The role of metabolic states in development and disease

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Abstract

During development, animal cells must adopt distinct metabolic strategies to support growth, produce energy and to synthesize and secrete key products. Some of these metabolic states maintain a very constrained program of nutrient utilization regardless of the external environment, while others allow for tremendous metabolic flexibility in support of homeostasis as a means to couple developmental progression with nutrient availability. Here we discuss recent advances in our understanding of metabolic programs, and how they support specific aspects of animal development. During phases of rapid proliferation such as during embryogenesis or during an immune response, a general metabolic program that includes aerobic glycolysis and glutaminolysis provides the building blocks and essential metabolites to support growth. As development progresses, metabolic programs are modified to support the unique demands of the each mature tissue. These shifts are typically induced by changes in hormone signaling and mediated by nuclear receptors that can themselves sense nutrients. Finally, we discuss how model systems, such as *Drosophila* egg production, provide a versatile platform for the discovery of novel mechanisms that control changes in metabolism during development.

Introduction:

All metazoan cells must produce molecular building blocks and energy to sustain homeostasis, to grow when required, and to support mature tissue function. After decades of research, the genes, proteins and biochemical transformations that sustain catabolism and biosynthesis of many of the core metabolites within many cell types have been defined. Recent studies have begun to show that metabolic pathways are dynamically regulated in order to support developmental progression. While changes in metabolism during differentiation are now well documented, only recently has the relationship between metabolism and development begun to be elucidated. Developing animal cells transition through distinct metabolic states that transiently meet their energetic and biosynthetic demands [1–6]. In this review, we define the concept of a metabolic state and discuss how shifting between metabolic states supports specific demands of development. Moreover, we discuss how corrupting these developmental metabolic mechanisms may contribute to several aspects of human disease.

Metabolic States: A Balance Between Energy and Biosynthesis

The metabolic demands of a cell are a balance between its energetic demands and its biosynthetic requirements to support cellular function. Cell types requiring high-energy

production adopt a metabolic state that directs most nutrient flux into ATP production. For example, cardiac muscle consumes large amounts of fatty acids and glucose, which it then channels through fatty acid β -oxidation [7, 8] and glycolysis [9] to drive mitochondrial oxidative metabolism. Numerous studies have shown that the heart produces 70–80% of its ATP from lipids while glucose and other nutrients account for the remaining 20–30%. This type of flux produces large amounts of ATP to fuel heart contraction while maintaining enough biosynthetic capacity to ensure muscle homeostasis (Figure 1).

In contrast, other cell types display pronounced capacity for biosynthetic conversion of nutrients into cellular building blocks. Hepatocytes take-up carbohydrates, amino acids, as well as circulating lipids and use these nutrients to produce lipoprotein particles, serum proteins, and glucose while at the same time generating sufficient ATP [10] [11]. The liver then secretes these products in response to hormonal signals to maintain the levels of circulating nutrients that sustain peripheral tissue in the body (Figure 1).

While cardiomyocytes and hepatocytes represent opposite ends of the spectrum with regard to energy production and biosynthetic capacity, most cell types fall somewhere in between. Some cell types fulfill highly specialized biosynthetic roles to fulfill, such as CNS neurons and B-cells of the immune system, that channel nutrients into the production of neurotransmitters and antibodies respectively [12, 13] [14]. Each cell's mission impacts its balance of energy production and biosynthesis, highlighting the substantial diversity in metabolic states observed between different cell types.

Flexible vs constrained metabolic states

The metabolic state of many cell types is inherently flexible, allowing them to adjust to dynamic changes in metabolic resources and demands resulting from circadian cycles and environmental stresses [15]. For example, the liver responds to signals such as insulin generated by feeding and promotes the absorption of nutrients such as glucose, lipids, and amino acids. Conversely, under fasting conditions ghrelin and glucagon levels increase, and insulin levels drop leading to a dramatic shift in hepatocyte metabolic flux [16]. Upon fasting, hepatocytes cease lipid synthesis and begin to consume lipids by fatty acid oxidation for ATP production [17, 18]. Interestingly, fasting strongly suppresses glycolysis and induces glucose synthesis from amino acids through gluconeogenesis [19, 20]. This allows the liver to synthesize and secrete glucose into the circulation, which maintains blood sugar to feed tissues such as the CNS.

