

Aerobic Glycolysis: Meeting the Metabolic Requirements of Cell Proliferation

Yun Kyung Kwon¹ and Matthew G. Vander Heiden^{1,2}

¹Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139; email: ykk@mit.edu, mvh@mit.edu

²Dana-Farber Cancer Institute, Boston, Massachusetts 02115

Shortened running title: The role of aerobic glycolysis

Corresponding Author contact information:

Matthew Vander Heiden

Massachusetts Institute of Technology

77 Massachusetts Avenue, 76-561

Cambridge, MA 02139

(617) 715-4523

mvh@mit.edu

Contents

Introduction

Aerobic glycolysis is not selected for increased ATP production

A major function of aerobic glycolysis is to support macromolecular synthesis

Why do proliferating cells excrete so much lactate?

How is enough NADPH generated to support cell proliferation?

Glutamine is also important for anaplerosis and ATP production

Metabolic reprogramming for proliferation

Upstream regulation of glycolysis

Pyruvate kinase influences the fate of glucose

Conclusions and perspectives

Key words: Warburg effect, cell metabolism, cancer metabolism, biosynthesis

Abstract

Warburg's observation that cancer cells exhibit a high rate of glycolysis even in the presence of oxygen (aerobic glycolysis) sparked debate over the role of glycolysis in normal and cancer cells. While it has been established that defects in mitochondrial respiration is not the cause of cancer or aerobic glycolysis, the advantages of enhanced glycolysis in cancer remain controversial. Many cells ranging from microbes to lymphocytes use aerobic glycolysis during rapid proliferation, suggesting it may play a fundamental role in supporting cell growth. Here, we review how glycolysis contributes to the metabolic processes of dividing cells. We provide a detailed accounting of the biosynthetic requirements to construct a new cell, and illustrate the importance of glycolysis in providing carbons to generate biomass. We argue that the major function of aerobic glycolysis is to maintain high levels of glycolytic intermediates to support anabolic reactions in cells, providing an explanation for why increased glucose metabolism is selected in proliferating cells throughout nature.

Introduction

All cells need a source of energy to maintain homeostasis. Cellular maintenance involves non-spontaneous, energy-consuming processes such as generation of concentration gradients, cytoskeletal dynamics, DNA repair, basal transcription and translation, protein turnover, and vesicle trafficking. The laws of thermodynamics mandate a continuous inflow of energy to balance these enthalpic (positive) and entropic (negative) requirements of cell maintenance, and cells must therefore extract free energy from nutrients or sunlight. While maintaining homeostasis, proliferating cells have additional energetic requirements to grow and divide. Thus, proliferating cells must acquire more nutrients, convert them into biosynthetic building blocks, and coordinate the reactions necessary to transform them into macromolecules essential for constructing a new cell. We have yet to fully understand how cells regulate biochemical pathways to: 1) allocate nutrients to provide free energy, mostly in the form of ATP, to allow otherwise unfavorable biochemical reactions; and 2) fuel the anabolic processes needed to grow and make new cells. By definition, cancer involves the inappropriate proliferation of cells, and the metabolic phenotype of cancer cells must represent a solution to how metabolic pathways are regulated to achieve a balance between ATP production and biomass production. However, this solution is not unique to cancer, as many phenotypes found in cancer cells also exist in normal proliferating cells and fast growing unicellular organisms.

A major source of cellular energy and new cell mass is glucose. Glucose is metabolized via glycolysis to pyruvate, which can be oxidatively metabolized to CO_2 in the tricarboxylic acid (TCA) cycle to generate large amounts of ATP through the process of oxidative phosphorylation. Pyruvate can also be reductively metabolized to organic acids or alcohols (e.g., lactate, acetate, or ethanol), a process known as fermentation. Glucose fermentation does not require oxygen,

but it is far less efficient in generating ATP. Despite decreased efficiency in ATP production, many fast growing unicellular organisms rely primarily on glucose fermentation during proliferation regardless of oxygen availability. For example, fermentative yeasts like *S. cerevisiae* (baker's yeast), which prefer to ferment glucose to ethanol even under aerobic conditions, grow faster when using fermentation rather than respiration (Rolland et al 2002). Although *S. cerevisiae* depend on oxidative phosphorylation in glucose-poor environments with only non-fermentable carbon sources, they immediately arrest respiration and accelerate glycolysis if glucose becomes available (Woehrer & Roehr 1981), a phenomenon known as the Crabtree effect (Crabtree 1929). Another well studied example is blood stage malaria parasites: they predominantly use glucose fermentation instead of oxidative phosphorylation for ATP production (Sherman 1998). When fed ^{14}C -glucose, malaria parasites do not generate a significant amount of $^{14}\text{CO}_2$, suggesting that the majority of glucose is not oxidized in the TCA cycle despite high oxygen concentrations in blood; instead, a large amount of ^{14}C -glucose is metabolized to ^{14}C -lactate (Bryant et al 1964). Blood cells infected with malaria also exhibit much higher glucose uptake than normal blood cells (Bryant et al 1964). While glucose fermentation is a common feature of many proliferating cells, it is not required for proliferation; aerobic yeasts such as *Y. lipolytica* rely solely on respiration (Christen & Sauer 2010), and *E. coli* do not undergo fermentation under aerobic conditions, only excreting the fermentative product acetate when glucose uptake exceeds a maximum respiration rate (Valgepea et al 2010, Xu et al 1999). Thus, evolution has devised more than one metabolic solution to support cell proliferation.

Like many unicellular organisms, multicellular organisms also exhibit enhanced glycolysis during fast growth. In 1924, Otto Warburg observed that cancer cells consume much

larger quantities of glucose than their normal counterparts and metabolize it predominantly through glycolysis, producing high levels of lactate even in oxygen-rich conditions (Warburg 1956, Warburg et al 1924). This phenomenon of aerobic glycolysis (i.e., conversion of glucose to lactate even in the presence of sufficient oxygen to support glucose catabolism via the TCA cycle with oxidative phosphorylation) is not exclusive to cancer cells. Many non-transformed cells also exhibit high aerobic glycolysis during rapid proliferation. In proliferating mouse fibroblasts, the rate of glucose uptake and lactate production is highest during logarithmic growth (Munyon & Merchant 1959). Increased glucose uptake and lactate excretion has been observed in mitogen-stimulated normal human lymphocytes (Hedeskov 1968), mouse lymphocytes (Wang et al 1976), and rat thymocytes (Brand 1985, Hume et al 1978). Further, no difference in lactic acid production was observed during optimal growth between normal and transformed chick embryo cell cultures (Steck et al 1968). These studies suggest that aerobic glycolysis is a common phenomenon among many proliferating animal cells.

In these rapidly dividing cells, most of the glucose is converted to lactate, and very little is oxidized to carbon dioxide. This observation led Warburg to suggest that cancer cells arise from a defect in mitochondrial respiration that causes them to rely on enhanced glycolysis. However, it is now clear that respiration is not impaired in most cancer cells (Fantin et al 2006, Moreno-Sanchez et al 2007, Zu & Guppy 2004). Aerobic glycolysis has been observed in a wide variety of tumors that originate from different cell types, but most normal cells in adult tissues from which cancer cells arise generally do not utilize aerobic glycolysis. Thus, cancer cells revert to a metabolic phenotype that is characteristic of rapidly dividing cells, suggesting that aerobic glycolysis must provide advantages during proliferation.

Aerobic glycolysis is not selected for increased ATP production

Glycolysis is inefficient in terms of adenosine triphosphate (ATP) production, generating only 2 ATP molecules per molecule of glucose, whereas complete oxidation of one glucose molecule by oxidative phosphorylation can generate up to a maximum of 36 ATP molecules (Berg et al 2007). Despite its low efficiency in ATP yield per molecule of glucose, aerobic glycolysis could generate more ATP than oxidative phosphorylation by producing ATP at a faster rate (Pfeiffer et al 2001). As long as glucose supply is abundant, an inefficient yet faster pathway for ATP production may be preferred, and one proposed advantage of aerobic glycolysis is faster ATP production to meet the high demands of dividing cells. However, glycolysis is not the major contributor of ATP in most cells: a compilation of data for 31 cancer cell lines/tissues from studies that determine oxidative ATP production (by measuring O₂ consumption) and glycolytic ATP production (by measuring lactate excretion) show that the average percentage of ATP contribution from glycolysis is 17% (Zu & Guppy 2004). This collection of data does not support the hypothesis that cancer cells exhibit aerobic glycolysis to generate ATP faster, since in many cancer cells the majority of ATP is generated through oxidative phosphorylation. It is important to note that the glycolytic ATP contributions are entirely dependent on the cell context and have a wide range (0.31 – 64%) depending on cell/tissue type and experimental conditions (Zu & Guppy 2004). Cells exhibit different metabolic phenotypes depending on the growth environment (e.g., hypoxia) and phase of the cell cycle. For example, aerobic glycolysis (as measured by lactate excretion) in mitogen stimulated mouse lymphocytes changes throughout the cell cycle, reaching a peak in S phase (Wang et al 1976). Also, budding yeast *S. cerevisiae* display robust, periodic cycles of glycolytic and respiratory metabolism in nutrient-limited conditions (Tu et al 2005).

ATP is likely not limiting in proliferating cells. Estimations of biosynthetic and maintenance energy requirements suggest that biosynthesis to produce a new cell is not the major consumer of ATP (Kilburn et al 1969). In fact, the rate-limiting step in glycolysis (i.e., phosphofructokinase) of Ehrlich ascites tumor cells was shown to be limited by the consumption, not production, of ATP (Scholnick et al 1973). Additionally, cancer cells utilize the less active M2 splice isoform of pyruvate kinase, which catalyzes the step in glycolysis responsible for net ATP synthesis (Mazurek et al 2005). Further, PKM2-expressing cells can uncouple the production of ATP from the conversion of PEP to pyruvate by using an alternate pathway (Vander Heiden et al 2010), suggesting that glycolysis can occur in proliferating cells without generating ATP.

An important challenge to understanding proliferative cell metabolism may be resolving how enough ATP is consumed to support the observed high rate of glycolysis (Racker 1976). Consumption of ATP is needed to prevent inhibition of key rate limiting steps in glycolysis by a high ATP to adenosine monophosphate (AMP) ratio. In particular, the phosphofructokinase step of glycolysis is sensitive to the ATP/AMP ratio and controls the entry of glucose metabolites into the downstream steps of glycolysis (Berg et al 2007). A recent study reports a mechanism by which cells increase ATP consumption in order to drive glycolytic flux (Fang et al 2010), supporting the notion that ATP consumption can increase glycolytic flux

ATP may not be limiting for growth, but it is still required by proliferating cells for both cellular maintenance functions and biosynthetic reactions. ATP is necessary to maintain homeostasis and keep cells alive; loss of intracellular ATP results in cell necrosis or apoptosis (Tsujimoto 1997). Additional ATP is necessary for cell proliferation: macromolecular synthesis ($X_n + X \rightarrow X_{n+1}$) is a thermodynamically unfavorable process that only occurs when coupled to

ATP hydrolysis (to provide free energy). Elongation of DNA, RNA, and proteins requires an NTP (or dNTP) for every unit added (e.g., amino acid + ATP + tRNA → aminoacyl-tRNA + AMP + PP_i), and when ATP is converted to AMP, two ATP molecules are required to regenerate ATP from AMP. ATP is also needed for many reactions that generate biosynthetic precursors. For example, as shown in Table 1, nucleotide and lipid biosyntheses require a significant amount of ATP.

