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REVIEW

Revisiting the role of metabolism during development

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ABSTRACT

An emerging view emphasizes that metabolism is highly regulated in both time and space. In addition, it is increasingly being recognized that metabolic pathways are tightly connected to specific biological processes such as cell signaling, proliferation and differentiation. As we obtain a better view of this spatiotemporal regulation of metabolism, and of the molecular mechanisms that connect metabolism and signaling, we can now move from largely correlative to more functional studies. It is, therefore, a particularly promising time to revisit how metabolism can affect multiple aspects of animal development. In this Review, we discuss how metabolism is mechanistically linked to cellular and developmental programs through both its bioenergetic and metabolic signaling functions. We highlight how metabolism is regulated across various spatial and temporal scales, and discuss how this regulation can influence cellular processes such as cell signaling, gene expression, and epigenetic and post-translational modifications during embryonic development.

KEY WORDS: Metabolic dynamics, Metabolic signaling, Moonlighting enzymes, Sentinel metabolites

Introduction

Central carbon metabolism is well recognized for its indispensable bioenergetic functions that underlie all cellular processes (Fig. 1). However, recent studies have shed new light on previously unrecognized roles of central carbon metabolism in the regulation of specific biological processes, such as cell signaling, proliferation and differentiation (Pavlova and Thompson, 2016; Shyh-Chang et al., 2013). This emerging view is stimulating metabolic research in the field of developmental biology, which is uncovering the key roles of glucose metabolism during embryonic development in a range of species (Gandara and Wappner, 2018; Krejci and Tennessen, 2017). Considering the intimate connection between metabolism and the environment, these lines of research also have the potential of yielding a better understanding of developmental and phenotypic plasticity, which relies on the integration of both genotype and environmental cues.

To help address the often complex and interconnected functions of metabolism, we suggest that it is helpful to first distinguish between two types of metabolic functions: bioenergetic functions and metabolic signaling functions (Fig. 2). Here, we define a bioenergetic function as one carrying out canonical metabolic activity, i.e. providing energy and/or cellular building blocks. An emerging view emphasizes that bioenergetic activities are highly regulated, in both time and space, in order to match context-dependent cellular demands (Vander Heiden and DeBerardinis, 2017). A well-known example of such a canonical yet specialized function of metabolism is the Warburg effect (Warburg, 1956). This particular metabolic state, which is characterized by increased glycolytic activity regardless of oxygen conditions, has been found in various highly proliferating cells, including cancer cells (Vander Heiden et al., 2009). A common view on this particular metabolic state is that, even though ATP generation from carbon sources is less efficient compared with oxidative phosphorylation (OXPHOS), aerobic glycolysis efficiently meets the metabolic demands for the production of macromolecules, such as nucleic acids, in order to facilitate cell proliferation (Christofk et al., 2008a; Lunt et al., 2015; Vander Heiden and DeBerardinis, 2017). How cells acquire such a specialized metabolic state is not entirely clear and has been the focus of intense research for decades. Nonetheless, this intricate regulation of a particular metabolic program exemplifies the tight adjustment of energy metabolism to specific metabolic demands.

Moreover, it is becoming clear that metabolic pathways can also play modulatory or instructive roles in the regulation of cellular programs, which can be summarized as metabolic signaling functions. In addition to known mechanisms, such as the activation of redox signaling by reactive oxygen species (ROS), recent findings emphasize the roles of metabolites as rate-limiting substrates for epigenetic modifications and protein post-translational modifications (PTMs). Furthermore, it is increasingly appreciated that metabolic enzymes also play non-bioenergetic 'moonlighting' functions (e.g. non-enzymatic nuclear roles of glycolytic enzymes) (Boukouris et al., 2016; Jeffery, 1999). These non-canonical signaling functions of metabolism can establish unexpected and direct ties between seemingly distant cellular processes.

In this Review, we first provide examples of how metabolism can be compartmentalized with regard to space and time. We then discuss the molecular mechanisms by which spatiotemporally regulated metabolism signals to cellular pathways, focusing on metabolic intermediates as rate-limiting substrates for epigenetic and protein modifications, and on the non-canonical signaling functions of metabolic enzymes and metabolites. Finally, we highlight recent studies that address the spatiotemporal regulation of metabolism and its signaling roles in various developmental contexts.

Metabolic regulation in time and space

In contrast to the general view of metabolism as being homogeneous within and between cells, recent findings indicate striking spatial compartmentalization of metabolism at both the intercellular/tissue and subcellular levels. In addition, it is becoming clear that energy metabolism is dynamically regulated not only in space, but also in time, across many scales. Below, we highlight a few examples of such spatiotemporal compartmentalization of metabolism.

Regulation at the intercellular/tissue level

Metabolic activity is spatially regulated at the cellular and tissue level. In the brain, for example, glucose metabolism is

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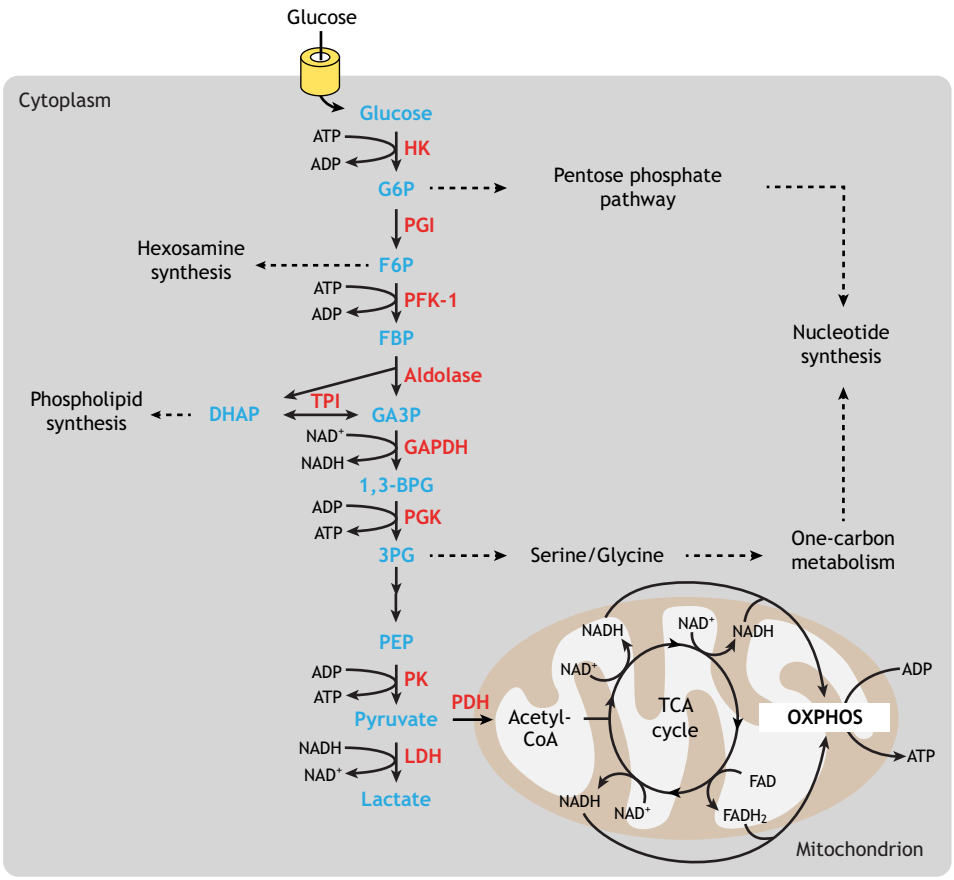