In contrast, certain cell types must maintain their metabolic state despite changes in dietary nutrient to ensure normal cellular function. Numerous studies have shown that CNS neurons are exceptionally dependent on glucose for ATP production and biosynthesis. Neurons typically absorb large amounts of glucose as well as lactate, and amino acids (glutamine particularly) from the circulation to generate ATP from glycolysis and oxidative phosphorylation [21–23]. The neuron's inflexible metabolic program may result from the relatively invariant demands on these cells to produce neurotransmitters. Neurotransmitters, such as Acetylcholine, GABA, and glutamate, are synthesized from TCA cycle intermediates as well as amino acids. As a result, the metabolic state of neurons must

provide these intermediates with little variation. Not surprisingly, neurons maintain their metabolic program upon fasting. If fasting is prolonged sufficiently to impact circulating glucose and amino acids, neurons compensate by increasing the uptake and utilization of ketone bodies [16, 23]. Ketone bodies, acetoacetate and β -hydroxybutyrate, are converted to acetyl-CoA, which is used to produce ATP and supplement glucose as a source of fuel [23, 24] (Figure 2).

In addition to the production of neurotransmitters, the constrained metabolic state of neurons may also be important for other aspects of neuronal function. Neurons live longer than other body cells and their reliance on glycolysis may facilitate cellular longevity by minimizing the accumulation of oxidative damage.

The presence of cells with both flexible and constrained metabolic programs provides great benefit to the organism. A constrained metabolic state likely allows cells to adopt highly specialized roles that come with precise biosynthetic and energetic demands. The metabolic flexibility of other cells, such as hepatocytes (Figure 2) ensures that constrained tissues like CNS neurons and cardiac muscle continue to receive the nutrient fuel they require regardless of dietary conditions. Interestingly, recent studies of mammalian muscle tissue argue that a loss of metabolic flexibility underlies the pathologies associated with metabolic syndrome [25–27]. A better understanding of the interplay between cells with flexible and constrained metabolic programs will likely provide key insights into the molecular basis for human diseases that display a strong metabolic component.

Metabolic states that drive growth and proliferation.

During development cells undergoing rapid growth and proliferation require large amounts of amino acids, nucleotides, and lipid building blocks. As a result, many actively proliferating cells utilize a metabolic state known as aerobic glycolysis (Warburg metabolism) that relies upon a unique subset of metabolic mechanisms to provide the raw material for growth [28, 29] [30]. Glycolysis is the process where glucose is metabolized through series enzymatic reactions into pyruvate. This process generates ATP for energy as well as the electron carrier NADH that functions as a key substrate for many biosynthetic reactions. With conventional glycolysis, the resulting pyruvate is transported into the mitochondria where it feeds the TCA cycle to produce energy. In contrast, cells actively growing via aerobic glycolysis increase glucose uptake and glycolytic flux but convert the resulting pyruvate into lactate and secrete it from the cell. This strategy supports pentose phosphate shunt activity, which generates large amounts of the NADPH (an electron carrier used by many biosynthetic reactions) and helps regenerate cytosolic NAD⁺ (Figure 3). Thus, aerobic glycolysis allows cells to produce enough ATP while satisfying the high demands for carbon and NADH required to generate the building for growth [31, 32]. While aerobic glycolysis has been best characterized in cancer cells [33] and in highly proliferative *in vitro* cell culture models [32] recent studies have begun to define its role *in vivo* during development.

Recent studies of metabolism during *Drosophila* development documented the importance of aerobic glycolysis in larvae during the period when mass increases at a maximal rate [1, 2].

The switch to aerobic glycolysis is triggered during mid-embryogenesis by the nuclear receptor, *ERR*, which upregulates the expression of multiple genes in the glycolytic and pentose phosphate pathways. *ERR* mutants fail to establish a state of aerobic glycolysis, grow poorly and arrest at the 2nd larval stage [1, 2]. These studies demonstrate that aerobic glycolysis functions normally during development to support rapidly growing tissues. Thus, cancer cells may acquire the metabolic state that supports their rapid growth simply by re-activating a normal metabolic program.