It is important to remember that mitochondria are not defective in most proliferating cells. They are quite active and generate the majority of ATP in many normal and cancer cells (Zu & Guppy 2004). In fact, mitochondrial activity is enhanced in stimulated lymphocytes when compared with resting lymphocytes, and maximal activity correlates with peak DNA synthesis during S phase (Darzynkiewicz et al 1981). In breast cancer cells, knockdown of p32, a protein overexpressed in certain cancer cells, shifts metabolism from oxidative phosphorylation to glycolysis but impairs tumorigenesis, suggesting that enhanced glycolysis cannot support tumor growth without adequate oxidative phosphorylation (Fogal et al 2011). Further, cancer cells are susceptible to mitochondrial inhibitors, and mitochondria are emerging as chemotherapeutic targets (Fantin & Leder 2006, Neuzil et al 2007). It has been suggested that under conditions of hypoxia or fluctuating oxygen availability in which mitochondria cannot generate enough ATP, aerobic glycolysis may give tumors a growth advantage (Postovit et al 2002, Pouyssegur et al 2006). However, oxygen is not limiting for electron transport until levels are extremely low: even at an O₂ concentration of 25 μM, electron transport is reduced only by 33% (Chandel et al 1996).

ATP generated from aerobic glycolysis is undoubtedly important for cellular function and plays an important role in biosynthesis for at least some proliferating cells. However, as we

describe below, the importance of aerobic glycolysis for proliferating cells likely extends beyond rapid ATP production to also allow nutrient assimilation into biosynthetic precursors and facilitate biomass accumulation.

A major function of aerobic glycolysis is to support macromolecular synthesis

Generating new daughter cells requires the replication of all cellular contents, including DNA, RNA, proteins, and lipids. Glucose can provide the precursors for the chemical constituents (e.g., nucleotides, amino acids, and lipids) that are used to build macromolecules essential for cell division. Therefore, a main function of upregulated glycolysis in proliferating cells may be to maintain levels of glycolytic intermediates needed to support biosynthesis (Hume & Weidemann 1979, Vander Heiden et al 2009). Understanding the role of glycolysis during proliferation requires a thorough analysis of central carbon metabolism and its myriad connections to macromolecular biosynthesis. Figure 1 shows the connections between glycolysis and the generation of many biosynthetic precursors.

DNA and RNA, which are made of nucleotides, account for a significant portion of cell mass. DNA and RNA make up 24-28% of the total dry weight in *E. coli* (Lewin 1997, Neidhardt & Umberger 1996). This is mostly due to ribosomes, which make up approximately 30% of the mass of an *E. coli* cell (Lewin 1997), and this percentage is likely similar in eukaryotic cells, as ribosome structure and function are highly conserved across all organisms (Nakamoto 2009, Wilson & Noller 1998). By mass, the ribosome is composed of approximately 70% RNA in prokaryotes and 56-62% in eukaryotes (Morgan et al 2000). Thus, approximately 80% of the RNA synthesis in proliferating cells goes to generating ribosomes (Ecker & Kokaisl 1969) and represents the major site of incorporation for newly synthesized nucleotides.

Each purine nucleotide (ATP, GTP, dATP, and dGTP) synthesized by the cell requires the assimilation of 10 carbons from the extracellular environment (Figure 1 and Table 2). Half of the purine nucleotide carbon is derived from 5-phosphoribosyl- α -pyrophosphate (PRPP), an activated version of ribose-5-phosphate, which ultimately is derived from carbohydrate nutrients, with glucose being the major carbohydrate available to most animal cells. The activity of PRPP synthetase, an enzyme that converts ribose-5-phosphate to PRPP, increases 2-10 fold in lymphocytes following mitogen stimulation (Chambers et al 1974), highlighting the importance of increased PRPP generation for nucleotide biosynthesis during cell growth. Two carbons in purines come from glycine, which can come from the extracellular environment or be synthesized from the glycolytic intermediate 3-phosphoglycerate. Two molecules of N^{10} -formyl-tetrahydrofolate (N^{10} -formyl-THF), one of many folates that can accept or donate one-carbon units, provide 2 carbons in purine synthesis. Folates acquire most of their carbon units from either serine or glycine, both of which can be derived from 3-phosphoglycerate (folates can also obtain one carbon units from formate). Thus, through glycolysis, glucose is a significant carbon source for purine nucleotides, providing a minimum of 5 carbons and up to 9 carbons. Purine nucleotides can also be synthesized by salvage pathways (e.g., guanine + PRPP \rightarrow GMP + PP_i), but this requires an exogenous source of the purine base to allow a net increase in cell mass. Generating nucleotides via the salvage pathway still requires PRPP that is largely derived from glycolysis, but the salvage pathway considerably decreases the amount of ATP required. Inhibitors of *de novo* purine biosynthesis inhibit lymphocyte growth, suggesting purine salvage pathways are insufficient to support nucleotide synthesis (Hovi et al 1976). In addition to serving as building blocks of nucleic acids, purines are necessary for cofactor biosynthesis, as adenine is present in FAD(H), NAD(H), NADP(H), and coenzyme A (CoA).

Next, consider the biosynthetic precursors that provide the 9 carbons in pyrimidine nucleotides (CTP, UTP, dCTP, and dTTP; see Figure 1 and Table 2). Glycolysis is also a major source of carbons for biosynthesis of these nucleotides, as the majority (5 out of 9) of carbons comes from PRPP. Three of the remaining carbons in pyrimidine nucleotides come from aspartate, which can be acquired directly from the environment or generated from the TCA cycle intermediate oxaloacetate. Carbons in oxaloacetate can be derived from glucose or provided by glutamine, as will be discussed in later sections. Unlike other pyrimidines, thymidine contains one additional carbon, which comes from folates. The folate species 5,10-methylene-tetrahydrofolate provides this carbon to convert dUMP to dTMP, which is phosphorylated to dTTP for incorporation into DNA. This folate-dependent production of dTMP is the target of current chemotherapeutic agents such as methotrexate, 5-fluorouracil, and pemetrexed, highlighting the importance of these biosynthetic reactions for proliferating cells.

Experimental evidence supports the notion that glycolysis is necessary for DNA synthesis, especially during S phase of the cell cycle. In mitogen-stimulated mouse lymphocytes, the exponential increase in lactate production for the first 50 hours and the subsequent decrease exactly parallels ³H-thymidine incorporation into DNA (Wang et al 1976), suggesting a link between aerobic glycolysis and DNA synthesis. Consistent with this observation, ³H-thymidine incorporation into DNA increases with increasing glucose concentration in mitogen stimulated rat thymocytes (Hume et al 1978). In stimulated human peripheral lymphocytes, glycolytic enzyme activities, lactate production, and DNA synthesis reach maximum levels in S phase of the cell cycle (Marjanovic et al 1988). Further, studies of cell proliferation in the absence of glucose have demonstrated that HeLa cells, mouse L cells, early passage human fibroblasts, and primary chicken embryo fibroblasts can all grow

indefinitely at near maximal growth rates without glucose as long as they are supplemented with glutamine and uridine (Wice et al 1981). Uridine (uracil attached to a ribose ring) can be converted to PRPP (uridine + ATP \rightarrow UMP + ADP; UMP + PPi \rightarrow PRPP + uracil) to support nucleotide biosynthesis. While not directly examined in these studies, additional nutrients (such as amino acids and vitamins) present in mammalian tissue culture media used for these experiments are also likely required to support proliferation in the absence of glucose. A more recent study demonstrated cells could grow in size using the amino acid glutamine as the only major carbon source, but were unable to proliferate unless a metabolite capable of entering glycolysis was available (Wellen et al 2010), presumably to support nucleic acid synthesis. These findings support the importance of glycolysis in providing nucleotide precursors under physiological cell growth conditions for animal cells where glucose is the major nutrient available.

In addition to supporting nucleotide biosynthesis, glycolysis is also a major source of carbon for lipid precursors. Lipid-containing cell membranes comprise ~10% of the mass of an *E. coli* cell (Lewin 1997, Neidhardt & Umbarger 1996), and this percentage is likely larger for eukaryotic cells, which contain complex internal membrane systems in addition to the plasma membrane (Alberts et al 2002). In Ehrlich ascites tumor cells, > 93% of triacylglycerol fatty acids are synthesized *de novo* (Ookhtens et al 1984) and the synthesis of most fatty acids from external nutrients is a common feature shared by most proliferating cells (Menendez & Lupu 2007). Further, increased activity of lipogenesis enzymes are implicated in tumor growth: fatty acid synthetase (FASN) is overexpressed in cancer cells (Kuhajda 2000), and inhibition of FASN activity has been shown to kill cancer cells and hinder the growth of tumors in xenograft models (Lupu & Menendez 2006). Tumor growth can also be impaired by inhibition of ATP citrate

lyase (ACL), the enzyme that cleaves citrate to generate acetyl-CoA for fatty acid synthesis (Hatzivassiliou et al 2005).

The glycolytic intermediate dihydroxyacetone phosphate (DHAP) is the precursor to glycerol-3-phosphate, which is crucial for the biosynthesis of phospholipids and triacylglycerols that serve as major structural lipids in cell membranes. Elevated levels of glycerol and glycerol-3-phosphate have been reported in human peripheral lymphocytes after mitogen stimulation (Roos & Loos 1970), and elevated levels of choline phospholipids have been found in brain tumors (Preul et al 1996) and breast cancer tumors (Katz-Brull et al 2002). DHAP is also the precursor for lipids such as cardiolipin, an important component of mitochondrial membranes. The glycolytic intermediate 3-phosphoglycerate is the precursor of sphingolipids, another major class of lipids in cells. In addition to being important membrane lipids, sphingolipids play a role in signaling, as these lipids are implicated in cell growth, differentiation, senescence, apoptosis, and cancer (Futerman & Hannun 2004, Ogretmen & Hannun 2004).

A large proportion of the cell membrane carbon is derived from acetyl-coA. Acetyl-coA provides the carbon for synthesis of fatty acyl chain components of the various lipid classes and also provides the carbon to synthesize mevalonate, a precursor for cholesterol and related molecules (Figure 1). Glucose can be a major source of acetyl-coA production, but acetyl-coA derived from glucose is not a direct product of glycolysis. Oxidative decarboxylation of pyruvate to acetyl-CoA occurs in mitochondria, but mitochondrial acetyl-coA is not accessible for lipid biosynthesis in the cytosol. Instead, the acetyl-coA enters the TCA cycle where it can be exported to the cytosol as citrate, from which acetyl-coA can be recovered via the action of ATP citrate lyase. The ability to generate cytosolic acetyl-coA is critical for cell proliferation (Hatzivassiliou et al 2005).