Fig. 1. An overview of glucose metabolism. Glucose, transported into cells via glucose transporters, is catabolized in a series of enzymatic reactions, eventually yielding pyruvate. Pyruvate is then either converted into lactate or transported into mitochondria and metabolized into acetyl-CoA, fueling the TCA cycle. NADH and $FADH_2$, produced through glycolysis and the TCA cycle, are used by the mitochondrial electron transport chain for generating an electrochemical proton gradient, which drives OXPHOS for ATP production. Glycolytic metabolites (shown in blue) also feed into metabolic pathways that branch from glycolysis. These include the pentose phosphate pathway, the one-carbon metabolism pathway, and the hexosamine and phospholipid synthesis pathways. Enzymes are shown in red. 1,3-BPG, 1,3-bisphosphoglyceric acid; 3PG, 3-phosphoglyceric acid; DHAP, dihydroxyacetone phosphate; F6P, fructose 6-phosphate; G6P, glucose 6-phosphate; GA3P, glyceraldehyde 3-phosphate; HK, hexokinase; PEP, phosphoenolpyruvic acid; PGI, phosphoglucose isomerase; PK, pyruvate kinase; TPI, triose phosphate isomerase.

compartmentalized between neurons and astrocytes. Neurons show a lower glycolytic activity than astrocytes because of the constant degradation of the enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatese-3 (PFKFB3), which produces a potent allosteric activator of a key glycolytic enzyme phosphofructokinase 1 (PFK-1) (Almeida et al., 2004; Herrero-Mendez et al., 2009). Such intercellular compartmentalization generates a gradient of the glycolytic end-product lactate from astrocytes to neurons (Mächler et al., 2016), which leads to lactate flow from astrocytes to neurons via facilitated transport. Neurons, in turn, oxidize lactate into CO_2 through the tricarboxylic acid (TCA) cycle, facilitating generation of ATP via OXPHOS (Pellerin and Magistretti, 2012). This astrocyte-neuron lactate shuttle allows neurons to use glucose preferentially for the maintenance of cellular redox balance, rather than for energy production. Neurons preferentially metabolize glucose through the pentose phosphate pathway (PPP), which ensures production of the reducing agent NADPH (Herrero-Mendez et al., 2009). When neurons are forced to activate glycolysis at the expense of glucose flux via the PPP, the lack of reducing equivalents leads to neuronal apoptosis due to oxidative stress (Herrero-Mendez et al., 2009). A similar metabolic interaction has been observed in intestinal organoids; in this case, the shuttling of lactate from Paneth cells to intestinal stem cells promotes mitochondrial respiration and ROS production to drive crypt formation (Rodríguez-Colman et al., 2017).

Another striking example of spatial intercellular metabolic differences is observed during the asymmetric cell division of T cells. Upon T-cell activation by antigen-producing cells, Myc, a regulator of glycolysis and glutaminolysis (Dang, 2017; Wang et al., 2011), is asymmetrically partitioned into daughter cells, leading to a metabolic asymmetry (Verbist et al., 2016). It has been suggested that this Myc asymmetry is maintained by the differential activation of mammalian target of rapamycin complex 1 (mTORC1) between daughter cells via the asymmetric distribution of amino acid transporters during cell division. Functionally, daughter cells with high Myc levels show elevated glycolysis and glutaminolysis compared with cells that have low Myc levels and are more prone to differentiate into actively proliferating effector T cells than into memory T cells (Verbist et al., 2016). This metabolic switch to a high glycolytic state also has important functional consequences (discussed below).

Regulation at the subcellular level

Cellular energy metabolism is also highly compartmentalized at the subcellular level. Although glycolytic reactions are mediated by soluble glycolytic enzymes, classical studies have suggested that these enzymes are not uniformly distributed throughout the cytoplasm, but rather are assembled into protein complexes named glycolytic metabolons, which facilitate the channeling of glycolytic intermediates (Clarke and Masters, 1975; Kurganov et al., 1985; Menard et al., 2014). Whereas the assembly of a glycolytic metabolon has not yet been confirmed *in vivo*, it has been demonstrated that glycolytic enzymes bind to cellular structures, including the cytoskeleton and intracellular vesicles. In a striking case, glycolytic enzymes were shown to be sequestered in an organelle, the glycosome, in Kinetoplastea, a large group of flagellated free-living and parasitic protozoans (Szöör et al., 2014). Such subcellular compartmentalization of enzymes enables local and efficient energy production at sites of the highest energy demand, as well as rapid adaptation of metabolism to environmental changes.

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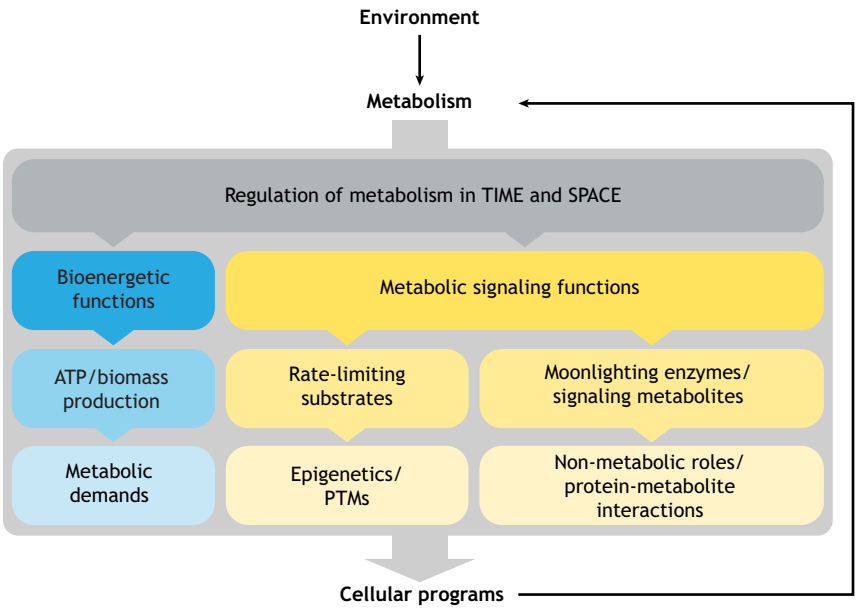

