well as in normal hematopoiesis, is interpreted as a way of blocking glycolysis (Christofk et al., 2008). The stall of the otherwise upregulated glycolytic flux at this point of the pathway is thought to raise the steady-state concentration of glycolytic metabolites required for anabolism. LDHA is often considered the isoenzyme with the highest efficiency to catalyze the conversion

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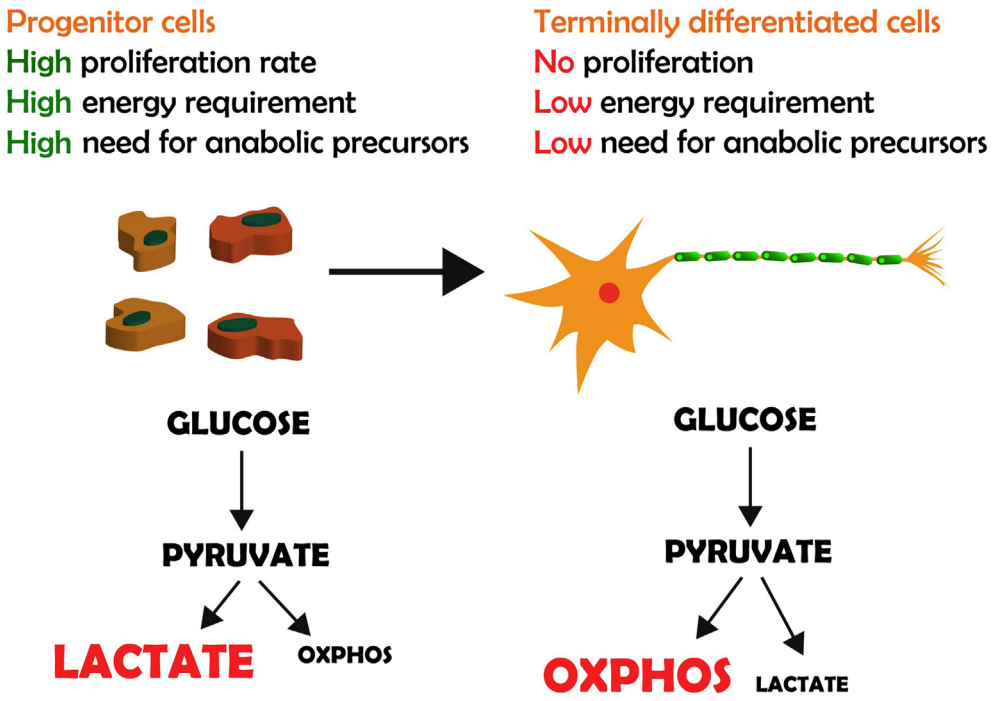

Fig. 2. Whilst differentiated cells employ oxidative phosphorylation (OXPHOS) and efficiently catabolize carbon chains, cells undergoing rapid growth and proliferation must adopt a glycolytic profile to provide anabolism with both carbon chains and ATP at high rates.

of pyruvate to lactate, which explains why it is needed under highly glycolytic conditions (Miao et al., 2013). Remarkably, forced oxidative phosphorylation achieved by either PKM2 or LDHA ablation impairs the capacity of hematopoietic progenitors to proliferate and mediate repopulation upon bone marrow transplantation.

A recent report by Zheng et al. (2016) provided a deeper mechanistic characterization of the metabolic switch. The authors proved in a neural differentiation model in cell culture that pluripotent stem cells require a metabolic switch from aerobic glycolysis to oxidative phosphorylation in order to differentiate into mature neurons. They employed *induced pluripotent stem cells* (iPSCs), which are derived from differentiated cells that have been genetically reprogrammed to an embryonic stem cell–like state by forced expression of genes needed to maintain stemness-related properties. Then, neural differentiation was induced by modifying the culture conditions (growth factors, extracellular matrix, *etc.*) (Qiang et al., 2013). Although these cells meet the criteria to be defined as pluripotent stem cells, it is unclear to what extent the cellular physiology of iPSCs and embryonic stem cells can actually be compared. This set-up provided a suitable model to conduct pharmacological or genetic interventions to assess the role of metabolism in differentiation. According to the authors, the molecular basis of the metabolic switch to oxidative phosphorylation involves several key events: Downregulation of LDHA, isotype switch from PKM2 to PKM1, downregulation of the glucose transporters GLUT1/3, downregulation of hexokinase 2 (HK2), and an increase of PDH activity mediated by diminished pyruvate dehydrogenase kinase (PDHK) activity. It is noteworthy that all these genes had been previously identified as metabolism-related genes (Table 1). Astonishingly, overexpression in these cells of LDHA and HK2 (Fig. 1) was sufficient to prevent the metabolic switch, provoking total blockade of neuronal differentiation (Fig. 2). Besides glycolysis inhibition, increased mitochondrial biogenesis also occurred during differentiation, and was attributed mainly to upregulation of two transcription factors: peroxisome-proliferator-activated receptor γ co-activator-1α (PGC-1α) and the estrogen related-receptor γ (ERRγ). PGC-1α is a transcriptional coactivator involved in several aspects of mitochondrial biogenesis. For example, it promotes transcription of nuclear transcription factors 1 and 2, which in turn activate the mitochondrial transcription factor A, leading to transcription and replication of mitochondrial DNA (Jornayvaz and Shulman, 2010). ERRγ is an orphan nuclear receptor that belongs to the ERR family and regulates transcription of several components of the mitochondrial oxidative phosphorylation pathway and other key mitochondrial genes (Alaynick et al., 2007). Transcriptional activity of ERRs is dependent on PGC-1α and β that function as coactivator proteins.