Selecting a metabolic program of rapid growth can have dramatic consequences on cellular interactions and cell survival. Undifferentiated adult progenitor cells within developing imaginal discs elevate glycolysis during mid-embryogenesis as they begin a period of rapid proliferation [34]. During imaginal disc growth, cells compete with each other to promote tissue growth. Cells with high *Myc* expression upregulate glycolytic enzymes leading to increased glucose uptake and glycolytic flux. High levels of *Myc* expression induces aerobic glycolysis and allows them to grow more rapidly compared to cells with relatively low *Myc* expression. This competitive growth advantage conferred by *Myc* may also contribute to the selective elimination of the slower growing cells [34]. Thus, switching to a state of aerobic glycolysis not only drives growth and proliferation but may indirectly lead to the elimination of less functional cells from the population.

Mammals also use aerobic glycolysis to promote cell proliferation during adulthood. During an adaptive immune response helper T-cells become active and must proliferate rapidly prior to secreting cytokines. T-cell activation and expansion are highly dependent on the T-cell's ability to adopt a aerobic glycolysis-like metabolic state [35, 36]. Upon T-cell activation, *Myc* and *ERRα* expression increases and the mTOR pathway is also up-regulated. These factors induce high levels of aerobic glycolysis and pentose phosphate shunt activity [37–39] [40]. Patients suffering from diabetes display an impaired immune response [41, 42], most likely because their T-cells cannot rapidly increase their glycolytic rate. Interestingly, both mTOR and *Myc* function downstream of insulin signaling providing a potential explanation for these links [18, 43, 44].

The roles of both *Myc* and *ERR* in promoting aerobic glycolysis appear to have been highly conserved as these transcription factors control growth and similar metabolic shifts in mammalian cell culture models [45, 46] [40–43]. Moreover, in many cancer cells, *Myc* [46] [44] and *ERRs* [47, 48] reactivate a state of aerobic metabolism whose normal function would be likely limited to periods of rapid cell growth during development.

By utilizing aerobic glycolysis for growth proliferating cells become dependent on high levels of the glutamine, compared to other amino acids, a process called glutaminolysis. In proliferating cells, when aerobic glycolysis is highly active, pyruvate is converted to lactate and no longer feeds the TCA cycle. Under these conditions, glutamine absorbed from the circulation is converted into glutamate, transported into mitochondria, and used to feed the TCA cycle to compensate for decreases in available pyruvate [49]. A significant portion of the metabolic flux from glutaminolysis is processed through malate and pyruvate to produce lactate. Glutaminolysis also allows citrate to be exported from the mitochondria to help drive the synthesis of fatty acids and other lipid species. Thus, glutaminolysis synergizes with the

metabolic changes inherent to high aerobic glycolysis and helps produce a metabolic state that can efficiently drive rapid growth (Figure 3).

Increasing evidence suggests that during development and in adulthood, glutaminolysis is part of the normal metabolic program that supports rapid growth, along with aerobic glycolysis. Both *Myc* and mTOR can stimulate glutaminolysis in normal tissues in the context of rapid growth and proliferation [50, 51]. Recent studies, of the adaptive immune system, show that glutaminolysis is required for growth and proliferation of B-cells and T-cells [52]. This suggests the activation of aerobic glycolysis and glutaminolysis together may be a common mechanism that supports the growth of many tissues. Moving forward, recent studies have proposed that dynamic shifts in mitochondrial function and single carbon metabolism may play an important role in T-cell activation [53, 54]. These studies suggesting that many other metabolic pathways outside of energy production play specific roles in growth and proliferation.

Transcriptional reprogramming of metabolic states during terminal differentiation.

After growth and proliferation have largely been completed tissue cells terminally differentiate and adopt their mature functions. This requires shifting away from a metabolic state that supports growth to an often very different metabolic program tailored to the demands of a particular mature tissue.