Fig. 2. Roles of metabolism: bioenergetic functions versus metabolic signaling functions. Scheme to categorize the distinct roles of metabolism, which can be regulated in time and space. Bioenergetic function: we define this as canonical metabolic activity providing energy and/or cellular building blocks to cover cellular demands, which differ within/across cells and tissues and dynamically change over time. Metabolic signaling: metabolic pathways are also known to exert signaling functions via diverse mechanisms, e.g. metabolic substrates can act as rate-limiting factors in modulating epigenetic modifications and protein PTMs, thereby affecting gene expression and signaling activities, respectively. Moreover, metabolic enzymes and metabolites can exert direct signaling functions in numerous ways, for example through non-metabolic moonlighting functions or via metabolite-protein interactions. Bioenergetic and metabolic signaling functions are, therefore, tightly and reciprocally linked to cellular and/or developmental programs, and also integrate environmental cues.

Subcellular compartmentalization of glycolysis is also prominent in neurons with arborization (branches). For example, the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) has been found to be anchored to intracellular vesicles (Zala et al., 2013). The downstream glycolytic enzyme phosphoglycerate kinase (PGK) also localizes to vesicles, and this is essential for local ATP production and axonal transport of vesicles (Zala et al., 2013). In addition, glycolytic enzymes have been found to form clusters near presynaptic sites to facilitate synaptic vesicle cycling upon energy stress (Jang et al., 2016).

Further examples of subcellular compartmentalization of glycolysis are found during blood vessel sprouting, in which glycolytic enzymes and activity are enriched in lamellipodia at the leading edge of migrating endothelial tip cells. The subcellular localization of these enzymes to lamellipodia generates ‘ATP hotspots’ that are proposed to meet the high energy demands associated with actin remodeling and cell motility (De Bock et al., 2013). Dynamic remodeling of the actin cytoskeleton is also accompanied by mobilization of active aldolase A from F-actin, thereby coupling acceleration of glycolysis to actin remodeling (Hu et al., 2016).

Temporal regulation of metabolism across scales

Cellular energy metabolism can be compartmentalized not only in space but also in time, over various time scales. A well known example includes the coordination of energy metabolism with the cell cycle in order to meet specific bioenergetic demands of each phase of the cell cycle (Salazar-Roa and Malumbres, 2017). At the G_1/S transition of the cell cycle, glycolysis is activated and mitochondria show hyperfused morphology, with greater ATP output than in any other cell cycle stages (Almeida et al., 2010; Bao et al., 2013; Mitra et al., 2009; Tudzarova et al., 2011). Mitochondrial respiration is also found to be enhanced at the G_2/M transition (Wang et al., 2014). These changes in energy metabolism during the cell cycle are mediated by the cell cycle machinery [e.g. cyclin-dependent kinases (CDKs) and E3 ubiquitin ligases]. Importantly, however, the link between the cell cycle and energy metabolism is bidirectional, with metabolic state functioning as a checkpoint of the cell cycle (Jones et al., 2005; Salazar-Roa and Malumbres, 2017).

Periodic metabolic rhythms are also linked to circadian clock activity, across the kingdoms of life (Eckel-Mahan et al., 2012; Qian and Scheer, 2016). In addition, circannual rhythms in metabolism are observed in hibernators (Dark, 2005). These rhythmic activity profiles of metabolism allow the coordination of physiology with external environmental cycles.

Finally, cells also show ultradian (i.e. shorter than 24 h period) rhythms and dynamics in metabolic activity, such as glycolytic oscillations, which were first identified in budding yeast decades ago (Ghosh and Chance, 1964; Richard, 2003). More recently it has been found that, during continuous culture in glucose-limited conditions, budding yeast exhibits robust cycles (with a period of ∼4 h) of oxygen consumption, designated the yeast metabolic cycle (YMC) (Cai et al., 2011; Tu et al., 2005, 2007). Interestingly, the iterations of oxidative and reductive phases are tightly coordinated with gene expression and cell proliferation/division (Cai et al., 2011; Papagiannakis et al., 2017). The YMC provides a striking example of the potential benefit of temporally compartmentalizing metabolic processes in order to enable optimal coordination with cellular programs (Tu et al., 2005). It has been shown that restricting DNA replication to the reductive phase minimizes the risk of oxidative DNA damage (Chen et al., 2007). Ultradian rhythms of metabolism have also been found in higher eukaryotic cells and in multicellular contexts. For example, glycolytic oscillations have been found in pancreatic β cells, and the link between these oscillations and pulsatile insulin secretion is being investigated (Merrins et al., 2013).

A direct consequence of spatiotemporal compartmentalization of metabolic activity is also that metabolite levels dynamically change over time and space. In turn, cellular levels of selected intermediate metabolites, such as acetyl coenzyme A (acetyl-CoA), can have a direct impact on epigenetic modifications, establishing an intriguing link between metabolic state and, for example, gene expression and cell signaling (see below). As technologies such as metabolite sensors (Paige et al., 2012; San Martín et al., 2014) and mass spectrometry imaging methods (Passarelli et al., 2017) keep improving at a fast pace, we expect that more examples of spatiotemporal compartmentalization of metabolism will be discovered in the coming years. Clearly, a major task will be to mechanistically link such spatiotemporally regulated metabolism to

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distinct cellular programs and, therefore, to developmental and/or physiological outcomes.

Metabolic regulation of cellular programs

In recent years, it has emerged that metabolic pathways, enzymes and products can influence cellular programs in numerous ways (Fig. 2). As we summarize in the following sections, these studies have revealed that links between metabolism and cellular processes can occur at various levels, ranging from effects on epigenetic modifications and PTMs, to regulation of mRNA transcription/translation and signaling pathways. This summary is certainly not complete and is intended to provide an overview. Reports on the impact of ROS in cell cycle progression (Han et al., 2018; Verbon et al., 2012) and embryonic segmentation (Ventre et al., 2015), and more generally the roles of redox signaling in stem cell biology and development, which have been already covered in several dedicated reviews (Coffman and Denegre, 2007; Shyh-Chang et al., 2013; Timme-Laragy et al., 2017), will not be discussed further here.