In another recent work, a thorough characterization of the metabolic profile at several points of neuronal differentiation was performed in cultured cells obtained from rat embryonic hippocampus (Agostini et al., 2016). This paper shows that glycolysis has a predominant role at an early phase of differentiation, whereas mitochondrial bioenergetics and glutamine are required at later stages of differentiation, including dendritic outgrowth and maturation (Fig. 2). This conclusion was achieved not only by a description of the metabolic profile throughout the successive neuron developmental stages, but also through pharmacological modulation of metabolism. Glycolysis blockade by addition of the competitive inhibitor 2-deoxy-D-glucose stalled differentiation at an early stage, whilst an impairment of the glutamine-fueled TCA cycle by addition of a selective inhibitor of glutaminase stalled differentiation at later stages. At a molecular level, mechanisms that promote glycolytic metabolism described in this report include induction of the glucose transporter GLUT3 and upregulation of phosphofructokinase (Fig. 1); both of these genes previously identified as Warburg-related genes (Table 1). In agreement with Zheng et al. (2016), PGC-1α was characterized in this work as the inducer of mitochondrial biogenesis.

According to the *anabolic interpretation* of the Warburg effect, the augmented glycolysis observed in proliferative cells does not need to be concomitant with downregulation of mitochondrial activity; glycolysis and mitochondrial oxidative metabolism just need to be uncoupled. In fact, a recent report (Sullivan et al., 2015) suggests that mitochondrial respiration plays a key role in proliferating cells by providing the electron acceptors needed for aspartate biosynthesis. This work shows in mammalian cultured cells that inhibition of the electron transport chain impairs growth and proliferation. However, cell proliferation is restored by providing α ketobutyrate, which acts as an exogenous electron acceptor that contributes neither to ATP production nor to

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metabolite synthesis. In most cultured cells, the carbon backbone used for *de novo* synthesis of aspartate is provided by anaplerotic glutamine (Fig. 1). Deamination of glutamine produces glutamate, which in turn can be converted into 2-OG by transamination (Hensley et al., 2013). Then, 2-OG can be converted into oxaloacetate, which is the α-ketoacid needed for aspartate biosynthesis. Oxaloacetate synthesis from 2-OG can be achieved using the TCA cycle enzymes either in an oxidative pathway (2-OG → succinate → fumarate → malate → oxaloacetate) or in a reductive one (2-OG → isocitrate → citrate → oxaloacetate). Both pathways require high levels of electron acceptors, and shortage of electron acceptors in respiration-inhibited cells accounts for the aspartate deficiency observed in these cells, and for the resulting impairment of proliferation. Strikingly, the provision of exogenous aspartate was enough to restore proliferation in cells treated with inhibitors of the electron transport chain.

A more complex outlook was achieved in a study that addressed the metabolic changes experienced by the mouse embryo after chorioallantoic branching (CB) (Miyazawa et al., 2017). CB is a process required for maternal-fetal exchange of gases and nutrients that occurs upon embryo implantation. Classical experiments from the 1970's (Clough and Whittingham, 1983; Tanimura and Shepard, 1970) indicated that early embryos inhibit glycolysis and accelerate the TCA cycle following CB, a stage of embryogenesis characterized by rapid embryonic growth. This observation would in principle contradict a Warburg-like model of development, as it shows downregulated glycolysis in cells undergoing fast growth and high proliferation. Due to the technical limitations of the time, the only measurements employed to characterize the metabolic state of these embryos were the assessment of extracellular lactate and release of CO_2. Therefore, the actual metabolic status of the embryo was not well characterized. State-of-the-art techniques, however, made possible a deeper analysis of this process. A recent paper (Miyazawa et al., 2017) shows that instead of simply inhibiting glycolysis, embryos undergoing CB divert the glycolytic flux towards the pentose phosphate pathway *via* suppression of the key glycolytic enzymes PFK-1 and aldolase (Fig. 1), thereby sustaining synthesis of biomass. Lactate synthesis is not downregulated, but instead it is increased, albeit lactate is not immediately secreted as previously thought. In fact, lactate intracellular distribution seems to be uneven in the embryo, although the biological relevance of this interesting observation has not been explored. Whatever the case may be, the metabolic changes observed during CB can now be understood according to the *anabolic interpretation* of the Warburg effect.

Even though the fruit fly *Drosophila melanogaster* is one of the most widely used model organisms in developmental studies, only a few works addressing the role of metabolic modulation in development in this species have been published so far. One of these works (Tennessen et al., 2011) shows that 12 h before hatching, the *Drosophila* embryo upregulates most glycolytic genes, including the *Drosophila* LDH orthologue known as ImpL3. The *Drosophila* Estrogen-Related Receptor (dERR) coordinates this embryonic metabolic transition, which allows newly hatched larvae to efficiently convert dietary carbohydrates into biomass. This metabolic condition is essential for the 200-fold increase in body mass that occurs during the 4 days of larval development. The inhibition of glycolysis observed in dERR or phosphofructokinase mutants produce a decrease in ATP concentration concomitant with elevated levels of circulating sugars. This might suggest an impaired glycolytic flux in these larvae, which die at the second instar. There is no evidence, however, that this lethality is due to impaired cell proliferation produced by glycolysis inhibition rather than a more general effect generated by the metabolic blockage in these mutant animals. Further research is certainly required to address this point.

