Published in final edited form as:

Semin Cell Dev Biol. 2015 July ; 43: 11–21. doi:10.1016/j.semcdb.2015.08.003.

MYC and metabolism on the path to cancer

Annie L. Hsieh^{a,b,c}, Zandra E. Walton^{a,b}, Brian J. Altman^{a,b}, Zachary E. Stine^{a,b}, and Chi V. Dang^{a,b,d,*}

^a Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

^b Abramson Cancer Center, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

^c Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

^d Division of Hematology–Oncology, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Abstract

The *MYC* proto-oncogene is frequently deregulated in human cancers, activating genetic programs that orchestrate biological processes to promote growth and proliferation. Altered metabolism characterized by heightened nutrients uptake, enhanced glycolysis and glutaminolysis and elevated fatty acid and nucleotide synthesis is the hallmark of *MYC*-driven cancer. Recent evidence strongly suggests that Myc-dependent metabolic reprogramming is critical for tumorigenesis, which could be attenuated by targeting specific metabolic pathways using small drug-like molecules. Understanding the complexity of *MYC*-mediated metabolic re-wiring in cancers as well as how *MYC* cooperates with other metabolic drivers such as mammalian target of rapamycin (mTOR) will provide translational opportunities for cancer therapy.

Keywords

Myc; Metabolism; Cancer; mTOR

1. Introduction

MYC was first discovered as a cellular homolog of v-*myc*, a retroviral gene that was found to induce tumorigenesis in birds [1,2]. Its role in human cancer was first recognized through observations of the pathognomonic chromosomal translocations, which juxtaposed the *MYC* gene next to constitutively active immunoglobulin enhancers, in human Burkitt's lymphoma [3,4].

The *MYC* proto-oncogene is aberrantly overexpressed in over half of human cancers [5]. It is one of the most frequently amplified oncogene in human cancers and a member of the

* Corresponding author at: Abramson Cancer Center, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. dangvchi@exchange.upenn.edu (C.V. Dang)..

larger MYC family, which includes MYCN and MYCL [6,7]. Dysregulations of *MYC*, such as amplification, chromosome translocation or loss of upstream repressors, have been found in many human cancers [8,9].

MYC encodes the Myc transcription factor, which dimerizes with Max – another helix–loop–helix leucine zipper protein – to bind DNA and alter gene expression. Myc belongs to the extended Myc transcriptional factor network that includes Max, Mxd proteins, Mga, Mnt, Mxl, Mxlp, and the carbohydrate response element binding protein (ChREBP) [10]. Myc, as a transcriptional factor, activates many genes that are involved in cellular processes, including transcription, translation, chromatin modification and protein degradation.

Cancer cells take advantage of Myc's broad reach to reprogram and augment the most critical processes for survival, particularly metabolism. Metabolism is comprised of networks of pathways that can be categorized into distinct biological functions: catabolism, anabolism, and redox homeostasis. Catabolism involves multiple processes that break down nutrients to generate ATPs and produce reductive power as NADPH, whereas anabolism import and transform nutrients for macromolecule biosynthesis pathways to support cell growth and proliferation. These metabolic pathways involve the mitochondrion and oxidative phosphorylation that generates the bulk of reactive oxygen species (ROS). ROS can serve as signaling molecules, but at very high levels ROS can be toxic and must be titrated by redox homeostatic pathways. The balance between catabolism and anabolism is largely determined by external nutrients availability and cell states. While resting cells can equilibrate their bioenergetic demands with their environment, rapidly growing cancer cells often have a biomass demand that outstrips sources and confront limited nutrients and oxygen in the tumor microenvironment. By enhancing both catabolism and anabolism, Myc allows cancer cells to meet these challenges and sustain growth and proliferation.

In this review, we provide an overview of how Myc regulates cellular metabolism to promote growth and cooperates with other major metabolic drivers, thus providing for the reader a comprehensive view of metabolic control in growing cancer cells.

2. Cellular homeostasis

The activation of metabolic pathways is very dynamic and highly dependent on the state of cell and the external cues, such as growth signals and nutrients. Mammalian cells exhibit two major states in their life: resting state or dividing state. The majority of cells in our body are differentiated and in the resting state, using energy to maintain basal metabolism. In contrast, a small population of cells in our body exists in the dividing state, including intestinal mucosal cells and hematopoietic cells. These cells have higher energy demands for growth and proliferation.