Metabolites as rate-limiting substrates for epigenetic and post-translational modifications

The intracellular concentrations of certain metabolites dynamically change over time and across cells and tissues, reflecting the spatiotemporal regulation of metabolic activity (Kochanowski et al., 2013). For several of these sentinel metabolites, it has been found that their levels impact downstream biochemical reactions and protein modifications, such as protein acetylation, glycosylation and methylation. For example, it has been shown that during the YMC, periodic changes in intracellular acetyl-CoA levels drive periodic changes in histone acetylation levels (Cai et al., 2011). Hence, acetyl-CoA is not only an important substrate for energy production by mitochondria, but its levels also impact the degree of epigenetic modifications occurring, providing an intriguing link between cellular metabolic state, epigenetics and gene expression (Cai et al., 2011). Similarly, the pronounced activation of glycolysis accompanying T-cell activation (Frauwirth et al., 2002; Wang et al., 2011) leads to increased acetyl-CoA and, therefore, histone acetylation levels (Peng et al., 2016). Again, it has been suggested that metabolic remodeling forms the basis of an epigenetic mechanism that promotes gene expression, including that of the proinflammatory cytokine interferon γ (Peng et al., 2016).

A related mechanism has also been observed in disease states. In cancer cells expressing a mutant form of isocitrate dehydrogenase (IDH), a TCA cycle-related enzyme, the oncometabolite 2-hydroxyglutarate (2-HG) is generated from α-ketoglutarate (αKG) (Dang et al., 2010; Ward et al., 2010). Because 2-HG is structurally similar to αKG, it acts as a competitive inhibitor of αKG-dependent enzymes including jumonji-C domain histone demethylases (JHDMs), which causes histone hypermethylation and promotes tumorigenesis (Lu et al., 2012). A recent study also showed the involvement of 2-HG in regulating the epitranscriptome (i.e. the chemical modifications of RNA) by inhibiting an αKG-dependent RNA demethylase (Su et al., 2018). Production of 2-HG can occur in cells with wild-type IDH upon exposure to hypoxia via malate dehydrogenase or via the promiscuous catalytic activity of lactate dehydrogenase (LDH) (Intlekofer et al., 2015; Oldham et al., 2015), thus affecting histone methylation (Intlekofer et al., 2015). The involvement of 2-HG in physiological development, as well as in pathological states, is discussed below.

Sentinel metabolites can also be rate-limiting factors for PTMs, by connecting the cellular metabolic state with cellular signaling pathways. For example, the hexosamine biosynthetic pathway (HBP) is connected with almost all central carbon metabolism pathways. The synthesis of the end product of the HBP pathway, UDP-acetylglucosamine (UDP-GlcNAc), requires glucose, glutamine, acetyl-CoA and UTP. Because levels of UDP-GlcNAc are found to mirror the cellular metabolic state, it has been suggested that UDP-GlcNAc functions as a metabolic sensor (Bond and Hanover, 2015; Hardivillé and Hart, 2014). It acts as the substrate for O-GlcNAcylation (the transfer of UDP-GlcNAc to a serine or threonine residue), which is known to often occur at, or proximal to, phosphorylation sites, thus competing with phosphorylation and affecting cell signaling outcomes (Bond and Hanover, 2015; Hardivillé and Hart, 2014). Along these lines, it has recently been shown that, in cancer cells, the TCA cycle enzyme fumarase is O-GlcNAcylated at the adenosine monophosphate-activated protein kinase (AMPK) phosphorylation site, impairing its interaction with ATF2, an AP-1 transcriptional factor family member (Wang et al., 2017). The importance of O-GlcNAcylation has also been reported in the context of *Caenorhabditis elegans* dauer development (Forsythe et al., 2006), *Drosophila* body pattern formation via polycomb proteins (Gambetta et al., 2009) and somatic cell reprogramming (Jang et al., 2012). These studies highlight that the modulation of PTMs by cellular metabolic state and levels of sentinel metabolites such as UDP-GlcNAc (Bond and Hanover, 2015; Hardivillé and Hart, 2014) can play a pivotal role in metabolic regulation of cell signaling.

Non-canonical moonlighting functions of metabolic enzymes

An additional distinct link between metabolism and cell signaling exists via the action of multi-functional or 'moonlighting' metabolic enzymes. Moonlighting enzymes are generally defined as those that carry out additional non-metabolic functions, for example in signaling or gene expression regulation (Jeffery, 1999). The discovery of moonlighting proteins has often been serendipitous but, more recently, guided approaches using, for example, protein localization information, have revealed a widespread occurrence of (potentially) moonlighting enzymes. Notably, essentially all glycolytic enzymes have been identified to be localized in the nucleus (Boukouris et al., 2016), raising the possibility that glycolysis might be active in the nucleus and/or that some of these proteins fulfill nuclear functions that are distinct from their roles in glycolysis (Boukouris et al., 2016; Yu and Li, 2016). Although evidence for the former possibility is still scarce, there are numerous and convincing studies that identify non-metabolic nuclear functions of glycolytic enzymes (see Boukouris et al., 2016 for a comprehensive overview).

For example, a specific splice isoform of pyruvate kinase, PKM2 (Christofk et al., 2008a), which is highly expressed in cancer cells, exhibits both enzymatic and non-enzymatic functions. As a glycolytic enzyme in the cytoplasm, PKM2 facilitates the flux of glycolytic intermediates into auxiliary glucose metabolism pathways, such as the PPP and one-carbon metabolism, in order to satisfy metabolic demands for biomass production (Anastasiou et al., 2011, 2012; Christofk et al., 2008a,b). At the same time, however, growth factor stimulation induces the translocation of PKM2 from the cytoplasm to the nucleus, where it acts as a transcriptional coactivator by interacting with β-catenin. PKM2 nuclear activity leads to increased transcription of cyclin D1 and *Myc*, which promotes cell cycle progression and glycolysis (Yang et al., 2011, 2012). Furthermore, because PKM2 expression is upregulated by Myc (Yang et al., 2012), this moonlighting function of PKM2 forms a positive feedback loop between glycolysis and growth factor signaling.

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Importantly, the link between moonlighting and canonical bioenergetic function can, in certain examples, be reciprocal (Jeffery, 1999). In these cases, metabolic activity has a direct impact on the ability of metabolic enzymes to engage in their moonlighting functions. One such example is GAPDH, which is an NAD^+-dependent dehydrogenase that not only functions as a canonical glycolytic enzyme, but also works as nuclear transcriptional co-activator (Zheng et al., 2003). Together with Oct-1 (also known as Pou2f1), GAPDH promotes histone H2B transcription during the S phase of the cell cycle (Zheng et al., 2003). The interaction between GAPDH and Oct-1 is modulated by the NAD^+/NADH redox status (Zheng et al., 2003), which provides a tight link between metabolic and moonlighting functions. Moreover, GAPDH has been identified as an RNA-binding protein, binding to the 3′-UTR of interferon γ mRNA and thereby suppressing its translation (Chang et al., 2013). During T-cell activation, cells switch to a highly glycolytic state and GAPDH becomes dedicated to glycolysis (NAD^+ binding). Concomitantly, its binding to the 3′-UTR of interferon γ mRNA is reduced, enabling efficient interferon γ translation. Mechanistically, this can be explained by the finding that the RNA-binding and NAD^+-binding domains of GAPDH overlap (Nagy et al., 2000). The post-transcriptional regulation of interferon γ expression is, therefore, directly linked to cellular metabolic state, providing another example of the intricate interconnection between cellular metabolic activity and gene expression via the moonlighting functions of glycolytic enzymes.