Much of the remaining mass of the cell is protein, which makes up 49-55% of dry cell mass in *E. coli* (Lewin 1997, Neidhardt & Umbarger 1996) and a similar percentage in mammalian cells (Alberts et al 2002). This high protein content imposes a large amino acid requirement on proliferating cells. Amino acid availability provides the dominant input for the cell growth signal transduction machinery. The target of rapamycin (TOR) protein (referred to as mTOR in mammalian cells) is a serine/threonine kinase that coordinates activation of the cell growth machinery with amino acid availability, and in metazoans also with the presence of growth signals and other metabolic conditions (Sengupta et al 2010, Wullschleger et al 2006). Glycolytic intermediates are direct precursors for the biosynthesis of some amino acids (Figure 1), and Table 1 shows the carbon precursors for nonessential amino acids. The carbons of four nonessential amino acids are derived from glycolytic intermediates: 3-phosphoglycerate provides the carbons for cysteine, glycine, and serine; and pyruvate provides the carbons for alanine. As mentioned previously, serine and glycine are involved in folate metabolism as carbon donors for many biosynthetic reactions, and serine is added directly to glycerol as the head group in phosphatidylserine biosynthesis.

Close examination of biosynthetic requirements and evidence linking glycolytic activity to macromolecular synthesis suggest that the major function of enhanced glycolysis in proliferating cells is to maintain constant levels of glycolytic intermediates as macromolecular precursors. This clearly illustrates that increased glycolysis in cancer cells, and other proliferating cells, provides a selective advantage for growth beyond rapid ATP generation.

Why do proliferating cells excrete so much lactate?

In rapidly dividing cells, much of the glucose is converted to lactate. If glycolysis is increased to supply carbon building blocks for biomass production, why is so much carbon excreted as lactate? The rate of utilization of glycolytic intermediates for biosynthesis of macromolecules accounts for less than 10% of the glycolytic rate: in stimulated rat thymocytes, the percentage of glucose uptake accounted for by macromolecule synthesis (DNA, RNA, protein, and lipids) is ~7% (Hume et al 1978), and in human glioma cells, lactate and alanine production accounts for ~93% of glucose uptake, leaving $\leq 7\%$ for macromolecule synthesis (DeBerardinis et al 2007).

Having a seemingly wasteful, high glycolytic flux to lactate and a low flux to biosynthetic precursors may contribute to the regulation of biomass production, allowing cells to ramp up biosynthesis only during cell proliferation. A low flux pathway (biosynthesis) branching from a high flux pathway (glycolysis) would be highly sensitive to a decrease in the high flux pathway (Newsholme et al 1985b): while biosynthesis can occur during periods of high glycolytic flux, it would quickly halt when glycolytic flux is low. This strategy allows for maximum biosynthesis of macromolecules from glycolytic intermediates only when precursor concentrations are maintained at adequate levels. Indeed, glycolytic intermediates are maintained at high levels in the G₁ phase of the cell cycle and at especially high levels in tumor cells (Eigenbrodt et al 1992), and this is supported by increased glycolytic flux with a high rate of lactate production. Additionally, a rapid accumulation of PRPP is observed in thymocytes after mitogenic stimulation (Hovi et al 1975), and this may be directly caused by increased intracellular concentrations of glucose-6-phosphate (Culvenor & Weidemann 1976) to support nucleotide biosynthesis. Thus, during cell growth, glycolytic flux may be enhanced to allow for

fast cell growth by maintaining the pool sizes of glycolytic intermediates as they are depleted for anabolic reactions.

Pyruvate, the end product of glycolysis, has three major fates in mammalian cells: 1) conversion to lactate via lactate dehydrogenase (LDH); 2) conversion to alanine via alanine aminotransferase (ALT) with the concomitant conversion of glutamate to α -ketoglutarate; or 3) conversion to acetyl-CoA in the mitochondria via the pyruvate dehydrogenase (PDH) complex to enter the TCA cycle (Figure 1). The high glycolytic flux in proliferating cells may be significantly greater than the maximum PDH activity (Curi et al 1988), leaving higher concentrations of cytosolic pyruvate for LDH and alanine aminotransferase. Though less well appreciated than lactate production, some cancer cells also excrete large amounts of alanine (DeBerardinis et al 2007), suggesting that the excretion of both molecules is a means to eliminate excess pyruvate. Consistent with this, pyruvate levels often mirror lactate levels in cells (Christofk et al 2008a).

Lactate generation has an important role in glycolysis: conversion of pyruvate to lactate via LDH regenerates NAD^+ . For glycolysis to continue, the NAD^+/NADH redox balance must be maintained; NAD^+ regeneration is necessary for continued flux through glycolysis, as NAD^+ is required for conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate. Maintenance of the cellular redox state is also important for regulating gene expression (Ladurner 2006). For example, the circadian clock is regulated by NAD(P) cofactors: the reduced forms (NADH and NADPH) activate transcription factors that control gene expression as a function of the light-dark cycle, and the oxidized forms (NAD^+ and NADP^+) inhibit DNA binding (Rutter et al 2001). Further, NADH levels regulate the transcriptional regulator C-

terminal binding protein, which is involved in cell growth, differentiation and transformation (Zhang et al 2002).

The mitochondrial electron transport chain can accept electrons from NADH to regenerate NAD^+ , but the electrons must be transported into the mitochondrial matrix by other metabolites (e.g., malate) via cytoplasmic shuttle systems (e.g., malate-aspartate shuttle). The malate-aspartate shuttle involves up to 6 separate reactions and is kinetically much slower than pyruvate to lactate conversion. LDH is an extremely efficient enzyme over a range of substrate conditions when the enzyme is abundant (Wuntch et al 1970). Many proliferating cells express LDH at high levels, and a high NADH/NAD^+ ratio resulting from elevated glycolysis may drive the production of lactate. NAD^+ is also necessary for nucleotide and amino acid biosynthesis (see Figure 1 and Table 1), suggesting that lactate production may allow faster glucose flux through glycolysis along with faster incorporation of glucose metabolites into biomass by efficiently regenerating NAD^+ . For most unicellular organisms, rapid proliferation has a selective advantage to outcompete other cells when nutrients are abundant. In animals, nutrients are rarely limiting for individual cells, but the ability to proliferate quickly (e.g., to mount an immune response or close a wound) provides a strong selection for rapid division. Viewed from this perspective, faster growth involving lactate loss is more important than the efficiency of carbon utilization for many cells (Vander Heiden et al 2009).

Lactate generation may have secondary benefits for tumors cells. Excreting high levels of lactate may support their survival, growth, and invasion by conditioning the tumor microenvironment (Koukourakis et al 2006), promoting tumor invasion (Gillies & Gatenby 2007, Swietach et al 2007), and suppressing anticancer T-cell immune response (Fischer et al 2007). While these sequelae could contribute to dysregulated tumor growth, these advantages

that arise from high lactate excretion are likely not the selective pressures responsible for aerobic glycolysis. The biosynthetic benefits of aerobic glycolysis (i.e., obtaining much of the carbons needed to replicate the entire contents of a cell) outweigh the invasive benefits provided by lactate. It is more likely that cells produce lactate to regenerate NAD^+ for continued glycolytic flux. Further, many normal proliferating cells utilize aerobic glycolysis but do not derive benefit from lactate. Thus, it is unlikely that aerobic glycolysis is selected for in cancer cells because of high lactate excretion.

How is enough NADPH generated to support cell proliferation?

NADPH (reduced nicotinamide adenine dinucleotide phosphate) is a cofactor important for cell proliferation. As shown in Figure 1 and Table 1, NADPH is necessary as a reducing agent for nucleotide, amino acid, and lipid biosynthesis. Lipid synthesis demands a great deal of NADPH. Generating palmitoyl-CoA, a fatty acyl-CoA, claims 14 molecules of NADPH (Vander Heiden et al 2009), and sphingolipid, phospholipid, and triacylglycerol biosynthesis employs one, two, and three molecules of fatty acyl-CoA's, respectively, imposing a large NADPH requirement. Cholesterol biosynthesis involves 26 molecules of NADPH. In fact, it has been proposed that NADPH production may be rate limiting for cell proliferation (Vander Heiden et al 2009). The major source of NADPH during cell growth is widely assumed to be the oxidative reactions of the pentose phosphate pathway (Feron 2009, Lehninger et al 1993). However, labeling experiments using 1,2- ^{13}C -labeled glucose in multiple cancer cell types have suggested that the pentose phosphate pathway cannot account for the NADPH requirements of proliferation in at least some cancer cells (Boros et al 1998, Boros et al 2000). Studies also suggest that the pentose phosphate pathway is a minor source for NADPH production in proliferating *E. coli*

(Csonka & Fraenkel 1977). Further, many patients deficient in glucose-6-phosphate dehydrogenase (one of the enzymes in the oxidative pentose phosphate shunt responsible for NADPH production) are asymptomatic, and there is no evidence they have decreased cancer risk (Cocco 1987).

It has been shown that metabolism of glutamine through malic enzyme (malate dehydrogenase (decarboxylating)) can be a significant source of NADPH in human glioma cells (DeBerardinis et al 2007). Each molecule of glutamine-derived lactate can generate up to one molecule of NADPH if glutamine is oxidatively metabolized to malate, which is converted to pyruvate via malic enzyme. Pyruvate can then re-enter the TCA cycle or be excreted as lactate. Estimating NADPH production from malic enzyme via isotopically labeled lactate produced from labeled glutamine is challenging, as cyclical flux between pyruvate and TCA cycle metabolites (e.g., pyruvate \rightarrow citrate \rightarrow malate \rightarrow pyruvate) do not allow unique isotope-labeling detection. Nonetheless, more than half of the glutamine taken up by glioblastoma cells is excreted as lactate, suggesting this pathway may be a significant source of NADPH production for some cancer cells (DeBerardinis et al 2007). However, not all cancers exhibit high glutamine uptake. Glutamine uptake and metabolism is regulated by the oncogene *MYC*, and *MYC*-transformed cells become addicted to glutamine through increased expression of glutamine transporters and glutamine catabolic enzymes (Dang 2010, Gao et al 2009, Wise et al 2008). Thus, other means of generating NADPH must exist for cancer cells less dependent on glutamine.

Cytosolic isocitrate dehydrogenase (IDH1) generates NADPH when converting isocitrate to α -ketoglutarate. IDH1 may be a significant source of NADPH for some cancer cells (Thompson 2009), and siRNA knockdown of IDH1 is toxic to a cancer cell line that expresses

wildtype IDH1 (Ward et al 2010). Cancer cells that rely on the reductive metabolism of glutamine also require IDH1; however, in these cells net flux appears to be production of isocitrate from glutamine, leading to NADPH consumption, not production. Furthermore, mutations in IDH1 are found in human cancers that result in a neomorphic activity to consume NADPH during conversion of α -ketoglutarate to 2-hydroxyglutarate (Dang et al 2009). IDH1 mutations always involve only one allele, but expression of the mutant enzyme has been reported to act as a dominant inhibitor of normal IDH1 provided by the remaining allele (Zhao et al 2009). Additional work is required to determine which cancer cells rely on NADPH production via IDH1, malic enzyme, and/or the oxidative pentose phosphate pathway. Furthermore, it remains possible that other sites of NADPH production are important for proliferation in some cells.