Another report (Tennessen et al., 2014) takes advantage of the large body of high-throughput data available in *Drosophila*. The modENCODE project includes a comprehensive transcriptional profile of staged *Drosophila* embryos obtained by RNA-seq. The authors used a mathematical tool, *singular value decomposition* (SVD) to identify in this database significant patterns of gene expression at different time points during embryogenesis. They found that, concomitantly with the embryonic metabolic transition described above, nearly 500 genes included in the category "*Generation of Precursor Metabolites and Energy*" were upregulated by mid-embryogenesis. Surprisingly, the upregulated genes within this category encompassed not only every gene that encodes a glycolytic enzyme, but also certain genes involved in mitochondrial metabolism, such as a PDH subunit, electron transport chain subunits, and enzymes of the TCA cycle (Fig. 1). However, a complementary metabolomic analysis of staged *Drosophila* embryos (Tennessen et al., 2014) revealed that oxidative phosphorylation appears to be attenuated during embryogenesis, as embryos exhibit a block of the TCA cycle that results in elevated levels of citrate, isocitrate, and 2 oxoglutarate (Fig. 1). Once again, this metabolic status of fly embryos is reminiscent of the Warburg effect of cancer cells, as glycolytic metabolism is required to sustain high anabolic rates. Nevertheless, the precise contribution of oxidative phosphorylation to the bioenergetics of *Drosophila* embryogenesis is still unclear.

Drosophila neural development has also been subjected to metabolic analysis (Homem et al., 2014). Larval neuroblasts (NBs) are stem-like progenitor cells that divide asymmetrically. This division gives rise to a larger cell that maintains stem cell identity and a smaller one committed to differentiation to neuron or glia (Ito and Hotta, 1992). The daughter cell that maintains NB identity also conserves the high anabolic rate that supports re-growth to its original size. Thus, NBs maintain a constant volume throughout larval development. During the pupal stage, NBs reduce their size progressively until they commit to terminal differentiation, leading to minimal or no-proliferation in the adult *Drosophila* brain (Fernández-Hernández et al., 2013). The work by Homem et al. (2014) shows that both the Ecdysone Receptor and the *Drosophila* Mediator complex are needed to trigger a metabolic switch towards mitochondrial oxidative phosphorylation, which in turn provokes NBs shrinkage. Interestingly, NBs in which key elements of the TCA cycle (α ketoglutarate dehydrogenase) or the electron transport chain (cytochrome *c* oxidase) have been knocked down, displayed increased cell size and extended lifespan. Presumably, NB shrinkage is a consequence of the inability of mitochondrial oxidative metabolism to sustain a high biosynthetic rate in early NBs. Ecdysone is an insect steroid hormone that triggers molting and pupariation (Ou and King-Jones, 2013). Thus, the fact that the metabolic switch is regulated by the ecdysone receptor is noteworthy, as it provides evidence of metabolic rewiring triggered by a well-established ontogenetic regulator.

2.3. Dissonant tunes in the bioenergetics toccata

We have summarized so far some of the most prevalent works that support the notion that active proliferation requires glycolytic metabolism to sustain biomass accumulation (Fig. 2). However, there are studies apparently contradicting this notion, as they report that cells undergo a switch towards glycolysis during terminal differentiation (Esteban-Martínez et al., 2017; Forni et al., 2016), as well as at least one study reporting that stem cells undergo active mitochondrial metabolism (Rodríguez-Colman et al., 2017).

Esteban-Martínez et al. (2017) reported a metabolic characterization of the murine retina development in a combined *ex vivo*/*in vivo* approach. Retinal ganglion cells (RGCs) are a retina-specific type of neurons that transmit image-forming and non-image forming visual information to the brain. This work shows that RGC differentiation depends on a switch to glycolytic metabolism defined by increased lactate production and elevated expression of glycolytic genes. Strikingly, the switch is achieved not by regulation of key enzymes that control the metabolic flux, but instead, through elimination of mitochondria by mitophagy. RGC differentiation is blocked upon pharmacological inhibition of mitophagy, implying that a net reduction of mitochondria is required for proper differentiation. Furthermore, the authors demonstrated that the embryonic mouse retina undergoes

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developmental hypoxia, which is expected to impair mitochondrial oxidative phosphorylation. This would explain the fact that mitochondria are dispensable for RGC differentiation, being mitochondrial biomass utilized to fuel other physiological processes. Pharmacological stabilization of HIF-1α, the main hypoxia response activator, provoked RGC differentiation, and the authors speculate that the metabolic reprogramming that occurs during RGC differentiation is triggered by the mainstream pathway of the hypoxia response.

Further evidence of a glycolytic switch during differentiation was reported in an *in vitro* model of mouse skin mesenchymal stem cells (msMSCs) (Forni et al., 2016). In this study, msMSCs were isolated and then differentiated to osteocytes, chondrocytes or adipocytes. Whilst differentiation commitment towards either adipocytes or osteocytes involves enhanced mitochondrial respiration, mitochondrial metabolism seems to decrease during chondrogenesis. In line with the work by Esteban-Martínez et al. (2017), the glycolytic profile is in this case also achieved by controlling the amount of mitochondria through LC3-dependent mitophagy. Notably, as occurs in RGCs, chondrocyte differentiation is also dependent on a hypoxic microenvironment (Araldi and Schipani, 2010). Thus, both differentiation of retinal cells and differentiation of msMSC into chondrocytes share remarkable metabolic features.