The growing list of documented moonlighting enzymes (Boukouris et al., 2016) bears intriguing potential to provide direct mechanistic insights into how metabolism is linked to cellular and/or developmental programs. For example, the number of RNA-binding metabolic enzymes that have been discovered has dramatically increased over recent years, with virtually all glycolytic enzymes identified as RNA-binders, from yeast to mammalian systems (Beckmann et al., 2015; Castello et al., 2012). Although the extent to which RNA-binding activity is dependent on metabolic activity remains to be tested for individual enzymes, the abundance of metabolic enzymes with RNA-binding properties has led to the suggestion of 'REM (RNA, enzyme, metabolite) networks' (Castello et al., 2012), in which metabolism and gene expression are mutually linked via moonlighting functions. Despite this progress, the roles of moonlighting enzymes in developmental contexts are only beginning to be explored (Pegoraro et al., 2015; Teixeira et al., 2015). One experimental challenge that remains is the dissection of canonical (i.e. bioenergetic) from non-canonical (i.e. moonlighting) functions. With an increase in mechanistic insight into the working principles of moonlighting proteins, new possibilities and refined approaches to overcome previous limitations become available. We therefore expect that the study of moonlighting proteins during development will be an attractive and interesting future field of research.

Metabolites as signaling molecules

Finally, yet another mechanism by which metabolism and cellular functions are linked is via the action of metabolic intermediates that harbor direct signaling functions. A classic example is xylulose 5-phosphate, a PPP intermediate that was shown to activate protein phosphatase 2A (Nishimura and Uyeda, 1995). Along related lines, several recent reports have provided support for the notion that metabolic intermediates are not only substrates for downstream biochemical reactions, but can also directly act as signaling molecules per se. One such notable 'moonlighting metabolite' is lactate, the end product, and therefore indicator, of glycolytic activity (Chang et al., 2015; Ho et al., 2015; Lee et al., 2015). Although lactate is often regarded as a metabolic waste product, it can itself act as a carbon source (Hui et al., 2017) and as a signaling molecule that mediates, for example, prolonged hypoxic responses by stabilizing a protein called NDRG3 (Lee et al., 2015).

Another glycolytic intermediate, fructose 1,6-bisphosphate (FBP), has also been suggested to exert moonlighting functions (Feng et al., 2014; Peeters et al., 2017; Zhang et al., 2017). For example, a recent study has shown that a decrease in FBP levels upon glucose starvation triggers acute AMPK activation in an aldolase-dependent manner (Zhang et al., 2017). Another study revealed that activation of the Ras proto-oncogene by increased glycolytic flux is mediated by direct binding of FBP to Cdc25/Sos1 (Peeters et al., 2017). These moonlighting functions of FBP are particularly interesting, as intracellular FBP levels are considered to be a glycolytic flux indicator and so reflect metabolic activity (Kochanowski et al., 2013; Zhang et al., 2017).

In summary, these recent studies further highlight the intimate and extensive link between energy metabolism and cellular programs (Fig. 2). As we obtain a better understanding of the molecular mechanisms that connect metabolism and signaling, we can now move from largely correlative to more functional studies. It is, therefore, a particularly promising era in which to reconsider how metabolic pathways, enzymes and products affect multiple aspects of animal development.

The emerging roles of metabolism during development

The investigation of the roles of metabolism in development has a long and complex history (reviewed by Blackstone, 2006; Witkowski, 1987). Pioneering studies by leading embryologists during the first half of the 20th century revealed striking correlations between metabolic activity and developmental potential (Child, 1941). These early studies already indicated that metabolic activities show remarkable spatiotemporal regulation during development, raising the possibility that metabolic gradients could be linked to developmental patterning (Child, 1925, 1943). Similarly, in a long series of studies, Nelson T. Spratt performed a detailed analysis of the nutritional requirements of early chick embryos by using defined *in vitro* culture conditions. By either varying carbon sources or by manipulating metabolism using chemical inhibitors, he found evidence showing 'differences in the metabolic processes underlying the formation and maintenance of the two different germ layer derivatives' (Spratt, 1950). These pioneering early studies, however, suffered from two constraints: the description of metabolic activities remained coarse grained and, importantly, a mechanistic insight into the cause-consequence relationship and evidence for an instructive function of metabolism remained largely elusive. As we gain a better understanding of not only the intricacies of metabolic pathways, but also their integration with other cellular processes, it is an exciting prospect to now link metabolic and developmental programs at a more mechanistic and functional level (Krejci and Tennessen, 2017). In the following sections, we discuss concrete examples that touch upon two key questions. First, how is metabolic activity coordinated in time and space with developmental programs and, second, what function does metabolism play in these developmental contexts?

Temporal control of metabolism during embryonic development

Recent technological advances in mass spectrometry offer opportunities to describe metabolic transitions during development

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with unprecedented resolution. Evidence is accumulating that suggests the rewiring of the cellular metabolic state is tightly coupled with the progression of embryonic development. Despite this, however, relatively little is known about the intrinsic and extrinsic cues that regulate the timely induction of metabolic changes in developing embryos.

It is known that the cellular metabolic state is closely linked to substrate availability, and that alterations in the growth environment can thus trigger metabolic changes. Indeed, during mammalian development, the rewiring of embryonic energy metabolism often accompanies alterations in oxygen availability (Clough, 1985; Dunwoodie, 2009). It is thought that oxygen availability in mouse embryos decreases around the implantation stage (Fischer and Bavister, 1993). At this stage, exposure to hypoxic conditions may lead to the stabilization of hypoxia-inducible factors (HIFs) for the induction of metabolic rewiring, as is observed *in vitro* during the transition from embryonic stem cells (ESCs) to epiblast stem cells (EpiSCs) (discussed below) (Zhou et al., 2012). After implantation, maternal oxygen supply to embryos increases, facilitated by placenta development and angiogenesis, and such environmental changes seem to trigger the acceleration of OXPHOS (Lange et al., 2016; Miyazawa et al., 2017; Morriss and New, 1979).