Glutamine is also important for anaplerosis and ATP production

In addition to serving as a source of NADPH production, glutamine contributes to bioenergetics, macromolecular synthesis, and glutathione production for protection against oxidative stress (DeBerardinis & Cheng 2010). In nonproliferating cells, the main role of the TCA cycle is to maximize ATP production, oxidizing substrates completely to CO₂. In proliferating cells, however, the TCA cycle serves as an important source of biosynthetic precursors (DeBerardinis et al 2008) in addition to providing ATP. Mitochondrial citrate is used for lipid biosynthesis while oxaloacetate and α -ketoglutarate are used to generate four nonessential amino acids (aspartate, asparagine, glutamate, and proline; see Figure 1). The efflux of these TCA cycle intermediates for biosynthesis is called cataplerosis. In order for cataplerosis to be sustainable, the TCA cycle intermediates must be replenished, and this process

is called anaplerosis. Anaplerosis of TCA cycle intermediates can be supported by pyruvate and various amino acids. Pyruvate is converted to oxaloacetate by pyruvate carboxylase in the mitochondrial matrix. Amino acids enter the TCA cycle via pyruvate, acetyl-CoA, acetoacetyl-CoA, α -ketoglutarate, succinyl-CoA, fumarate, and oxaloacetate (Owen et al 2002, Voet & Voet 2004). Glutamine, the most abundant amino acid in human plasma (Stein & Moore 1954), is a major contributor to anaplerotic flux. Labeling studies show that glutamine significantly contributes to anaplerosis in both rat and human glioma cells (DeBerardinis et al 2007, Portais et al 1996). Glutamine depletion induces MYC-dependent apoptosis of normal human fibroblasts, but apoptosis can be rescued by addition of pyruvate or oxaloacetate (Yuneva et al 2007). Pyruvate and oxaloacetate do not contain any nitrogen, suggesting that cell death induced by glutamine starvation is due to lack of glutamine-derived carbons. Glutamine anaplerotic flux may also be important for mitochondrial production of NADH and FADH, as NADH/NAD⁺ and FADH/FAD⁺ ratios are important for mitochondrial integrity and function (Jonas et al 2004).

In proliferating cells, glutamine carbons are converted to amino acids, TCA cycle intermediates, lactate, and CO₂ (Hanson & Parsons 1977, Lanks 1987, Newsholme et al 1985a, Watford et al 1979). Figure 2 provides an overall schematic for contributions of glucose and glutamine to biomass, lactate, and CO₂. Due to similarities with glycolysis in lactate production and partial oxidation to CO₂, this metabolism of glutamine is referred to as glutaminolysis (McKeehan 1982). By contributing to anaplerotic flux, glutamine provides carbons for TCA cycle intermediates that serve as precursors of many nonessential amino acids (Table 1). Glutamine is also an important source of carbon for fatty acids, as acetyl-CoA can also be derived from glutamine in addition to glucose (DeBerardinis et al 2007, Yoo et al 2004). Further, glutamine is a nitrogen source for nucleotides (Table 2), amino acids, and hexosamines

(amino sugars such as glucosamine, a precursor to glycosylated proteins and lipids). Glutamine can be converted to glutamate, which serves as a nitrogen donor to amino acids through transamination reactions. Glutamate is also important for folate metabolism: glutamates are added to folates to increase cell retention and regulate enzyme activity, and antifolates can stop cell growth by inhibiting glutamate addition to folates (Kwon et al 2008).

Glutamine carbons also contribute to fatty acid biosynthesis. In human glioma cells, 5% of ^{14}C -glutamine-derived carbon is incorporated into fatty acids (Wise et al 2008). This percentage may be higher under certain conditions, as reductive metabolism of glutamine can occur and lead to significant incorporation of glutamine carbon into acetyl-coA (Yoo et al 2008). Reductive glutamine metabolism is favored under low oxygen conditions and can be a major contributor of carbon to lipid synthesis.

Glutaminolysis has the capacity to generate more ATP than glycolysis. While ATP generation from glucose has been emphasized, glutamine's contribution to ATP production in proliferating cells may have been underappreciated. Glutamine is well known for its role in anaplerosis, protein biosynthesis, and nucleotide biosynthesis (as an amino group donor). However, glutamine may also be an important respiratory fuel for proliferating cells, significantly contributing to cellular ATP production. Isolated rat intestinal cells selectively uptake large amounts of glutamine from blood even in the presence of 10 to 15 mM glucose, amounts higher than physiological concentrations. The majority of ^{14}C -glutamine carbons (57%) were released back into the blood as $^{14}\text{CO}_2$ in this experiment, while only 14% appeared in tissue (Windmueller & Spaeth 1974). In mouse L-M strain fibroblasts grown in medium containing 11 mM glucose, 55% of ^{14}C -glutamine uptake is converted to $^{14}\text{CO}_2$, contributing up to 35% of the ATP requirement for these cells (Stoner & Merchant 1972). Similarly, glutamine provides

approximately 30% of the cellular ATP requirement in human diploid fibroblasts cultured in minimum essential medium (MEM) containing ≥ 5.5 mM glucose (Zielke et al 1978). In cultured HeLa cells, more than half of the ATP requirement (determined by comparing $^{14}\text{CO}_2$ production from ^{14}C -glutamine to $^{14}\text{CO}_2$ and ^{14}C -lactate production from ^{14}C -glucose) comes from glutamine even when a high concentration (10 mM) of glucose is present (Reitzer et al 1979). Thus, glutamine is a critical nutrient that serves myriad functions to support proliferative cell metabolism.

Metabolic reprogramming for proliferation

In metazoans, normal cells grow and divide only when signal transduction pathways are activated by growth factors. These signaling pathways control gene expression and cell physiology, leading to cell cycle progression. Additionally, they impact metabolism to allow for cell growth. The metabolic changes induced by cell growth signals are largely conserved between normal and cancer cells; however, cancer cells activate signaling pathways in the absence of normal extracellular cues, promoting a metabolic phenotype that allows inappropriate cell proliferation. A major regulator of glucose metabolism is the phosphoinositide 3-kinase (PI3K) signaling pathway. PI3K signaling through the protein kinase Akt can increase uptake of glucose by increasing expression of the glucose transporter Glut1 (Barthel et al 1999, Frauwirth et al 2002, Vander Heiden et al 2001), and maintaining its levels on the cell surface by preventing internalization (Wieman et al 2007). Akt activation enhances flux through glycolysis (Elstrom et al 2004) in part by maintaining hexokinase association with mitochondria (Gottlob et al 2001) and activating phosphofructokinase 2 (PFK-2) through phosphorylation (Deprez et al 1997). In normal cells, the PI3K pathway is tightly controlled to increase glucose uptake and

metabolism in response to growth signals (Cantley 2002). However, in cancer cells, various mutations activate PI3K in the absence of growth signals, suggesting that inappropriate activation of this pathway may be a major driver of aerobic glycolysis in cancer cells (DeBerardinis et al 2008).

Rapidly proliferating cells require close proximity to blood vessels for access to oxygen and nutrients. As tumors grow, cells may encounter hypoxic conditions leading to induction of the hypoxia inducible factor 1 (HIF-1) transcription factor. HIF-1 increases the expression of vascular endothelial growth factor (VEGF) to facilitate the growth of new blood vessels. HIF-1 also increases the transcription of glucose transporters, many glycolytic enzymes, and lactate dehydrogenase A (O'Rourke et al 1996, Semenza et al 1994). HIF-1 is a heterodimer composed of HIF-1 α and HIF-1 β (ARNT) subunits, and the biological activity of HIF-1 is determined by presence of the HIF-1 α subunit, whose stability is regulated by oxygen levels (Kaelin & Ratcliffe 2008). During periods of fast growth and rapid biomass synthesis, such as embryogenesis and tumorigenesis, local hypoxic conditions may arise stimulating HIF-1 to enhance glycolytic gene expression. HIF-1 is required for embryogenesis, as mice homozygous for a loss-of-function mutation in HIF-1 α or HIF-1 β die at midgestation (Iyer et al 1998, Maltepe et al 1997). Loss of HIF-1 α in tumor cells also dramatically slows their growth when injected into nude mice (Jiang et al 1997, Maxwell et al 1997, Ryan et al 1998). Even under normoxic conditions, HIF-1 α can be induced by the glycolytic metabolites pyruvate and lactate (McFate et al 2008), mTOR activation, NAD⁺ levels, reactive oxygen species, nitric oxide, many TCA cycle metabolites (reviewed in (Semenza 2010b)), and oncogene gain of function or tumor suppressor gene loss of function (reviewed in (Semenza 2010a)).

Activation of the PI3K pathway appears to be an important way to increase HIF-1 transcription in cancer (DeBerardinis et al 2008, Majumder & Sellers 2005). In addition to increasing the expression of glycolytic enzymes, increased HIF-1 activity promotes aerobic glycolysis by upregulating the expression of pyruvate dehydrogenase kinase (PDK). HIF-1 induced PDK activity inhibits PDH, which converts pyruvate to acetyl-CoA (Kim et al 2006, Papandreou et al 2006). Inhibition of PDH activity decreases pyruvate flux into the TCA cycle, promoting pyruvate conversion to lactate (i.e., aerobic glycolysis). Thus, activation of HIF resulting from hypoxia, PI3K activation or other mechanisms can promote glucose metabolism by aerobic glycolysis.

In addition to promoting glutamine metabolism as discussed above, MYC promotes transcription of glucose transporters and glycolytic enzymes (Ahuja et al 2010, Osthus et al 2000). MYC regulates the expression of LDH-A (Shim et al 1997), and MYC-dependent tumors cannot proliferate when LDH-A expression is knocked down (Le et al 2010). Further, MYC regulates enzymes in the nucleotide biosynthesis pathway, including thymidylate synthase, inosine monophosphate dehydrogenase 1 and 2, and phosphoribosyl pyrophosphate synthetase 2 (Tong et al 2009b). Additional transcription factors related to MYC, ChREBP and MondoA, control glycolytic enzyme expression and can promote anabolic metabolism in some contexts (Sloan & Ayer 2010, Tong et al 2009a). *RAS*, another oncogene widely implicated in human cancer, also promotes glucose metabolism by enhancing glucose uptake (Yun et al 2009). Mutation of p53 is another common genetic event in human cancer, and the p53 protein prevents tumor growth by suppressing metabolic pathways conducive to proliferation of stressed or damaged cells. p53 promotes mitochondrial respiration while inhibiting glycolysis by repressing glucose transporters, inhibiting the glycolytic enzyme phosphoglycerate mutase, and decreasing

the activity of PFK-1 through TIGAR (Cheung & Vousden 2010, Levine & Puzio-Kuter 2010). Thus, p53 loss of function in cancer can promote glycolysis, and signaling networks that regulate metabolism contribute to enhanced glycolysis observed in proliferating cells.

Upstream regulation of glycolysis

Glucose import into mammalian cells is facilitated primarily by five transmembrane transporters, GLUT1-5. While GLUT2, GLUT4, and GLUT5 are only found in specific tissues, GLUT1 and GLUT3 are expressed in nearly all mammalian cells and have the lowest K_M value (1 mM), allowing them to transport glucose into the cell at a nearly constant rate from serum where glucose concentration ranges from 4 mM to 8 mM. Tumor cells frequently overexpress GLUT1 and GLUT3 and have an increased capacity for glucose uptake (Au et al 1997, Suzuki et al 1999, Yamamoto et al 1990, Younes et al 1996). Suppression of GLUT1 expression in a human gastric cancer cell line can decrease the number of cells in S phase and inhibit tumor growth (Noguchi et al 2000).