A different deviation from the hypothesis that active glycolytic metabolism is required to sustain cell growth and proliferation, was recently reported in a study focused on mammalian intestinal stem cells (Rodríguez-Colman et al., 2017). Crypt base columnar cells (CBCs) are intestine stem cells that lie at the base of intestinal crypts, surrounded by terminally differentiated Paneth cells. The authors observed that freshly isolated Paneth cells exhibited prevalently glycolytic metabolism with high rate of lactate synthesis, whilst isolated CBCs displayed increased mitochondrial activity. Using a model of intestinal organoid development, they reported that inhibition of either glycolysis in Paneth cells or the electron transport chain in CBCs impaired stem cell function. Remarkably, Paneth cells treated with inhibitors of lactate transporters were inefficient in supporting organoid formation. This suggested that Paneth cells support stem cell function by providing lactate to sustain mitochondrial metabolism of CBCs. Traditional analysis of stem cell metabolism has highlighted the importance of downregulated mitochondrial activity to prevent ROS biogenesis and DNA oxidative damage, thereby avoiding mutations in daughter cells (Suda et al., 2011). Future works will probably need to explore possible functions of ROS as physiological mediators of signal transduction in stem cells, in order to integrate these apparently paradoxical results into our current conception of stem cell metabolism.

All the evidence described so far in this section highlights the fact that every particular cell lineage has its own energetic and metabolic requirements, so we should be cautious when generalizing about metabolic aspects of development.

3. An alternative view of the metabolic requirements of development

3.1. Metabolism in epigenetic regulation

Precise regulation of gene transcription is not possible without some degree of epigenetic control. Among other mechanisms, this can be achieved by DNA methylation and posttranslational modifications of histones. Over the last decade, the fact that the metabolic status of the cell is reflected in its epigenetic profile has been firmly established (Liu et al., 2013; Martinez-Pastor et al., 2013).

Histone acetylation is mediated by specific acetyl transferases (HATs), which catalyze the conjugation of an acetyl group derived from acetyl-CoA to a lysine residue of the histone. This modification alters high order chromatin structures and is widely considered as a pro-transcription epigenetic mark (Anastasiou et al., 2012). Acetyl-CoA is produced by the pyruvate dehydrogenase complex (PDH) in the mitochondria, using pyruvate as substrate (Fig. 1). However, the nuclear-cytoplasmic pool of acetyl-CoA required for histone acetylation is isolated from the mitochondrial pool, due to the inability of acetyl-CoA to diffuse across mitochondrial membranes. Instead, nuclear acetyl-CoA originates from the activity of the ATP-citrate lyase, which employs mitochondrial citrate as a precursor (Wellen et al., 2009) (Fig. 1). Citrate can freely diffuse from the mitochondria out to the cytosol and then into the nucleus, and its levels directly affect HAT activity (Wellen et al., 2009). The reverse process, histone deacetylation, is catalyzed by histone deacetylases (HDACs) and sirtuins. Deacetylation of histones is also under metabolic control, as sirtuins are able to sense the NAD^+/NADH ratio. These enzymes use NAD^+ as cofactor, and a decreased NAD^+/NADH ratio, like the one observed in cells with high glycolytic flux, inhibits sirtuin activity (Martinez-Pastor et al., 2013; Ryall et al., 2015).

Histone mono-, di-, or tri-methylation occurs on different lysine or arginine residues, and some of these histone methylation marks can activate transcription, while others mediate transcriptional inhibition (Teperino et al., 2010). Histone methylation is catalyzed by a family of histone methyltransferases (HMTs) that employ S adenosylmethionine (SAM) to transfer a methyl group onto the proper residues in histone tails. SAM is produced by the enzyme S adenosyl methionine transferase (MAT) in a reaction that requires methionine and ATP as substrates (Teperino et al., 2010). Thus, SAM levels are indeed dependent on the metabolic status of the cell (Chiang et al., 2009). The reverse reaction, histone demethylation, is catalyzed by histone demethylases (HDMs), which can belong to either the LSD enzyme family or the JmjC family (Shi and Tsukada, 2013). Whilst LSDs employ flavin adenine dinucleotide (FAD^+) as a co-factor for the catalytic reaction, the JmjCs family of HDMs uses 2-OG and O_2 as co-substrates (Loenarz and Schofield, 2008). 2-OG is a TCA intermediate metabolite synthesized and consumed by enzymes that work far from the equilibrium (isocitrate dehydrogenase and 2-OG dehydrogenase respectively (Voet and Voet, 2004); (Fig. 1), and therefore, 2-OG levels are thought to depend on the metabolic flux of the TCA Cycle. Changes in the diet that affect SAM, FAD or 2-OG concentrations in either the mitochondria or the cytoplasm have therefore been shown to affect histone methylation through the modulation of LSD or JmjC family enzymes (Teperino et al., 2010). Notably, many of the JmjC enzymes are not only dependent on 2-OG levels, but also inhibited by succinate (Loenarz and Schofield, 2008), which is the oxidation product of 2-OG in the TCA cycle. Thus, it is actually the 2-OG/succinate ratio which exerts control over the activity of these enzymes.