In addition to these extrinsic environmental changes, intrinsic developmental programs play important roles in the coordination of development and metabolic remodeling. For example, even in *in vitro* culture with atmospheric oxygen conditions, ESCs and EpiSCs show distinct metabolic signatures, with the latter exhibiting a higher glycolytic activity, induced by the activity of HIFs (Zhou et al., 2012). The extent to which this *in vitro* metabolic remodeling mimics the situation *in vivo* remains to be fully investigated. Intrinsic regulation of cellular metabolism is also observed during mammalian development *in vivo*. Developing embryos redirect glucose carbon flow into the PPP via suppression of aldolase and PFK-1 following placental development (chorioallantoic branching) (Miyazawa et al., 2017). This metabolic rewiring is accompanied by downregulation of Lin28a (Balzer et al., 2010; Miyazawa et al., 2017), an RNA-binding protein that is associated with pluripotency and stem cell self-renewal (Tsialikas and Romer-Seibert, 2015). It has been shown that Lin28a downregulation constitutes a developmental cue that controls metabolic rewiring in embryos that are undergoing placental development (Miyazawa et al., 2017; Shinoda et al., 2013). Metabolic transitions that are regulated by intrinsic developmental programs have also been identified in other developmental and/or organismal contexts (Agathocleous et al., 2012; Bulusu et al., 2017; Homem et al., 2014; Oginuma et al., 2017; Slaninova et al., 2016; Tennessen et al., 2011). It therefore appears that the timing of metabolic rewiring is coordinated with developmental progression via the integration of both extrinsic and intrinsic cues. Interestingly, metabolic rewiring can, in turn, feedback onto developmental processes, as discussed below.

Roles of the Warburg effect in development

The Warburg effect, which is observed in highly proliferating cancer cells (discussed above), is also widely observed in developing embryos (Agathocleous et al., 2012; Bulusu et al., 2017; Homem et al., 2014; Miyazawa et al., 2017; Oginuma et al., 2017; Slaninova et al., 2016; Tennessen et al., 2011). In fruit flies, for example, systemic metabolic remodeling is induced via the *Drosophila* estrogen-related receptor to activate aerobic glycolysis during mid-embryogenesis (Tennessen et al., 2011). It has been proposed that this switch to aerobic glycolysis sustains the dramatic increase in body mass observed during subsequent developmental larval stages. Similarly, proliferating neuroblast cells in the *Drosophila* larval brain are highly dependent on aerobic glycolysis, yet during their cell cycle exit and cell differentiation, metabolism switches from aerobic glycolysis to OXPHOS (Homem et al., 2014). Of note, this metabolic switch, which is dictated by signaling via the insect molting hormone ecdysone, is required for terminating the proliferative phase of neuroblast cells. A similar metabolic switch and role for aerobic glycolysis is also found during *Xenopus* retina development (Agathocleous et al., 2012). These examples support the idea that aerobic glycolysis underlies canonical proliferative metabolism, which facilitates biomass production, also during embryo development. However, as we highlight below, increasing evidence indicates that aerobic glycolysis plays not only a canonical role for biomass production, but also exerts specific signaling functions in diverse developmental contexts.

Glycolytic gradients linked to signaling gradients

The development of analytical tools to assess spatiotemporal patterns of metabolism is now paving the way for visualizing specific metabolic activities in developing embryos in time and space (Bailey et al., 2015; Bulusu et al., 2017; Miyazawa et al., 2017). Using such approaches, two recent papers provide evidence that, during axis development in vertebrate embryos, glycolysis might serve functions beyond its bioenergetic roles (Bulusu et al., 2017; Oginuma et al., 2017). These studies analyzed metabolic activity during the formation of somites, the precursors of muscle and vertebrae, which bud off periodically from the presomitic mesoderm (PSM). Whereas new somites form at the anterior end of the PSM, cells proliferate throughout the PSM and are also continuously produced from the posterior tail bud region, in which neuromesodermal progenitors (NMPs) reside (Hubaud and Pourquié, 2014; Wilson et al., 2009). In addition to the dynamic cell signaling that takes place within the PSM (Hubaud and Pourquié, 2014), it was found that metabolic activities are also highly regulated in a spatiotemporal manner within the PSM (Bulusu et al., 2017; Oginuma et al., 2017). By using fluorescence- and/or mass-labeled glucose and a Förster resonance energy transfer (FRET)-based pyruvate sensor mouse reporter line, these studies showed that glycolytic activity is graded along the PSM, with cells in the posterior, more undifferentiated PSM exhibiting higher (aerobic) glycolytic activity than those in the anterior PSM (Fig. 3). As cell proliferation was found to be uniform across the PSM, the high glycolytic activity in the posterior PSM appears not to be linked to cell proliferation activity per se. Interestingly, graded glycolytic activity found in the PSM *in vivo* reflects an intrinsic program, as this metabolic signature is also found to be established *de novo* in an *in vitro* two-dimensional segmentation assay system with uniform culture conditions (Bulusu et al., 2017). Furthermore, it was found that glycolytic activity is tightly linked to growth factor signaling (Oginuma et al., 2017); whereas graded fibroblast growth factor (FGF) signaling controls the expression of glycolytic enzymes, glycolytic activity in turn impacts the activity of another signaling pathway – the Wingless/Integrated (WNT) signaling pathway – in the chick PSM (Fig. 3). Combined, these activities constitute a closed regulatory network with reciprocal feedback between growth factor signaling and metabolic activity, in this case glycolysis (Oginuma et al., 2017). Excitingly, it was also shown that manipulating glycolytic activity altered the differentiation of NMPs, with the inhibition of glycolysis favoring differentiation into a neural fate rather than into a mesodermal fate (Oginuma et al., 2017). This is consistent with the positive effect of WNT signaling

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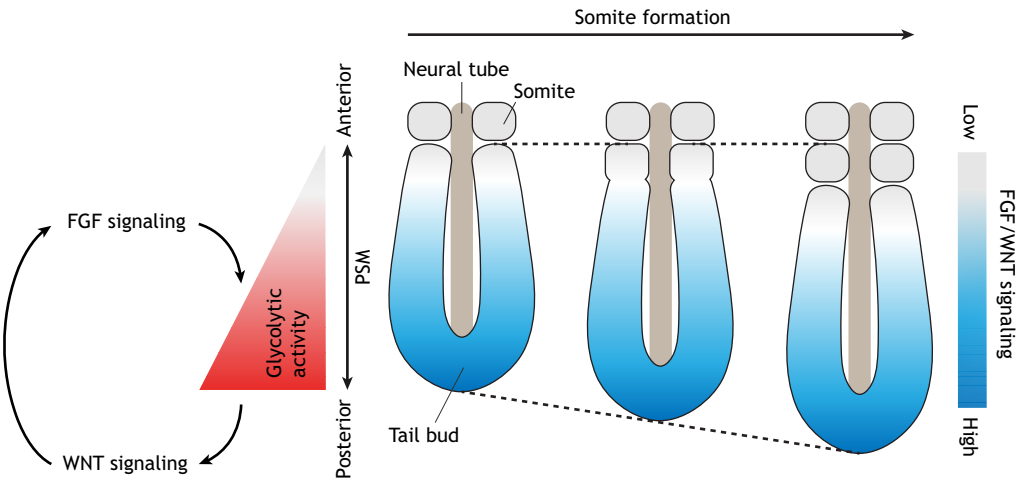