Once in the cell, glucose must be converted to glucose-6-phosphate by hexokinase to prevent its transport out of the cell and to prime it for metabolism in subsequent reactions (Berg et al 2007). There are four known mammalian hexokinase isoforms (HK I-IV), and HK I is expressed in most normal cells and at particularly high levels in brain tissue (Wilson 2003). HK II expression is more limited and is normally found mainly in skeletal muscle and adipose tissue. However, cancer cells frequently overexpress HK II (Mathupala et al 2006), and at least some glioblastoma cells are specifically dependent on HK II over other isoforms of the enzyme (Wolf et al 2011). Both HK I and HK II are associated with the voltage dependent anion channel (VDAC) on the cytosolic side of the outer mitochondrial membrane. This VDAC-hexokinase

association may inhibit mitochondria-induced apoptosis (Majewski et al 2004) and give hexokinase preferential access to mitochondria-generated ATP (Arora & Pedersen 1988). Preference for mitochondria-bound HK II in cancer cells remains unclear.

While hexokinase traps glucose inside the cell, PFK-1 controls its commitment to glycolysis and is therefore highly regulated. PFK-1, which irreversibly converts fructose-6-phosphate to fructose-1,6-bisphosphate (FBP), is overexpressed in various human cancer cell lines (Vora et al 1985). PFK-1 is allosterically inhibited by high levels of ATP (Berg et al 2007), and relieving this ATP inhibition is an important means to increase glucose metabolism in proliferating cells (Fang et al 2010, Israelsen & Vander Heiden 2010, Scholnick et al 1973). PFK-1 inhibition by ATP is diminished by fructose-2,6-bisphosphate, a metabolite synthesized from fructose-6-phosphate by PFK-2. Regulation of PFK-2 expression or activity has been proposed as an important way to couple growth signals with regulation of glucose metabolism in proliferating cells (Christofk et al 2008a, Marsin et al 2000, Telang et al 2006). In human tissues, PFK-1 subunit composition, a complex mixture of homotetramers or heterotetramers composed of up to three different subunits, can vary depending on tissue type. Each subunit (C, L, M) differs in sensitivity to allosteric effectors; thus, the kinetic and regulatory properties of PFK-1 are determined by subunit composition (Dunaway et al 1988). PFK subunits overexpressed in rat thyroid carcinomas and human gliomas are less sensitive to the allosteric inhibitors ATP and citrate (Meldolesi et al 1976, Oskam et al 1985, Staal et al 1987). In addition to controlling glucose commitment to glycolysis, PFK-1 may regulate the amounts of glucose-6-phosphate available for nucleotide biosynthesis.

Pyruvate kinase influences the fate of glucose

Pyruvate kinase (PK) catalyzes the final irreversible step in glycolysis, generating pyruvate and ATP. PK serves as a critical point in controlling the levels of glycolytic intermediates for biosynthesis and the availability of pyruvate for mitochondrial ATP production. When the cell energy charge (ATP/AMP ratio) is high, ATP inhibits PK. PK is activated by FBP, the product of the glycolytic rate-controlling enzyme PFK-1. Alanine, which can be synthesized from the PK product pyruvate, is an allosteric inhibitor of PK.

Four PK isoforms with different kinetic properties exist in mammals (L, R, M1 and M2). The L and R isoforms are expressed in the liver and red blood cells, respectively. The M1 isoform is found in most adult tissues while the M2 isoform is found in rapidly proliferating fetal tissues. L, R, and M2 isoforms are regulated by FBP, ATP, and phosphorylation, but the M1 isoform is largely unregulated (Jurica et al 1998). The M2 isoform can also be inhibited as a consequence of tyrosine kinase signaling in tumor cells (Christofk et al 2008b). Many cancer cells exclusively express the M2 isoform of PK (Mazurek et al 2005), and PKM2 expression is important for tumor growth (Christofk et al 2008a). PKM2 is less active than PKM1 both *in vitro* and in cells (Vander Heiden et al 2010), and inactivation of PKM2 appears to be mediated by phospho-tyrosine catalyzed release of FBP from the enzyme (Christofk et al 2008b, Hitosugi et al 2009). Cancer cells may revert to the fetal M2 isoform because the highly regulated M2 isoform can switch between active and inactive forms, controlling the flow of glycolytic carbons between biosynthesis and mitochondrial ATP production during different phases of the cell cycle or different physiological contexts. An inactive PKM2 would support increased biosynthesis during periods that require higher concentrations of triose phosphates for amino acid (serine, glycine, and cysteine), lipid (triacylglycerol, phospholipid, and sphingolipid), and nucleotide

synthesis. In contrast, active pyruvate kinase promotes oxidative phosphorylation, and this ability to switch rapidly from biosynthesis to promoting ATP production may explain why PKM2 is selected for in all cancer cells studied.

Paradoxically, proliferating cells that selectively express the less active isoform of pyruvate kinase (M2) produce more lactate. Human cancer cell lines engineered to express PKM1 produce less lactate than the same cells expressing PKM2 (Christofk et al 2008a). Also, PKM2 inhibition by prolactin increases lactate content and stimulates proliferation in human cell lines (Varghese et al 2010). One potential explanation for this paradox is that the overall increase in glycolytic flux results in higher flux through pyruvate kinase despite expression of the less active isoform. Also, an alternative metabolism of phosphoenolpyruvate to pyruvate can compensate for decreased pyruvate kinase activity in proliferating cells, allowing for glucose conversion to pyruvate without producing ATP (Vander Heiden et al 2010). Finally, lactate production regenerates NAD^+ , a cofactor necessary to maintain high glycolytic flux, suggesting that lactate production itself may facilitate faster glucose metabolism.

Conclusions and perspectives

Cell proliferation requires increased uptake of nutrients (e.g., glucose and glutamine), elevated flux through biosynthetic pathways (e.g., nucleotide, lipid, and protein biosynthesis), maintenance of metabolic intermediates (e.g., glucose-6-phosphate, fructose-6-phosphate, glyceraldehydes-3-phosphate, dihydroxyacetone phosphate, 3-phosphoglycerate and acetyl-CoA) and continued regeneration of cofactors needed to provide free energy or reducing equivalents for reactions (e.g., ATP, NADPH and NADH). Aerobic glycolysis allows the cell to meet these requirements of proliferation, and this may be why aerobic glycolysis is a common feature of

many proliferating cells, including cancer cells. Although aerobic glycolysis also generates lactate and ATP, these products are likely not the main reason for enhanced glucose metabolism. Accounting of ATP production by glycolysis versus oxidative phosphorylation has been done many times in various cells and tissues, and all have concluded that oxidative phosphorylation remains the main contributor of ATP in most cells under aerobic conditions. An understanding of how cells meet the other requirements of proliferation is needed. Which nutrients provide all the carbons, nitrogens, and other atoms necessary to double the mass of a cell? How much does each nutrient contribute to biomass? How is enough reducing power generated for cell maintenance and proliferation? Which pathways provide the considerable amounts of NADPH required for lipid biosynthesis? How does a cell balance the requirements of growth and homeostasis? A comprehensive accounting of the complete metabolic requirements of a cell has never been done. The technology exists to begin answering these questions and move forward in understanding metabolism in cancer and other proliferating cells. Examining the requirements of cell growth in its entirety will shed new light on the role of aerobic glycolysis in cells.

Acknowledgements

We thank Eric Bell, Sarah-Maria Fendt, Brian Fiske, Will Israelsen, Katie Mattaini, and Christian Metallo for thoughtful discussions and critical reading of the manuscript. MVH acknowledges support from the Burrough's Wellcome Fund, the Smith Family Foundation, the Starr Cancer Consortium, and the Damon Runyon Cancer Research Foundation.

Literature cited

- Ahuja P, Zhao P, Angelis E, Ruan H, Korge P, et al. 2010. Myc controls transcriptional regulation of cardiac metabolism and mitochondrial biogenesis in response to pathological stress in mice. *J Clin Invest* 120: 1494-505
- Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. 2002. *Molecular biology of the cell*. New York: Garland Science. xxxiv, [1548] p. pp.
- Arora KK, Pedersen PL. 1988. Functional significance of mitochondrial bound hexokinase in tumor cell metabolism. Evidence for preferential phosphorylation of glucose by intramitochondrially generated ATP. *J Biol Chem* 263: 17422-8
- Barthel A, Okino ST, Liao J, Nakatani K, Li J, et al. 1999. Regulation of GLUT1 gene transcription by the serine/threonine kinase Akt1. *J Biol Chem* 274: 20281-6
- Berg JM, Tymoczko JL, Stryer L. 2007. *Biochemistry*. New York: W.H. Freeman
- Boros LG, Lee PW, Brandes JL, Cascante M, Muscarella P, et al. 1998. Nonoxidative pentose phosphate pathways and their direct role in ribose synthesis in tumors: is cancer a disease of cellular glucose metabolism? *Med Hypotheses* 50: 55-9
- Boros LG, Torday JS, Lim S, Bassilian S, Cascante M, Lee WN. 2000. Transforming growth factor beta2 promotes glucose carbon incorporation into nucleic acid ribose through the nonoxidative pentose cycle in lung epithelial carcinoma cells. *Cancer Res* 60: 1183-5
- Brand K. 1985. Glutamine and glucose metabolism during thymocyte proliferation. Pathways of glutamine and glutamate metabolism. *Biochem J* 228: 353-61
- Bryant C, Voller A, Smith MJH. 1964. The Incorporation of Radioactivity from [14C] Glucose into the Soluble Metabolic Intermediates of Malaria Parasites. *Am J Trop Med Hyg* 13: 515-9
- Cantley LC. 2002. The phosphoinositide 3-kinase pathway. *Science* 296: 1655-7
- Chambers DA, Martin DW, Jr., Weinstein Y. 1974. The effect of cyclic nucleotides on purine biosynthesis and the induction of PRPP synthetase during lymphocyte activation. *Cell* 3: 375-80
- Chandel NS, Budinger GR, Schumacker PT. 1996. Molecular oxygen modulates cytochrome c oxidase function. *J Biol Chem* 271: 18672-7
- Cheung EC, Vousden KH. 2010. The role of p53 in glucose metabolism. *Curr Opin Cell Biol* 22: 186-91
- Christen S, Sauer U. 2010. Intracellular characterization of aerobic glucose metabolism in seven yeast species by (13) C flux analysis and metabolomics. *FEMS Yeast Res*
- Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, et al. 2008a. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature* 452: 230-3
- Christofk HR, Vander Heiden MG, Wu N, Asara JM, Cantley LC. 2008b. Pyruvate kinase M2 is a phosphotyrosine-binding protein. *Nature* 452: 181-6
- Cocco P. 1987. Does G6PD deficiency protect against cancer? A critical review. *J Epidemiol Community Health* 41: 89-93
- Crabtree HG. 1929. Observations on the carbohydrate metabolism of tumours. *Biochem J* 23: 536-45
- Csonka LN, Fraenkel DG. 1977. Pathways of NADPH formation in Escherichia coli. *J Biol Chem* 252: 3382-91