Additionally, 2-OG can also regulate DNA demethylation. The ten-eleven-translocation (TET) family of 5 methylcytosine (5mC) hydroxylases involved in DNA demethylation shares features with the JmjC family of histone demethylases (Loenarz and Schofield, 2008). They also require 2-OG as a co-substrate for catalysis, and thus they are subjected to the same kind of metabolic control than JmjC demethylases. As mentioned above, 2-OG is generated at the TCA cycle by the isocitrate dehydrogenase (Fig. 1). Many cancer-related mutations in this enzyme lead to the synthesis of an alternative product, D 2 hydroxyglutarate (D-2HG), which is commonly assumed as an onco-metabolite as it is rarely found in normal cells. D-2HG is in fact a competitive inhibitor of 2-OG dependent dioxygenases, including JmjC and TET enzymes, resulting in alterations of the histone methylation profile (Xu et al., 2011). These epigenetic alterations elicited by 2-HG are thought to contribute to tumorigenesis.

3.2. Metabolic-driven epigenetic changes are required for development

The previous section described how the metabolic status of the cell impacts on epigenetics, and thus regulates transcription (Fig. 3). Strikingly, recent studies have begun to explore whether the epigenetic changes observed during development are in fact under metabolic control.

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Pluripotent stem cells (PSCs) cultured *in vitro* are characterized by a glycolytic metabolism (Panopoulos et al., 2012), which may explain the high levels of acetyl-CoA present in these cells. Earlier studies have shown that histone acetylation plays a central role in maintenance of the open chromatin structure that characterizes PSCs (Melcer et al., 2012), even though the notion that cell differentiation may depend directly on acetyl-CoA levels has been addressed only recently (Moussaieff et al., 2015). This study shows that reduction of acetyl-CoA levels, and concomitant reduction of histone acetylation are required during murine and human embryonic stem cell differentiation in culture. Decreased expression of pluripotency markers was observed upon pharmacological inhibition of ATP-citrate lyase, the enzyme that synthesizes cytosolic acetyl-CoA (Fig. 1). Consistent with this, exogenously provided acetate delayed differentiation in a dose-dependent manner. In fact, the addition of acetate prevented the loss of histone acetylation observed during differentiation, leading instead to hyperacetylation of histones upon exposure to high acetate doses. The effect of exogenous acetate was antagonized by anacardic acid, a histone acetyl transferase inhibitor. Thus, it seems clear that proper PSC differentiation requires a metabolic shift leading to decreased acetyl-CoA levels in the cytoplasm, provoking reduction of histone acetylation.

The metabolic facets of PSC differentiation have also been studied in a recent work by TeSlaa et al. (2016). This paper provides evidence for 2-OG dependent control of both DNA and histone methylation during PSC differentiation. The authors manipulated cytoplasmic levels of 2-OG and succinate by employing the membrane-permeable analogues of these two metabolites, dimethyl 2 oxoglutarate and dimethyl-succinate, respectively. Whilst a high 2-OG/succinate ratio accelerated differentiation, elevated succinate levels led to blockage. Consistent with this, inhibition of succinate dehydrogenase A, the enzyme that converts succinate into fumarate in the TCA cycle, delayed PSC differentiation. Strikingly, pharmacological inhibition of JmjC and TET activity by addition of dimethyloxalylglycine (DMOG) prevented differentiation too. Thus, these results point to a central role in PSC differentiation of both TET and JmJC, two families of enzymes controlled by the 2-OG/succinate ratio in the cell.

Whilst the above works described metabolic control of epigenetic marks that contribute to cell differentiation, Nagaraj et al. (2017) reported that this mechanism also plays a role in zygotic-genome activation (ZGA) of murine two-cell embryos. ZGA comprises the degradation of maternal transcripts, a process known as *maternal clearance*, as well as the start of zygotic transcription. Previous works reported that activation of zygotic transcription relies on chromatin structural and epigenetic changes (Weaver et al., 2009). Nagaraj et al. showed that, during ZGA, transient nuclear localization of PDHK as well as of the first enzymes of the TCA cycle (citrate synthase, aconitase 2 and isocitrate dehidrogenase 3) (Fig. 1), allows for acetyl-CoA and 2-OG localized synthesis in the nucleus, where the enzymes responsible of the epigenetic marks exert their activity. In the absence of the whole enzymatic machinery needed to complete the TCA cycle, a fourth enzyme, not usually considered as a TCA cycle-related enzyme, the pyruvate carboxylase (Fig. 1), is also required in the nucleus to mediate pyruvate conversion to oxaloacetate. This spatial reorganization of metabolic enzymes leads to altered nuclear levels of acetyl-CoA and 2-OG, which in turn regulate histone acetylation and methylation patterns. Inhibition of this metabolic rewiring impaired ZGA, and blocked development at the two-cell embryonic stage. Thus, it seems clear that metabolic control of development occurs even at the earliest stages of embryogenesis.