For example, as hepatocytes develop and mature they must adopt a metabolic state that supports the diverse functions of the liver. As the early liver bud forms, hepatocyte precursors begin to express the nuclear receptor HNF4 α , which is often viewed as a master regulator of hepatic gene expression [55]. HNF4 α , like ERR, is a ligand-regulated transcription factor called a nuclear receptor. Nuclear receptors have a ligand-binding domain which in the case of HNF4 allows it to sense dietary nutrients such as fatty acids [56] [55]. Once activated HNF4 induces the expression of many factors required for the normal function of the liver such as genes involved in lipid metabolism, apolipoprotein synthesis, amino acid metabolism, gluconeogenesis, and xenobiotic detoxification [55, 57]. *HNF4a* mutants, which don't establish this metabolic state, display abnormalities in systemic glucose and lipid homeostasis [58, 59]. Interestingly, metabolic flexibility of the liver is likely in part result from the fact that HNF4 functions in concert with multiple other nutrient-regulated transcription factors such as *LXR*s [60, 61], *HNF1a* [62], *FXR* [63, 64], *Foxo*(1–3) [65, 66], *PPAR*s [67], and SREBPs [68] [69] to regulate subsets of the liver's diverse metabolic repertoire. Indeed, this network of transcription factors and the fact they are individually nutrient-dependent provides a model for how metabolic flexibility is established the molecular level.

Interestingly, studies of heart development have shown that during embryogenesis immature cells rely on glycolysis and have a fairly low levels of mitochondrial oxidative metabolism. This state supports growth during a time when the embryo is largely supplied by glucose from the mother. Later in embryogenesis the developing heart transitions towards its final

metabolic state, which is highly reliant on lipid oxidation and mitochondrial oxidative metabolism to produce the abundant ATP needed for cardiac function [70, 71].

Once again, *ERR* nuclear receptors, regulate metabolic specialization during tissue development [72]. *ERR* γ directly binds and regulates the promoter regions of many nuclear-encoded mitochondrial genes. Activation of *ERR* γ targets increases mitochondrial number, alters respiratory activity, and decreases lactate build up due to more efficient pyruvate utilization [71]. *ERR* γ mutants display excess lactate build-up, cardiac contractility defects, and die less than a week after birth [71]. Recent studies show that *ERR* γ promotes a very similar shift in mitochondrial oxidative metabolism during pancreatic beta cell development suggesting that *ERRs* reprogram mitochondrial metabolism during the development of multiple tissues [6]. In contrast to many nuclear receptors, *ERRs* appear to induce target gene expression independent of a ligand [73]. This suggests that *ERRs* are predominantly regulated at the level of expression and post-translation modification. The ligand-independent regulation of *ERRs* may contribute to the constrained metabolic state observed in the mammalian heart [72].

Some tissues, such as white and brown adipose tissue, require multiple steps to establish a mature metabolic state that supports tissue function. Early in development adipose progenitors transition to a metabolic program that supports lipid synthesis and storage [74–77]. Later, as brown and white adipose are specified, pre-adipocytes destined to become brown adipose increase their levels of mitochondrial oxidative metabolism via a PPAR γ /PRDM16 mediated shift in metabolic gene expression [5, 78]. In contrast, pre-adipocyte destined become white adipose suppress lipid oxidation through a PPAR γ /TLE3 mediated suppression of mitochondrial oxidative metabolism [4, 79]. Taken together, despite coming from a common developmental origin and utilizing several common factors during their differentiation, white and brown adipose acquire very distinctive metabolic states during the late stages of development.

Interestingly, recent studies have suggested that disruptions in mitochondrial function and the levels of cellular metabolites can influence differentiation in germline stem cells of the *Drosophila* ovary as well as *in vitro* mammalian cell culture models [80–83]. Metabolic intermediates such as NAD and acetyl-CoA are known factors required by specific classes of chromatin remodelers to mediate changes in histone tail modifications. This suggests that programmed changes in cellular metabolites may serve as regulatory cues to trigger progression through development. However, it remains unclear if these metabolites change sufficiently during *in vivo* development to function as instructive cues to regulate differentiation or if they are simply required permissively to allow for differentiation to occur.