- Culvenor JG, Weidemann MJ. 1976. Phytohaemagglutinin stimulation of rat thymus lymphocytes glycolysis. *Biochim Biophys Acta* 437: 354-63
- Curi R, Newsholme P, Newsholme EA. 1988. Metabolism of pyruvate by isolated rat mesenteric lymphocytes, lymphocyte mitochondria and isolated mouse macrophages. *Biochem J* 250: 383-8
- Dang CV. 2010. Rethinking the Warburg effect with Myc micromanaging glutamine metabolism. *Cancer Res* 70: 859-62
- Dang L, White DW, Gross S, Bennett BD, Bittinger MA, et al. 2009. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* 462: 739-44
- Darzynkiewicz Z, Staiano-Coico L, Melamed MR. 1981. Increased mitochondrial uptake of rhodamine 123 during lymphocyte stimulation. *Proc Natl Acad Sci U S A* 78: 2383-7
- DeBerardinis RJ, Cheng T. 2010. Q's next: the diverse functions of glutamine in metabolism, cell biology and cancer. *Oncogene* 29: 313-24
- DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. 2008. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab* 7: 11-20
- DeBerardinis RJ, Mancuso A, Daikhin E, Nissim I, Yudkoff M, et al. 2007. Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. *Proc Natl Acad Sci U S A* 104: 19345-50
- Deprez J, Vertommen D, Alessi DR, Hue L, Rider MH. 1997. Phosphorylation and activation of heart 6-phosphofructo-2-kinase by protein kinase B and other protein kinases of the insulin signaling cascades. *J Biol Chem* 272: 17269-75
- Dunaway GA, Kasten TP, Sebo T, Trapp R. 1988. Analysis of the phosphofructokinase subunits and isoenzymes in human tissues. *Biochem J* 251: 677-83
- Ecker RE, Kokaisl G. 1969. Synthesis of protein, ribonucleic acid, and ribosomes by individual bacterial cells in balanced growth. *J Bacteriol* 98: 1219-26
- Eigenbrodt E, Reinacher M, Scheefers-Borchel U, Scheefers H, Friis R. 1992. Double role for pyruvate kinase type M2 in the expansion of phosphometabolite pools found in tumor cells. *Crit Rev Oncog* 3: 91-115
- Fang M, Shen Z, Huang S, Zhao L, Chen S, et al. 2010. The ER UDPase ENTPD5 promotes protein N-glycosylation, the Warburg effect, and proliferation in the PTEN pathway. *Cell* 143: 711-24
- Fantin VR, Leder P. 2006. Mitochondriotoxic compounds for cancer therapy. *Oncogene* 25: 4787-97
- Fantin VR, St-Pierre J, Leder P. 2006. Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. *Cancer Cell* 9: 425-34
- Feron O. 2009. Pyruvate into lactate and back: from the Warburg effect to symbiotic energy fuel exchange in cancer cells. *Radiother Oncol* 92: 329-33
- Fischer K, Hoffmann P, Voelkl S, Meidenbauer N, Ammer J, et al. 2007. Inhibitory effect of tumor cell-derived lactic acid on human T cells. *Blood* 109: 3812-9
- Fogal V, Richardson A, Karmali PP, Scheffler IE, Smith JW, Ruoslahti E. 2011. Mitochondrial p32 Protein is a Critical Regulator of Tumor Metabolism via Maintenance of Oxidative Phosphorylation. *Mol. Cell. Biol.*: MCB.01101-09
- Frauwirth KA, Riley JL, Harris MH, Parry RV, Rathmell JC, et al. 2002. The CD28 signaling pathway regulates glucose metabolism. *Immunity* 16: 769-77

- Futerman AH, Hannun YA. 2004. The complex life of simple sphingolipids. *EMBO Rep* 5: 777-82
- Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, et al. 2009. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. *Nature* 458: 762-5
- Gillies RJ, Gatenby RA. 2007. Hypoxia and adaptive landscapes in the evolution of carcinogenesis. *Cancer Metastasis Rev* 26: 311-7
- Hanson PJ, Parsons S. 1977. Metabolism and transport of glutamine and glucose in vascularly perfused small intestine rat. *Biochem J* 166: 509-19
- Hatzivassiliou G, Zhao F, Bauer DE, Andreadis C, Shaw AN, et al. 2005. ATP citrate lyase inhibition can suppress tumor cell growth. *Cancer Cell* 8: 311-21
- Hedekov CJ. 1968. Early effects of phytohaemagglutinin on glucose metabolism of normal human lymphocytes. *Biochem J* 110: 373-80
- Hitosugi T, Kang S, Vander Heiden MG, Chung TW, Elf S, et al. 2009. Tyrosine phosphorylation inhibits PKM2 to promote the Warburg effect and tumor growth. *Sci Signal* 2: ra73
- Hovi T, Allison AC, Allsop J. 1975. Rapid increase of phosphoribosyl pyrophosphate concentration after mitogenic stimulation of lymphocytes. *FEBS Lett* 55: 291-3
- Hovi T, Smyth JF, Allison AC, Williams SC. 1976. Role of adenosine deaminase in lymphocyte proliferation. *Clin Exp Immunol* 23: 395-403
- Hume DA, Radik JL, Ferber E, Weidemann MJ. 1978. Aerobic glycolysis and lymphocyte transformation. *Biochem J* 174: 703-9
- Hume DA, Weidemann MJ. 1979. Role and regulation of glucose metabolism in proliferating cells. *J Natl Cancer Inst* 62: 3-8
- Israelsen WJ, Vander Heiden MG. 2010. ATP consumption promotes cancer metabolism. *Cell* 143: 669-71
- Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, et al. 1998. Cellular and developmental control of O₂ homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev* 12: 149-62
- Jiang BH, Agani F, Passaniti A, Semenza GL. 1997. V-SRC induces expression of hypoxia-inducible factor 1 (HIF-1) and transcription of genes encoding vascular endothelial growth factor and enolase 1: involvement of HIF-1 in tumor progression. *Cancer Res* 57: 5328-35
- Jonas EA, Hickman JA, Chachar M, Polster BM, Brandt TA, et al. 2004. Proapoptotic N-truncated BCL-xL protein activates endogenous mitochondrial channels in living synaptic terminals. *Proc Natl Acad Sci U S A* 101: 13590-5
- Jurica MS, Mesecar A, Heath PJ, Shi W, Nowak T, Stoddard BL. 1998. The allosteric regulation of pyruvate kinase by fructose-1,6-bisphosphate. *Structure* 6: 195-210
- Kaelin WG, Jr., Ratcliffe PJ. 2008. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol Cell* 30: 393-402
- Katz-Brull R, Lavin PT, Lenkinski RE. 2002. Clinical Utility of Proton Magnetic Resonance Spectroscopy in Characterizing Breast Lesions. *J. Natl. Cancer Inst.* 94: 1197-203
- Kilburn DG, Lilly MD, Webb FC. 1969. The energetics of mammalian cell growth. *J Cell Sci* 4: 645-54
- Kim JW, Tchernyshyov I, Semenza GL, Dang CV. 2006. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* 3: 177-85

- Koukourakis MI, Giatromanolaki A, Harris AL, Sivridis E. 2006. Comparison of metabolic pathways between cancer cells and stromal cells in colorectal carcinomas: a metabolic survival role for tumor-associated stroma. *Cancer Res* 66: 632-7
- Kuhajda FP. 2000. Fatty-acid synthase and human cancer: new perspectives on its role in tumor biology. *Nutrition* 16: 202-8
- Kwon YK, Lu W, Melamud E, Khanam N, Bognar A, Rabinowitz JD. 2008. A domino effect in antifolate drug action in *Escherichia coli*. *Nat Chem Biol* 4: 602-8
- Ladurner AG. 2006. Rheostat control of gene expression by metabolites. *Mol Cell* 24: 1-11
- Lanks KW. 1987. End products of glucose and glutamine metabolism by L929 cells. *J Biol Chem* 262: 10093-7
- Le A, Cooper CR, Gouw AM, Dinavahi R, Maitra A, et al. 2010. Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. *Proc Natl Acad Sci U S A* 107: 2037-42
- Lehninger AL, Nelson DL, Cox MM. 1993. *Principles of biochemistry*. New York, NY: Worth Publishers. xli, 1013, [77] p. pp.
- Levine AJ, Puzio-Kuter AM. 2010. The control of the metabolic switch in cancers by oncogenes and tumor suppressor genes. *Science* 330: 1340-4
- Lewin B. 1997. *Genes VI*. Oxford ; New York: Oxford University Press. xviii, 1260 p. pp.
- Lupu R, Menendez JA. 2006. Pharmacological Inhibitors of Fatty Acid Synthase (FASN)-Catalyzed Endogenous Fatty Acid Biogenesis: A New Family of Anti-Cancer Agents? *Current Pharmaceutical Biotechnology* 7: 495-502
- Majewski N, Nogueira V, Bhaskar P, Coy PE, Skeen JE, et al. 2004. Hexokinase-mitochondria interaction mediated by Akt is required to inhibit apoptosis in the presence or absence of Bax and Bak. *Mol Cell* 16: 819-30
- Majumder PK, Sellers WR. 2005. Akt-regulated pathways in prostate cancer. *Oncogene* 24: 7465-74
- Maltepe E, Schmidt JV, Baunoch D, Bradfield CA, Simon MC. 1997. Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. *Nature* 386: 403-7
- Marjanovic S, Wielburski A, Nelson BD. 1988. Effect of phorbol myristate acetate and concanavalin A on the glycolytic enzymes of human peripheral lymphocytes. *Biochim Biophys Acta* 970: 1-6
- Mathupala SP, Ko YH, Pedersen PL. 2006. Hexokinase II: cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria. *Oncogene* 25: 4777-86
- Maxwell PH, Dachs GU, Gleadle JM, Nicholls LG, Harris AL, et al. 1997. Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth. *Proc Natl Acad Sci U S A* 94: 8104-9
- Mazurek S, Boschek CB, Hugo F, Eigenbrodt E. 2005. Pyruvate kinase type M2 and its role in tumor growth and spreading. *Semin Cancer Biol* 15: 300-8
- McFate T, Mohyeldin A, Lu H, Thakar J, Henriques J, et al. 2008. Pyruvate dehydrogenase complex activity controls metabolic and malignant phenotype in cancer cells. *J Biol Chem* 283: 22700-8
- McKeehan WL. 1982. Glycolysis, glutaminolysis and cell proliferation. *Cell Biol Int Rep* 6: 635-50