A different metabolic modulation of the epigenetic profile was reported in *Drosophila* development (Li et al., 2017). We have discussed in Section 2.2 that *Drosophila* larvae exhibit a prevalently glycolytic metabolism triggered by dERR, that supports the 200-fold increase in body mass that occurs during this stage (Tennessen et al., 2011). Li et al. (2017) reported that *Drosophila* larvae synthesize an uncommon metabolite, L 2 hydroxyglutarate (L-2HG), which affects the DNA methylation pattern of cells that will form adult tissues. L-2HG is the enantiomer of the above-mentioned onco-metabolite D-2HG. It is commonly considered as a metabolic aberrant product synthesized by malate dehydrogenase, lactate dehydrogenase A (LDHA) and lactate dehydrogenase C (LDHC) (Fig. 1). However, in *Drosophila* L-2HG seems to be synthesized exclusively by LDH, as inferred from both genetic and biochemical experiments (Li et al., 2017). Upon pupariation, L-

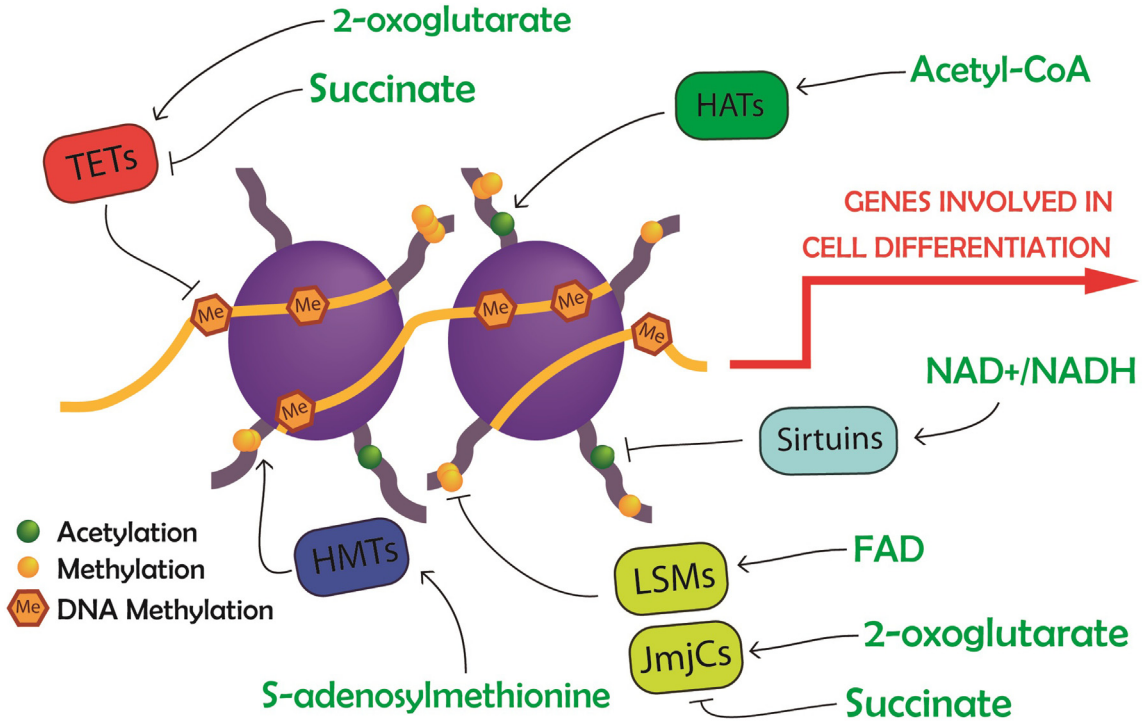

Fig. 3. Metabolism can impact on the epigenetic profile of the cell on many ways. Acetyl-CoA is needed for histone acetylation. SAM, FAD and the 2-OG/succinate ratio control histone methylation and demethylation. The 2-OG/succinate ratio can also alter the DNA methylation status.

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2HG levels are drastically reduced by the action of L 2 hydroxyglutarate dehydrogenase, reflecting an unexpected physiological regulation of this metabolite. Like its dextrorotatory enantiomer, L-2HG is thought to affect the activity of 2-OG dependent oxygenases, including the Tet-family DNA methylases. Although larval N 6 methyldeoxyadenosine (6mdA) levels were not affected in LDH mutants unable to synthesize L-2HG, its abundance in adult genomic DNA decreased in the absence of larval LDH activity. Therefore, this finding suggests that L-2HG production during larval development does impact on DNA methylation, although only in tissues that will be represented in the adult fly. In mammalian cells, 2HG accumulation on a cancer context seems to impact on epigenetic marks and chromatin architecture (Shim et al., 2014), but no studies have been reported so far addressing a possible physiological role of this metabolite in vertebrate development. It remains to be investigated as whether the function of L-2HG that has just begun to be explored in *Drosophila* is conserved in mammals.