Fig. 3. Graded glycolytic activity in the presomitic mesoderm during vertebrate somite formation. A posterior-to-anterior gradient of glycolytic activity (red) is found in the PSM of vertebrate embryos during somite formation and axis elongation. The metabolic gradient correlates with FGF- and WNT-signaling gradients in the PSM. FGF signaling regulates glycolytic activity, which in turn increases WNT-signaling activity. This constitutes a closed regulatory network with reciprocal feedback between growth factor signaling and glycolytic activity.

on the mesodermal fate choice of NMPs (Kimelman, 2016). Although the precise molecular link between glycolysis and FGF/WNT signaling remains to be revealed, these studies suggest that aerobic glycolysis plays important roles in developmental signaling and differentiation processes.

Aerobic glycolysis linked to the regulation of gene expression in *Drosophila* larvae

Recent findings have also uncovered links between aerobic glycolysis and the regulation of gene expression, mediated by the metabolite 2-HG. Until recently, 2-HG was considered as an oncometabolite that accumulates only in disease states (as discussed above). For example, accumulation of 2-HG is a characteristic of 2-hydroxyglutaric acidurias, an autosomal recessive neurometabolic disorder that causes developmental delay, epilepsy and cerebellar ataxia (Steenweg et al., 2010). Excitingly, however, a recent study (Li et al., 2017) has highlighted the need to inspect the function of 2-HG and potentially other oncometabolites during physiological development. In this study, it was shown that 2-HG production occurs at the specific stage of development when aerobic glycolysis is promoted in *Drosophila* larvae during normal development (Li et al., 2017). It was further shown that 2-HG, which is produced in a specific window of development by the activity of glycolytic enzyme LDH, can function as a signaling molecule that affects heterochromatin formation, therefore providing a mechanistic link between metabolic, developmental and gene expression programs (Li et al., 2017).

Aerobic glycolysis during cell competition

Cell competition is a cell-cell interaction mechanism that leads to the selection of ‘winner’ cells at the expense of ‘loser’ cells (Di Gregorio et al., 2016). Cell competition has classically been studied in *Drosophila* wing discs, in which Myc-overexpressing cells (supercompetitors) induce the death of neighboring wild-type cells (de la Cova et al., 2004). It has been reported that these supercompetitor cells boost glycolysis dramatically in a non-cell-autonomous manner under competitive conditions (de la Cova et al., 2014). This enhanced glycolysis then promotes the proliferation of supercompetitor cells, which suggests that intercellular heterogeneity of metabolism facilitates cell competition. Further supporting a key role of metabolism during cell competition, it has recently been reported that non-cell-autonomous changes in mitochondrial and glycolytic activity underlie cell competition events in RasV12-transformed cells (Kon et al., 2017). Interestingly, in this context, higher glycolytic activity is required in loser cells for their elimination; the underlying mechanism of this remains unknown. Cell competition has also been described during mouse gastrulation, leading to the elimination of unfit epiblast cells (Clavería et al., 2013; Sancho et al., 2013), so it would be of particular interest to investigate whether metabolic changes also underlie cell competition in this context.

Metabolic regulation of epigenetics in development

A major remaining challenge is the identification of detailed mechanistic links between metabolism and development. One such mechanism that has been highlighted in several recent studies, and touched upon in some of the examples above, is the metabolic control of the epigenetic state during developmental processes. We anticipate that many more such examples will emerge in the near future, but below we highlight two developmental contexts in which clear links between metabolism and epigenetics have been described.

Metabolic control of epigenetics during cell differentiation

Stem cells primarily depend on glycolysis and undergo a metabolic switch to OXPHOS during cell differentiation, whereas somatic cells activate glycolysis and suppress OXPHOS upon reprogramming (Mathieu and Ruohola-Baker, 2017). Remarkably, the remodeling of energy metabolism during somatic cell reprogramming precedes the upregulation of pluripotency marker genes (Folmes et al., 2011; Kida et al., 2015). Moreover, lineage-specific metabolic rewiring is essential for commitment to a specific lineage (Cliff et al., 2017; Zheng et al., 2016). These examples suggest the possibility that cellular energy metabolism plays an instructive role in cell fate decisions. Indeed, it has been shown that cellular metabolism controls cell differentiation via epigenetic regulation (Carey et al., 2015; Moussaieff et al., 2015). For example, active glycolysis in naïve ESCs facilitates histone acetylation by maintaining high intracellular acetyl-CoA levels, ultimately promoting the expression of pluripotent genes (Moussaieff et al., 2015). Accordingly, the suppression of glycolytic production of acetyl-CoA promotes early differentiation of ESCs, whereas supplementation of acetate, a precursor for acetyl-CoA, delays it. Levels of another TCA cycle metabolite, αKG, also affect the cellular epigenetic state of ESCs via regulation of αKG-dependent dioxygenases, such as JHDMs and TET family DNA demethylases. Naïve ESCs control the flux of glucose- and glutamine-derived

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carbons to maintain a high αKG to succinate ratio, facilitating DNA/histone demethylation and thus enabling maintenance of pluripotency-related gene expression (Carey et al., 2015).

Metabolic control of zygotic gene activation in mouse embryos

The tight regulation of metabolic programs has been well described in the context of early mouse development. Whereas pyruvate supports embryo development upon fertilization, glucose catabolism starts to be enhanced at the eight-cell stage (Leese and Barton, 1984). The inhibition of glycolysis occurs at the level of the hexokinase and the PFK-1 reactions (Barbehenn et al., 1978) and multiple mechanisms have been implicated (reviewed by Dumollard et al., 2009). Interestingly, although early embryos require pyruvate as a carbon source for normal development, they only oxidize a fraction of the pyruvate into carbon dioxide for energy production (Brown and Whittingham, 1991; Lane and Gardner, 2000). The physiological roles of this particular metabolic state remained unclear for a long time. Excitingly, a recent study has now linked this unique metabolic state to the regulation of epigenetic changes that are associated with embryonic (zygotic) gene activation (EGA/ZGA) (Nagaraj et al., 2017). This study revealed that, in developing mouse embryos, the mitochondrial enzymes that are responsible for the generation of acetyl-CoA and αKG are also transiently localized to the nucleus during the cleavage stages (Fig. 4) (Nagaraj et al., 2017). In particular, it was shown that, whereas the mitochondrial pyruvate dehydrogenase complex (PDH), which is a rate-limiting enzyme complex that regulates entry of pyruvate into the TCA cycle, is phosphorylated and thereby inhibited in cleavage-stage embryos, nuclear PDH remains unphosphorylated and active, suggesting subcellular compartmentalization of PDH activity. It was also reported that αKG, which appears to be actively produced in the nucleus, is required for proper EGA/ZGA by impacting epigenetic histone modifications (Fig. 4). Overall, this study provides another example of how the remodeling of metabolism, epigenetics and gene activity is tightly interlinked during the early stages of mammalian development.