- Meldolesi MF, Macchia V, Laccetti P. 1976. Differences in phosphofructokinase regulation in normal and tumor rat thyroid cells. *J Biol Chem* 251: 6244-51
- Menendez JA, Lupu R. 2007. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nat Rev Cancer* 7: 763-77
- Moreno-Sanchez R, Rodriguez-Enriquez S, Marin-Hernandez A, Saavedra E. 2007. Energy metabolism in tumor cells. *FEBS J* 274: 1393-418
- Morgan DG, Menetret JF, Radermacher M, Neuhof A, Akey IV, et al. 2000. A comparison of the yeast and rabbit 80 S ribosome reveals the topology of the nascent chain exit tunnel, inter-subunit bridges and mammalian rRNA expansion segments. *J Mol Biol* 301: 301-21
- Munyon WH, Merchant DJ. 1959. The relation between glucose utilization, lactic acid production and utilization and the growth cycle of L strain fibroblasts. *Experimental Cell Research* 17: 490-8
- Nakamoto T. 2009. Evolution and the universality of the mechanism of initiation of protein synthesis. *Gene* 432: 1-6
- Neidhardt FC, Umberger HE. 1996. Chemical Composition of *Escherichia coli*. In *Escherichia coli and Salmonella : cellular and molecular biology*, ed. FC Neidhardt, R Curtiss, pp. 13-6. Washington, D.C.: ASM Press
- Neuzil J, Dyason JC, Freeman R, Dong LF, Prochazka L, et al. 2007. Mitocans as anti-cancer agents targeting mitochondria: lessons from studies with vitamin E analogues, inhibitors of complex II. *J Bioenerg Biomembr* 39: 65-72
- Newsholme EA, Crabtree B, Ardawi MS. 1985a. Glutamine metabolism in lymphocytes: its biochemical, physiological and clinical importance. *Q J Exp Physiol* 70: 473-89
- Newsholme EA, Crabtree B, Ardawi MS. 1985b. The role of high rates of glycolysis and glutamine utilization in rapidly dividing cells. *Biosci Rep* 5: 393-400
- Noguchi Y, Saito A, Miyagi Y, Yamanaka S, Marat D, et al. 2000. Suppression of facilitative glucose transporter 1 mRNA can suppress tumor growth. *Cancer Lett* 154: 175-82
- Ogretmen B, Hannun YA. 2004. Biologically active sphingolipids in cancer pathogenesis and treatment. *Nat Rev Cancer* 4: 604-16
- Ookhtens M, Kannan R, Lyon I, Baker N. 1984. Liver and adipose tissue contributions to newly formed fatty acids in an ascites tumor. *Am J Physiol* 247: R146-53
- Oskam R, Rijksen G, Staal GE, Vora S. 1985. Isozymic composition and regulatory properties of phosphofructokinase from well-differentiated and anaplastic medullary thyroid carcinomas of the rat. *Cancer Res* 45: 135-42
- Osthus RC, Shim H, Kim S, Li Q, Reddy R, et al. 2000. Deregulation of glucose transporter 1 and glycolytic gene expression by c-Myc. *J Biol Chem* 275: 21797-800
- Owen OE, Kalhan SC, Hanson RW. 2002. The key role of anaplerosis and cataplerosis for citric acid cycle function. *J Biol Chem* 277: 30409-12
- Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC. 2006. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab* 3: 187-97
- Pfeiffer T, Schuster S, Bonhoeffer S. 2001. Cooperation and competition in the evolution of ATP-producing pathways. *Science* 292: 504-7
- Postovit LM, Adams MA, Lash GE, Heaton JP, Graham CH. 2002. Oxygen-mediated regulation of tumor cell invasiveness. Involvement of a nitric oxide signaling pathway. *J Biol Chem* 277: 35730-7

- Pouyssegur J, Dayan F, Mazure NM. 2006. Hypoxia signalling in cancer and approaches to enforce tumour regression. *Nature* 441: 437-43
- Preul MC, Caramanos Z, Collins DL, Villemure J-G, Leblanc R, et al. 1996. Accurate, noninvasive diagnosis of human brain tumors by using proton magnetic resonance spectroscopy. *Nat Med* 2: 323-5
- Racker E. 1976. Why do tumor cells have a high aerobic glycolysis? *J Cell Physiol* 89: 697-700
- Reitzer LJ, Wice BM, Kennell D. 1979. Evidence that glutamine, not sugar, is the major energy source for cultured HeLa cells. *J Biol Chem* 254: 2669-76
- Rolland F, Winderickx J, Thevelein JM. 2002. Glucose-sensing and -signalling mechanisms in yeast. *FEMS Yeast Res* 2: 183-201
- Roos D, Loos JA. 1970. Changes in the carbohydrate metabolism of mitogenically stimulated human peripheral lymphocytes. I. Stimulation by phytohaemagglutinin. *Biochim Biophys Acta* 222: 565-82
- Rutter J, Reick M, Wu LC, McKnight SL. 2001. Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science* 293: 510-4
- Ryan HE, Lo J, Johnson RS. 1998. HIF-1 alpha is required for solid tumor formation and embryonic vascularization. *EMBO J* 17: 3005-15
- Scholnick P, Lang D, Racker E. 1973. Regulatory mechanisms in carbohydrate metabolism. IX. Stimulation of aerobic glycolysis by energy-linked ion transport and inhibition by dextran sulfate. *J Biol Chem* 248: 5175
- Semenza GL. 2010a. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene* 29: 625-34
- Semenza GL. 2010b. HIF-1: upstream and downstream of cancer metabolism. *Curr Opin Genet Dev* 20: 51-6
- Sengupta S, Peterson TR, Sabatini DM. 2010. Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Mol Cell* 40: 310-22
- Sherman IW. 1998. Carbohydrate Metabolism of Asexual Stages. In *Malaria, Parasite Biology, Pathogenesis and Protection*, ed. IW Sherman, pp. 135-43: ASM Press
- Shim H, Dolde C, Lewis BC, Wu CS, Dang G, et al. 1997. c-Myc transactivation of LDH-A: implications for tumor metabolism and growth. *Proc Natl Acad Sci U S A* 94: 6658-63
- Sloan EJ, Ayer DE. 2010. Myc, Mondo, and Metabolism. *Genes Cancer* 1: 587-96
- Staal GE, Kalff A, Heesbeen EC, van Veelen CW, Rijkse G. 1987. Subunit composition, regulatory properties, and phosphorylation of phosphofructokinase from human gliomas. *Cancer Res* 47: 5047-51
- Steck TL, Kaufman S, Bader JP. 1968. Glycolysis in chick embryo cell cultures transformed by Rous sarcoma virus. *Cancer Res* 28: 1611-9
- Stein WH, Moore S. 1954. The free amino acids of human blood plasma. *J Biol Chem* 211: 915-26
- Stoner GD, Merchant DJ. 1972. Amino acid utilization by L-M strain mouse cells in a chemically defined medium. *In Vitro* 7: 330-43
- Swietach P, Vaughan-Jones RD, Harris AL. 2007. Regulation of tumor pH and the role of carbonic anhydrase 9. *Cancer Metastasis Rev* 26: 299-310
- Thompson CB. 2009. Metabolic enzymes as oncogenes or tumor suppressors. *N Engl J Med* 360: 813-5

- Tong X, Zhao F, Mancuso A, Gruber JJ, Thompson CB. 2009a. The glucose-responsive transcription factor ChREBP contributes to glucose-dependent anabolic synthesis and cell proliferation. *Proc Natl Acad Sci U S A* 106: 21660-5
- Tong X, Zhao F, Thompson CB. 2009b. The molecular determinants of de novo nucleotide biosynthesis in cancer cells. *Curr Opin Genet Dev* 19: 32-7
- Tsujimoto Y. 1997. Apoptosis and necrosis: intracellular ATP level as a determinant for cell death modes. *Cell Death Differ* 4: 429-34
- Tu BP, Kudlicki A, Rowicka M, McKnight SL. 2005. Logic of the yeast metabolic cycle: temporal compartmentalization of cellular processes. *Science* 310: 1152-8
- Valgepea K, Adamberg K, Nahku R, Lahtvee PJ, Arike L, Vilu R. 2010. Systems biology approach reveals that overflow metabolism of acetate in *Escherichia coli* is triggered by carbon catabolite repression of acetyl-CoA synthetase. *BMC Syst Biol* 4: 166
- Vander Heiden MG, Cantley LC, Thompson CB. 2009. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324: 1029-33
- Vander Heiden MG, Locasale JW, Swanson KD, Sharfi H, Heffron GJ, et al. 2010. Evidence for an Alternative Glycolytic Pathway in Rapidly Proliferating Cells. *Science* 329: 1492-9
- Vander Heiden MG, Plas DR, Rathmell JC, Fox CJ, Harris MH, Thompson CB. 2001. Growth factors can influence cell growth and survival through effects on glucose metabolism. *Mol Cell Biol* 21: 5899-912
- Varghese B, Swaminathan G, Plotnikov A, Tzimas C, Yang N, et al. 2010. Prolactin inhibits activity of pyruvate kinase M2 to stimulate cell proliferation. *Mol Endocrinol* 24: 2356-65
- Voet D, Voet JG. 2004. *Biochemistry*. New York: J. Wiley & Sons. 2 v. (xv, 1560 p.) pp.
- Vora S, Halper JP, Knowles DM. 1985. Alterations in the activity and isozymic profile of human phosphofructokinase during malignant transformation in vivo and in vitro: transformation- and progression-linked discriminants of malignancy. *Cancer Res* 45: 2993-3001
- Wang T, Marquardt C, Foker J. 1976. Aerobic glycolysis during lymphocyte proliferation. *Nature* 261: 702-5
- Warburg O. 1956. On the origin of cancer cells. *Science* 123: 309-14
- Warburg O, Posener K, Negelein E. 1924. Ueber den stoffwechsel der tumoren. *Biochem Z* 152: 319-44
- Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, et al. 2010. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. *Cancer Cell* 17: 225-34
- Watford M, Lund P, Krebs HA. 1979. Isolation and metabolic characteristics of rat and chicken enterocytes. *Biochem J* 178: 589-96
- Wellen KE, Lu C, Mancuso A, Lemons JM, Ryczko M, et al. 2010. The hexosamine biosynthetic pathway couples growth factor-induced glutamine uptake to glucose metabolism. *Genes Dev* 24: 2784-99
- Wice BM, Reitzer LJ, Kennell D. 1981. The continuous growth of vertebrate cells in the absence of sugar. *J Biol Chem* 256: 7812-9
- Wieman HL, Wofford JA, Rathmell JC. 2007. Cytokine stimulation promotes glucose uptake via phosphatidylinositol-3 kinase/Akt regulation of Glut1 activity and trafficking. *Mol Biol Cell* 18: 1437-46

- Wilson JE. 2003. Isozymes of mammalian hexokinase: structure, subcellular localization and metabolic function. *J Exp Biol* 206: 2049-57
- Wilson KS, Noller HF. 1998. Molecular movement inside the translational engine. *Cell* 92: 337-49
- Windmueller HG, Spaeth AE. 1974. Uptake and metabolism of plasma glutamine by the small intestine. *J Biol Chem* 249: 5070-9
- Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang XY, et al. 2008. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. *Proc Natl Acad Sci U S A* 105: 18782-7
- Woehrer W, Roehr M. 1981. Regulatory aspects of bakers' yeast metabolism in aerobic fed-batch cultures. *Biotechnology and Bioengineering* 23: 567-81
- Wolf A, Agnihotri S, Micallef J, Mukherjee J, Sabha N, et al. 2011. Hexokinase 2 is a key mediator of aerobic glycolysis and promotes tumor growth in human glioblastoma multiforme. *J Exp Med*
- Wullschleger S, Loewith R, Hall MN. 2006. TOR signaling in growth and metabolism. *Cell* 124: 471-84
- Wuntch T, Chen RF, Vesell ES. 1970. Lactate dehydrogenase isozymes: kinetic properties at high enzyme concentrations. *Science* 167: 63-5
- Xu B, Jahic M, Enfors SO. 1999. Modeling of overflow metabolism in batch and fed-batch cultures of *Escherichia coli*. *Biotechnol Prog* 15: 81-90
- Yoo H, Antoniewicz MR, Stephanopoulos G, Kelleher JK. 2008. Quantifying reductive carboxylation flux of glutamine to lipid in a brown adipocyte cell line. *J Biol Chem* 283: 20621-7
- Yoo H, Stephanopoulos G, Kelleher JK. 2004. Quantifying carbon sources for de novo lipogenesis in wild-type and IRS-1 knockout brown adipocytes. *J Lipid Res* 45: 1324-32
- Yun J, Rago C, Cheong I, Pagliarini R, Angenendt P, et al. 2009. Glucose deprivation contributes to the development of KRAS pathway mutations in tumor cells. *Science* 325: 1555-9
- Yuneva M, Zamboni N, Oefner P, Sachidanandam R, Lazebnik Y. 2007. Deficiency in glutamine but not glucose induces MYC-dependent apoptosis in human cells. *J Cell Biol* 178: 93-105
- Zhang Q, Piston DW, Goodman RH. 2002. Regulation of corepressor function by nuclear NADH. *Science* 295: 1895-7
- Zielke HR, Ozand PT, Tildon JT, Sevdalian DA, Cornblath M. 1978. Reciprocal regulation of glucose and glutamine utilization by cultured human diploid fibroblasts. *J Cell Physiol* 95: 41-8
- Zu XL, Guppy M. 2004. Cancer metabolism: facts, fantasy, and fiction. *Biochem Biophys Res Commun* 313: 459-65

Figure captions and Tables

Figure 1. Metabolic pathways active in proliferating cells. This schematic represents our current understanding of how glycolysis, oxidative phosphorylation, the pentose phosphate pathway, and glutamine metabolism contribute to biomass precursors. Enzymes that control critical steps and are often overexpressed or mutated in cancer cells are shown in blue. Nucleotides that can be incorporated into DNA and RNA are highlighted in light blue; representative lipids are highlighted in green; and nonessential amino acids are highlighted in orange. Some key metabolites that serve as important precursors for biomass production are boxed. Glucose and glutamine, the major carbon sources in most proliferating cells, are shown; however, other metabolites can serve as carbon sources if available. In the mitochondria, conversion between glutamate and α -ketoglutarate requires NAD(P)/NAD(P)H only if catalyzed by glutamate dehydrogenase.