4. Metabolism and signaling: a deeper look into metabolic aspects of development

We have already discussed the role metabolism plays in providing both energy and carbon chains required for proper development, as well as the fact that certain metabolic shifts observed during development could be coupled to epigenetic regulations needed in embryogenesis. However, recent reports indicate that there are still other mechanisms by which metabolism regulates developmental programs. A study by Pegoraro et al. (2015) shows that 6 phosphofructo 2 kinase/fructose 2,6 biphosphatase 4 (PFKFB4), which has classically been characterized as a glycolysis-inducing enzyme (Rider et al., 2004), acts instead by promoting AKT phosphorylation in the dorsal ectoderm of gastrulating *Xenopus* embryos. PFKFB4 is a bifunctional kinase/phosphatase that mediates the reversible conversion of fructose 6 phosphate, a glycolytic metabolite, into fructose 2,6 biphosphate (Rider et al., 2004) (Fig. 1). This metabolite does not belong to the glycolytic pathway, but instead acts as an allosteric activator of the phosphofructokinase 1, a key enzyme of glycolysis. This PFKFB4-dependent activation of AKT constitutes a checkpoint required for progenitors of the dorsal ectoderm to differentiate, whatever their cell fate may be. Thus, alteration of this checkpoint provokes local developmental arrest followed by apoptosis. Strikingly, pharmacological inhibition of glycolysis does not impair PFKFB4/AKT-dependent developmental checkpoint in *Xenopus* dorsal ectoderm, indicating that the AKT phosphorylation activated by PFKFB4 is independent of the role of this enzyme in glycolysis activation. The molecular mechanism by which PFKFB4 activity leads to AKT phosphorylation has not been deciphered as yet.

However, the authors continued to explore PFKFB4 function in *Xenopus* dorsal ectoderm, discovering a role of this enzyme in neural crest (NC) development (Figueiredo et al., 2017). By silencing PFKFB4 in a temporally controlled fashion, the authors defined two different functions of PFKFB4 in this developmental context. At the neurula stage, PFKFB4 is required for NC specification, as its depletion severely compromised the expression of the gene twist. Later on, at tailbud stage, PFKFB4 is required again for NC migration in a cell-autonomous manner, being required for n-cadherin expression. Both functions depend on PFKFB4-dependent phosphorylation of AKT described in their previous work (Pegoraro et al., 2015). In this case, pharmacological inhibition of glycolysis did impair NC migration, so glycolysis activation mediated by PFKFB4 is apparently required for NC migration. PFKFB4 levels increase during NC development and this increase apparently depends on the classical NC specification pathways, such as FGF8, Wnt or BMP, albeit a deeper research is required to further elucidate the molecular mechanism.

The vertebrate presomitic mesoderm (PSM) has also been the subject of recent studies which widen our current knowledge on how metabolism and development are intertwined. Bulusu et al. (2017) analyzed the metabolic profile of the PSM in mouse embryos, and discovered a glycolytic gradient within this region: Whilst the undifferentiated mesenchymal cells of the posterior region of the PSM are highly glycolytic, the more differentiated cells of the anterior PSM display active oxidative phosphorylation. This metabolic switch observed within the PSM during differentiation is achieved by transcriptional downregulation of most glycolytic genes, being aldolase A (AldoA), triose phosphate isomerase (TPI), and glyceraldehyde 3 phosphate dehydrogenase (GAPDH) particularly affected (Fig. 1). *Ex vivo* culture of PSM explants in a medium containing pyruvate as the only carbon source (which inhibits glycolysis) caused severe developmental anomalies, including impaired somite formation.

Although the above results could be interpreted in favor of the hypothesis that rapid growth and proliferation requires a glycolytic metabolism, the proliferation rate at the anterior and posterior regions of the PSM have been measured previously using a variety of approaches, and was found to remain relatively stable after differentiation (Gomez et al., 2008; Venters et al., 2008). A deeper understanding of this phenomenon was provided by a complementary work by Oginuma et al., (2017), which shows that the glycolytic gradient is controlled by FGF signaling. This study carried out in chick shows that pharmacological inhibition of FGF signaling dissipates the lactate gradient and downregulates key glycolytic enzymes on the posterior region of the PSM. Pharmacological inhibition of glycolysis affected neither cell death nor cell proliferation, but instead impaired random cell motility, a process required for posterior elongation movements in the chick embryo. In tumor microenvironments, the motility of malignant cells depends directly on their glycolytic metabolism. Lactate secretion that characterizes glycolytic metabolism acidifies the extracellular milieu, which in turn activates metalloproteinases needed to remodel the extracellular matrix during cell metastasis (Parks et al., 2013). To test if the same mechanism applies to PSM development, Oginuma et al. interfered with extracellular acidification by culturing embryos on alkaline plates, and observed a similar impairment of cell motility. Therefore, glycolysis seems to be required to modify the external pH and alter the extracellular matrix, thereby allowing for the morphogenetic movements that drive PSM development (Oginuma et al., 2017).

Interestingly, this work (Oginuma et al., 2017) reports another unexpected role of glycolysis in PSM undifferentiated cells: Upon pharmacological inhibition of glycolysis, activity of the Wnt pathway was impaired, as β-catenin nuclear localization, as well as transcription of two Wnt targets (AXIN2 and BRACHYURY) were severely compromised. Thus, glycolysis induced by FGF signaling seems to promote activation of the Wnt pathway in chick PSM, albeit the precise mechanism by which the metabolic profile of the cell affects Wnt signaling remains elusive. These results highlight a second mechanism by which metabolism affects cell differentiation and behavior: Instead of supplying the developmental process with energy or carbon chains, aerobic glycolysis directly affects cell physiology, either by regulating signaling pathways or by affecting cell motility (Fig. 4).