Conclusions and perspectives

In this Review, we have highlighted the extensive connections between central carbon metabolism and cellular programs, such as gene regulation and cell signaling, in several developmental contexts. However, a major future task will be to demonstrate a clear causal relationship between metabolism and cellular and/or developmental programs. To address the often complex and interconnected roles of metabolism, we think the categorization into bioenergetic and metabolic signaling functions (Fig. 2) could be useful. Therefore, we can first ask whether, in a given context of interest, metabolic activity primarily serves to cover particular cellular metabolic demands (i.e. in a bioenergetic role) or whether

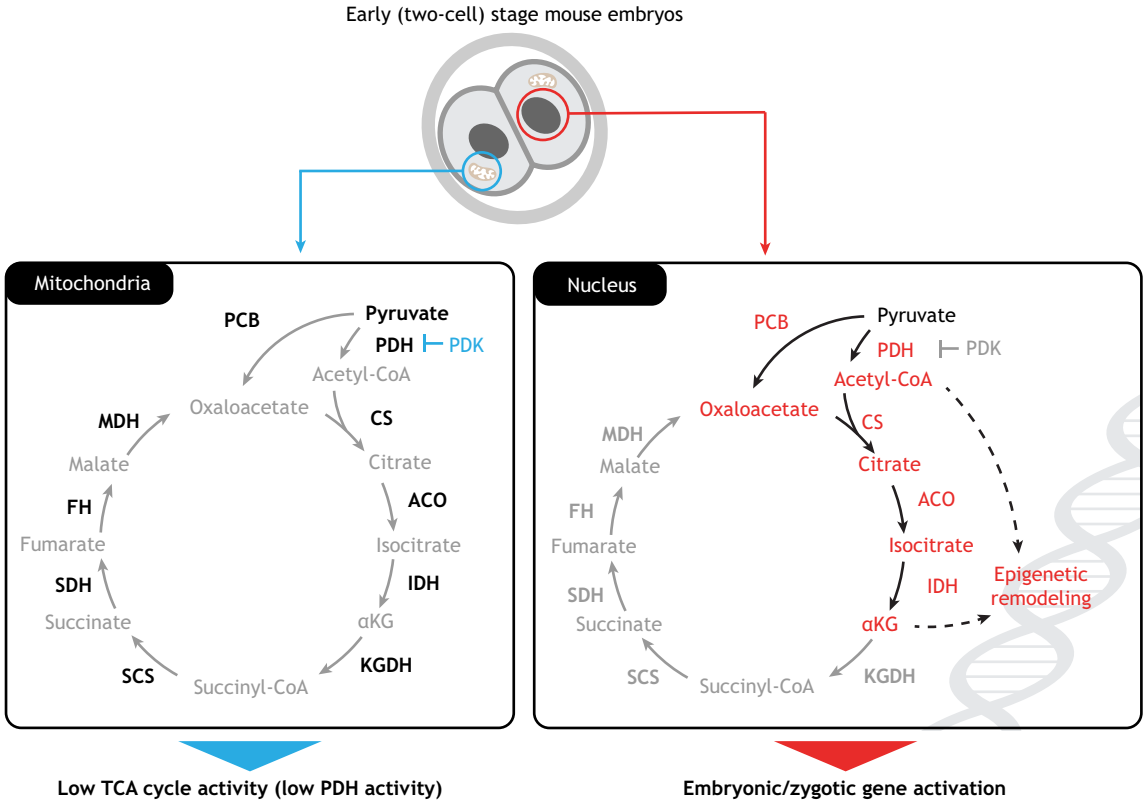

Fig. 4. Metabolic regulation of epigenetics during embryonic/zygotic gene activation. During the early stages of mammalian embryo development, TCA cycle enzymes involved in the production of acetyl-CoA and αKG localize to the nucleus as well as to mitochondria, whereas other TCA cycle enzymes (marked in gray) are rarely detected in the nucleus. Importantly, PDH, which regulates the entry of pyruvate into the TCA cycle, is in an active state in the nucleus, whereas it is inactivated by pyruvate dehydrogenase kinases (PDK) in mitochondria. This allows for nuclear production of TCA cycle metabolites (e.g. acetyl-CoA, αKG), which are used for epigenetic modifications and hence promote embryonic/zygotic gene activation. ACO, aconitase; CS, citrate synthase; FH, fumarate hydratase; IDH, isocitrate dehydrogenase; KGDH, α-ketoglutarate dehydrogenase; MDH, malate dehydrogenase; PCB, pyruvate carboxylase; SCS, succinyl-CoA synthetase; SDH, succinate dehydrogenase.

metabolic enzymes and/or products also have signaling functions. To obtain answers to these questions, the experimental strategy has to be aligned accordingly. This might entail manipulating metabolic activity in a specific subcellular compartment or, alternatively, specifically challenging the moonlighting functions of metabolic enzymes, for example by rescue experiments using catalytic-dead proteins. Combined with recent technological progress in metabolite sensors and analytic technologies (Paige et al., 2012; Passarelli et al., 2017; San Martín et al., 2014), this refined mechanistic approach will help to disentangle the bioenergetic and signaling functions of metabolism.

A particularly exciting research area will be the investigation of metabolic signaling when considering the impact of environmental conditions on developmental outcome. Across the animal kingdom, developing embryos show developmental plasticity that depends on environmental cues, e.g. nutrients and temperature (Gilbert and Epel, 2015). As environmental cues directly impact on metabolic activities, it is likely that a comprehensive mechanistic understanding of the link between metabolism and developmental programs will also reveal the basis for environmentally induced and/or modified phenotypes. Whereas the ability of developing embryos to react to an environmental input with a change in phenotype varies, depending on the species and context, environmental and metabolic cues likely play a vastly understudied role in most developmental settings. Phenotype, as already defined by Johannsen in 1909, integrates both the genotype and the environment (Johannsen, 1909). Although the last few decades have brought enormous insight into the role of genotype, it appears that we now have the possibility to close a major gap and reveal how, and to what extent, environment and metabolism impact on developmental outcome and phenotype.

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