Figure 2. Schematic showing the approximate contributions of glucose carbons and glutamine carbons and nitrogens to biomass, lactate, and CO₂ in a proliferating cell.

Table 1. Cofactor requirements for *de novo* nucleotide, amino acid, and lipid biosynthesis from indicated precursors. Cofactors required for glucose import and precursor synthesis are not included. Ribose-5-phosphate requires 2 NADP⁺ molecules if generated oxidatively from glucose; however, it can also be made non-oxidatively, and there is evidence that this is a major pathway used in at least some cancer cells (Boros et al 1998). At least one NADPH is required for the reaction cycle involving thymidylate synthase (which uses 5,10-methylene-THF) in dTTP synthesis and this requirement is included. Additional NADP(H) requirements for folate metabolites used in the synthesis of purine bases and amino acids can vary and are not included. Cofactor requirements for lipids that incorporate fatty acyl-CoA's (e.g., phospholipids, triacylglycerols, and sphingolipids) vary and are not included.

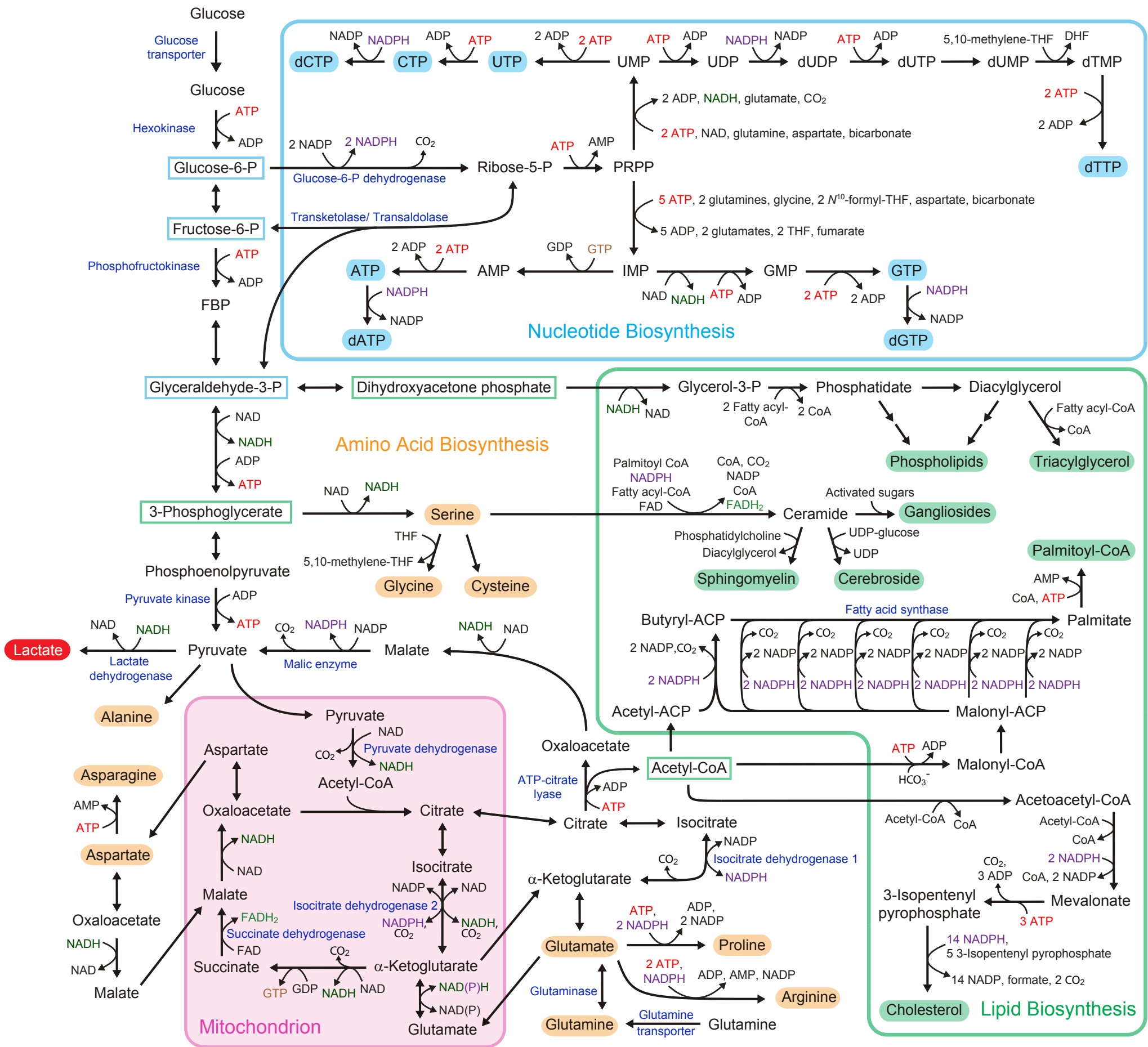
	Metabolite	Precursor	ATP	NAD ⁺	NADPH	GTP
NUCLEOTIDES	ATP	Ribose-5-P	8	--	--	1
	GTP	Ribose-5-P	9	1	--	--
	CTP	Ribose-5-P	6	1	--	--
	UTP	Ribose-5-P	4	1	--	--
	dATP	Ribose-5-P	8	--	1	1
	dGTP	Ribose-5-P	9	1	1	--
	dCTP	Ribose-5-P	6	1	1	--
	dTTP	Ribose-5-P	7	1	2	--
NONESSENTIAL AMINOACIDS	Cysteine	3-Phosphoglycerate	--	1	--	--
	Glycine	3-Phosphoglycerate	--	1	--	--
	Serine	3-Phosphoglycerate	--	1	--	--
	Alanine	Pyruvate	--	--	--	--
	Asparagine	Oxaloacetate	1	--	--	--
	Aspartate	Oxaloacetate	--	--	--	--
	Glutamate	α -Ketoglutarate	--	--	--*	--
	Glutamine	α -Ketoglutarate	1	--	--*	--
	Proline	α -Ketoglutarate	1	--	2*	--
Arginine	α -Ketoglutarate	1	--	1*	--	
Tyrosine	Phenylalanine	--	--	--	--	
LIPIDS	Palmitoyl-CoA	Acetyl-CoA	8	--	14	--
	Cholesterol	Acetyl-CoA	18	--	26	--

*An additional NAD(P)H is required if the α -ketoglutarate to glutamate conversion is catalyzed by mitochondrial glutamate dehydrogenase.

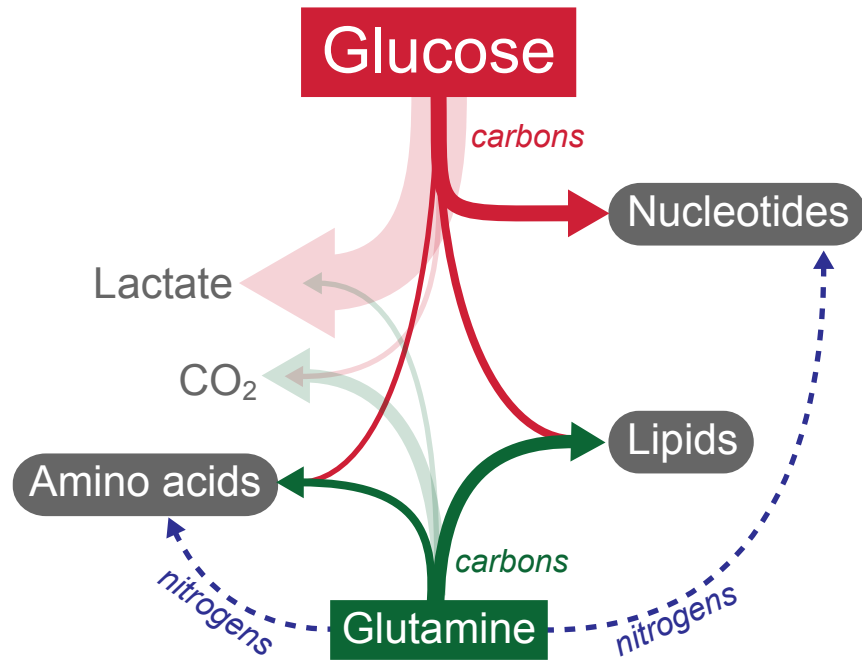
Table 2. Biosynthetic precursors that provide the carbons and nitrogens in NTPs and dNTPs.

The majority of carbons in nucleotides are derived from 5-phosphoribosyl- α -pyrophosphate (PRPP), a metabolite derived from glycolytic intermediates. Thus, glycolysis is a major carbon contributor to nucleotide biosynthesis.

dNTP	#	Precursor	Carbon contribution	Nitrogen contribution
ATP/ dATP	1	5-Phosphoribosyl- α -pyrophosphate	5	--
	2	N^{10} -formyl-tetrahydrofolates	2	--
	1	Bicarbonate	1	--
	1	Glycine	2	1
	1	Aspartate	--	2
	2	Glutamines	--	2
Total			10	5
GTP/ dGTP	1	5-Phosphoribosyl- α -pyrophosphate	5	--
	2	N^{10} -formyl-tetrahydrofolates	2	--
	1	Bicarbonate	1	--
	1	Glycine	2	1
	1	Aspartate	--	1
	2	Glutamines	--	3
Total			10	5
UTP/ CTP/ dCTP	1	5-Phosphoribosyl- α -pyrophosphate	5	--
	1	Bicarbonate	1	--
	1	Aspartate	3	1
	1	Glutamine	--	1
Total			9	2
dTTP	1	5-Phosphoribosyl- α -pyrophosphate	5	--
	1	Bicarbonate	1	--
	1	Aspartate	3	1
	1	Glutamine	--	1
	1	5,10-methylene-tetrahydrofolate	1	1
Total			10	2



Kwon and Vander Heiden Figure 1



Kwon and Vander Heiden Figure 2