5. Conclusions and future directions

We have discussed three non mutually-exclusive ways by which metabolism and developmental processes dialogue with each other:

A. A Warburg-inspired model of development, in which metabolic rewiring towards aerobic glycolysis is required in highly proliferating cells to cope with the demands of anabolic precursors and energy, whereas mitochondrial activity tends to increase during differentiation (Fig. 2). This model is supported by metabolic analysis of *Xenopus* retinal cell differentiation (Agathocleous et al., 2012), murine embryos undergoing chorioallantoic branching (Miyazawa et al., 2017), mammalian hematopoiesis (Wang et al., 2014), mammalian neuronal differentiation (Agostini et al., 2016; Zheng et al.,

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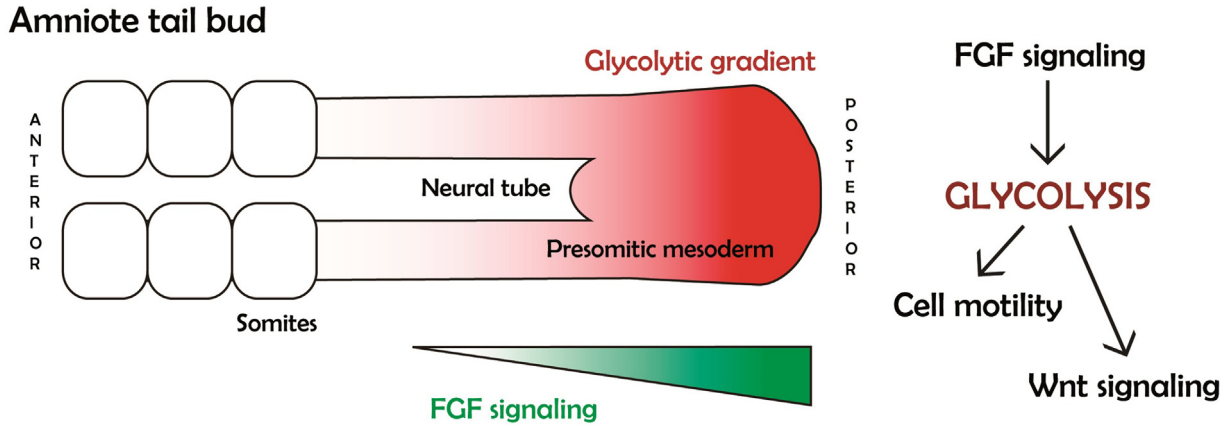

Fig. 4. Metabolic pathways as signal transducers. The glycolytic profile in undifferentiated cells of the presomitic mesoderm is required not for energetic reasons but to convey FGF signaling to activate the Wnt pathways.

2016), *Drosophila* neuroblast differentiation (Homem et al., 2014), *Drosophila* embryogenesis (Tennessen et al., 2014) and overall *Drosophila* larval development (Tennessen et al., 2011). However, the converse situation applies to some other developmental events, as there are results showing that cells undergo a switch towards glycolysis during terminal differentiation. This has been shown during differentiation of mammalian retinal ganglion cells (Esteban-Martínez et al., 2017) and skin mesenchymal stem cells (Forni et al., 2016).

B. A metabolically-controlled epigenetic reconfiguration that triggers cell differentiation and certain developmental milestones (Fig. 3). Evidence supporting this model derives from studies on human and murine embryonic stem cell differentiation in culture (Moussaieff et al., 2015; TeSlaa et al., 2016), embryonic murine zygotic-genome activation (Nagaraj et al., 2017) and *Drosophila* larval development (Li et al., 2017).

C. A still obscure mechanism by which metabolism, or at least metabolic enzymes, may influence a signaling pathway, as was reported for AKT in *Xenopus* dorsal ectoderm, as well as for the Wnt pathway in the vertebrate PSM (Bulusu et al., 2017; Oginuma et al., 2017) (Fig. 4).

New technologies are leading to the notion that aspects of metabolism hitherto considered particularities are instead widespread phenomena. For example, most glycolytic enzymes can act as RNA-binding proteins (RBPs) (Beckmann et al., 2015), hence receiving the name of enigmRBPs. Noteworthy, RNA-binding activity of at least two of them, Human Aconitase I (Castello et al., 2015) and GAPDH (Chang et al., 2013), was reported to play physiological functions. An exciting idea in current cell biology is the REM (RNA-Enzymes-Metabolites) network hypothesis, which proposes that gene expression and metabolism are linked through the regulatory activity of moonlighting RNA-binding metabolic enzymes. So far, no evidence of REM dynamics governing developmental processes has been provided, although the possibility is appealing. Another interesting aspect of metabolism that is just starting to be unveiled is the fact that metabolites themselves can act as signaling molecules by direct binding to target proteins. Lactate has been recently reported to bind and regulate NDRG3, a protein involved in angiogenesis and cell growth (Lee et al., 2015). It would not be surprising if future works report that certain metabolites bind and regulate key activators of developmental processes.

Setting speculations aside, developmental biologists are starting to consider metabolism as much more than a housekeeping process and to discuss biochemistry vigorously. The definition of "metabolite" is functional, not structural. The fact that a molecule belongs to a metabolic pathway does not exclude it from fulfilling regulatory roles in cell physiology and development. Improved metabolomic technologies, real-time single-cell metabolite imaging and new techniques for modelling metabolic networks will be useful tools in forthcoming times, as will hopefully contribute to shed light on the metabolic forces that govern growth and development. Developmental biology is experiencing a new *amalgamation* in which biochemistry and metabolism are fully being integrated to the field.

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