***Drosophila* oogenesis: a model to dissect the links between metabolic states and development.**

Across all developmental processes gamete development is among the most sensitive to nutrition and systemic metabolism. This can easily be seen in humans where malnutrition and starvation cause a complete arrest in female reproductive cycles and a block in oocyte

development [84, 85]. In contrast, diabetes, obesity, and other metabolic syndromes are strongly associated with a wide variety of reproductive defects including polycystic ovary syndrome [86, 87]. Reproduction is likely to be so closely connected with nutrition and metabolism because oocytes, supplemented by the action of maternal trophic tissue, must acquire and store vast amounts of nutrients to support embryogenesis [3, 88–92].

Drosophila oogenesis provides an excellent system for investigating the connections between metabolism and reproduction. *Drosophila* females produce up to 100 yolk-rich eggs daily with nutrients, including protein, triglycerides, and glycogen, equal to half her body weight each day. The glycogen and triglycerides stored within the egg are consumed progressively during embryogenesis[2], and their utilization is essential for early development in many species [89, 90] [93] [94] [95] . To acquire an adequate nutrient depot, developing follicles pass through a fixed sequence of states, collectively known as vitellogenic stages, that provide tractable examples for studying how metabolic transitions are controlled.

The first step in nutrient loading involves the uptake of yolk proteins, a process controlled by a checkpoint requiring threshold systemic levels of insulin and the steroid hormone ecdysone [96] [97] . As follicles progress they next absorb and store large amounts of lipid from circulation [92] [98]. Lipid accumulation is stimulated locally in each follicle at the appropriate point by ecdysone produced in overlying somatic follicle cells [88] [99]. Ecdysone promotes lipid storage by inducing the activation of the highly conserved lipogenic transcription factor SREBP. Once activated SRBEP induces the expression of the LDL receptor homolog LPR2 that in turn facilitates lipid uptake [88, 98]. Interestingly, both steroid signaling and SREBP activation during oogenesis are regulated by dietary nutrients [88]. This provides a mechanism that allows an organism to coordinate the progression of egg development to nutrients levels and systemic lipid metabolism. Moreover, these studies provide a framework to understand the links between systemic metabolism and a number of reproductive diseases such as polycystic ovary disease and potentially ovarian cancer [86, 87] .

After lipid accumulation ceases, the oocyte prepares to stop all transcription and translation as it transitions into a quiescent state awaiting fertilization[100, 101]. During this same period, the follicle cells produce a multi-layered eggshell that cuts off further access to circulating nutrients. Intriguingly, during this transition, oocytes begin to store very large amount of carbohydrates in the form of glycogen [3, 102] . Glycogen accumulation is caused by a shift in the balance between glycolysis and gluconeogenesis [3]. Oocytes decrease glucose catabolism in part by decreasing the activity of the insulin-signaling pathway and, in turn, activating the serine-threonine kinase *GSK3*. Once activated *GSK3* induces a shift in mitochondrial protein content leading to remodeling of the electron transport chain (ETC) [3]. By remodeling the ETC the oocyte reduces mitochondrial oxidative metabolism, allows for glucose to accumulate, and ensures that stored nutrients are not consumed by the oocyte prior to fertilization. Maturing oocytes in many species undergo similar steps and end with low levels of mitochondrial oxidative metabolism [103, 104]. In addition, the transition to metabolic quiescence likely protects the oocyte nucleus from oxidative damage during storage, which can persist for up to two weeks. *GSK3*-mediated

ETC remodeling may be a conserved aspect of oocyte maturation in many species as well as a key step for generating a quiescent state in other cell types.

Studies of the *Drosophila* germline provide an ideal system to define the mechanisms that coordinate the establishment of metabolic states with the progression of cells through development. Each developing follicle is subjected to systemic and local hormonal signals that couple nutrient status of the animal with the metabolic transitions that occur during oocyte development. A similar diverse repertoire of metabolic states is likely to be present in many mammalian tissues. And disruptions in the ability to properly regulate the transition between states may provide the metabolic basis for many human diseases.

Intriguingly, studies of *Drosophila* oogenesis have found that metabolites will often be accumulated sequentially rather than synchronously. The fact that nutrients accumulate in distinct stages suggests that the metabolic states supporting the storage of particular nutrients are mutually incompatible. For example, cells synthesizing and storing large amounts of triglycerides require glucose to drive glycolysis to feed the TCA cycle and the pentose phosphate pathway to produce NADPH required to drive fatty acid synthesis. Under these conditions, glucose cannot be stored, at high levels, in the form of glycogen. Only when lipid synthesis is decreased can glucose be directed into glycogen storage to promote high levels of carbohydrate storage. Overall, studies of this system provide a platform for the discovery and characterization of novel mechanisms that regulate metabolic flux. Moreover, by understanding these types of dynamic changes in metabolism during *in vivo* development we can gain a more accurate picture of how these mechanisms may be corrupted in disease and contribute to the progression of conditions such as metabolic syndrome, neural degeneration, and cancer.

Conclusion

As cells undergo dramatic cellular and anatomical changes during development, cellular metabolism changes to support development. Highly conserved hormones and transcriptional regulators that date back to the evolution of multicellularity regulate these transitions in metabolic state. Many of these regulators are nuclear receptors that can sense nutrients directly. Intriguingly, it's possible that these transcription factors originally arose to allow free-living cells to adapt to different nutritional resources, by controlling a switch in cellular metabolism. By understanding how such regulatory pathways operate, we will likely gain a better understanding of how changes in cellular physiology drive the progression of a cell through development. Moreover, it allows us to determine if the metabolic basis for many diseases/disorders such as infertility, cancer, neural degeneration, and immune disease stems from dysfunctional regulation of developmental metabolic programs.

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Figure 1. Metabolic states: the examples of heart and liver

Diagrams show the major metabolic inputs and outputs of cardiac myocytes (A) and liver parenchymal cells (B). Cardiac cells primarily take up fatty acids and use them to generate ATP for muscle contraction. In contrast, liver parenchymal cells take up carbohydrates, lipids and amino acids from the circulation and convert them into a variety of outputs, including glycogen, fat, blood proteins and diverse biosynthetic products for storage or export to other organs throughout the body.

Figure 2. Flexible vs constrained metabolic states

This diagram depicts how nutrients are consumed by the hepatocytes(A) and neurons of the CNS (B). Upon fasting the highly flexible metabolic state of hepatocytes shifts dramatically and suppresses both glycolysis and the production of several biosynthetic products while at the same time activating gluconeogenesis. In contrast, upon fasting the constrained metabolic state of neurons changes very little by increasing the consumption of ketone bodies to compensate for reduced uptake of glucose.

Figure 3. Aerobic Glycolysis and Glutaminolysis work together to support rapid growth.

This diagram depicts a generalized metabolic program that supports growth. Under these conditions, aerobic glycolysis consumes glucose to produce cytosolic ATP for energy and NADH to feed main biosynthetic process in the cytosol. Upon activation of aerobic glycolysis carbon from glucose is shuttled into lactate production. To make up for the decreased pyruvate import into the TCA cycle glutaminolysis is activated to supplement the TCA cycle with carbon from glutamate. By supplementing the TCA cycle glutaminolysis helps maintain mitochondrial transport and support the biosynthesis of many compounds such as fatty acids and maintain transport in and out of the mitochondria.

Figure 4. Metabolic transitions during *Drosophila* germline development.

(A) The diagram shows the stepwise accumulation of yolk proteins, lipids, and glycogen during *Drosophila* oocyte development. (B) This diagram depicts how steroid signaling functions during mid-oogenesis to activate SREBP mediated lipid storage during stage 10 of oogenesis. After lipid loading during stage-10 AKT signaling is inactivated leading to a GSK3 mediated suppression of mitochondrial function and subsequent glycogen storage. (C) Depicts the how once activated GSK3 induces changes in the mitochondrial proteome that stimulate a remodeling of the ETC. The GSK3 mediate ETC remodeling cause mitochondria to enter a state of mitochondrial respiratory quiescence that suppresses the consumption of nutrients and promotes glycogen and lipid storage.