In resting cells, homeostasis is accomplished by generating sufficient energy from extracellular nutrients to sustain protein synthesis, maintain cellular membrane potentials, turn over or repair damaged organelles and macromolecules. To maintain a constant level of ATP, the major high-energy molecule in the cells, several key factors tightly regulate metabolic pathways in response to external nutrient availability. For example, in the nutrient-replete state, abundant amino acids activate mTOR complex, which stimulates anabolic

pathways including protein synthesis, lipid synthesis and ribosomal biogenesis [11]. Conversely, under the nutrient-depleted state, low energy induces AMP-activated protein kinase (AMPK), which phosphorylates and inhibits the majority of anabolic pathways while turning on catabolic, energy-producing pathways including glycolysis, oxidative phosphorylation and fatty acid oxidation [12]. Notably, inactivated mTOR and activated AMPK in response to fasting collectively induce autophagy (self-eating), allowing cells to digest their own organelles to produce energy and recycle cellular building blocks [11,12].

Because food availability and demands for energy-consuming organismal activity are not constant, many organisms have evolved systems to anticipate predictable changes and thereby optimize metabolic efficiency and enhance evolutionary fitness. One such example is circadian rhythm, an autonomous oscillation of biological processes with approximately 24-h period, coordinates feeding and sleeping in both unicellular and multicellular organisms. In humans and mice, autonomous cellular metabolic oscillation is synchronized with the day–night cycle through a central clock residing in the suprachiasmatic nucleus in hypothalamus [13]. The central clock synchronizes with light *via* signals from the retina and in turn sends neuronal and hormonal signals to synchronize clocks found in peripheral tissues and cells [13]. Virtually all cells possess a molecular clock circuitry with interlocking feedback loops that is comprised of transcriptional activators, such as Clock and Bmal1, and the transcriptional repressors, PERs, CRYs and REV-ERBs. Clock and Bmal1 form a complex Clock:Bmal1 that binds to the canonical DNA sequence 5'-CACGTG-3' (E-Box) to activate several circadian regulators including PERs, CRYs and REV-ERBs. PERs and CRYs form a complex in the cytoplasm and translocate into the nucleus to repress Clock:Bmal1 transcriptional activity, whereas REV-ERBs directly repress Bmal1 expression. These two feedback loops give rise to the observed 24-h oscillation [13]. Clock:Bmal1 diurnally drives a number of biological pathways, particularly metabolic pathways, to coordinate with feeding and sleeping [14].

Intriguingly, the “E-box” motif used by the Clock proteins could also be bound by other transcription factors that are involved in metabolism and growth. The carbohydrate-responsive element-binding protein (ChREBP), sterol regulatory element-binding protein (SREBP), microphthalmia-associated transcription factor (MITF), transcription factor EB (TFEB), transcription factor E3 (TFE3), and oncoprotein Myc [5,15–17] are helix–loop–helix proteins that have canonical E-box binding activities, suggesting the possible interplay between these transcription factors in regulating metabolism, cell homeostasis and growth.

We surmise that the E-box motif, which is highly enriched in regulatory sequences of the human genome provides a means for transcriptional orchestration of anabolic and catabolic metabolism with cell growth and homeostasis [18]. Circadian chromatin Immunoprecipitation Sequencing (ChIP-seq) and gene expression studies in the mouse liver have found oscillation of genes involved in glycolysis, oxidative phosphorylation, lipid synthesis and autophagy, regulated by diurnal Bmal1 E-box binding (Table 1) [19]. These Bmal1-regulated metabolic genes have also been documented as Myc target genes (Table 1) that exhibit increased expression in the Myc-driven murine liver cancer (Fig. 1) [20,21]. Together these observations suggest that, while Bmal1 diurnally regulates metabolism to maintain homeostasis, Myc may substitute for Bmal1 during proliferation to activate

metabolic genes in a sustained and enhanced manner that supports cell growth and division [5] (Fig. 2). Notably, several of these genes encode rate-limiting enzymes in the metabolic pathways, such as phosphofructokinase (PFK) in glycolysis, HMG-CoA reductase (HMGCR) in cholesterol synthesis pathway and NAMPT in NAD⁺ salvage pathways; thus by controlling the magnitude and temporal expression of these genes, Myc can effectively reprogram metabolism. Given these observations, we surmise that the ChREBP, SREBP, and MITF/TFE transcription factors are also likely to have inter-related activities to control carbohydrate and lipid metabolism and autophagy, respectively. These factors along with Clock/Bmal and the extended Myc transcription factor family form a tapestry of transcription factors that orchestrate metabolism and cell growth (Fig. 2).

3. Cell growth

Unlike resting cells, which strive to maintain homeostasis, rapid dividing cells undergo cell mass accumulation to promote growth and proliferation. Cell growth requires ATP, NADPH and a significant pool of building blocks, including fatty acids and cholesterol for cell membranes, nucleotides for DNA and RNA, amino acids for enzymes and structural proteins, and carbohydrates for post-translational modifications.

The fundamentals of cell growth have been extensively studied in simple organism such as the yeast species *Saccharomyces cerevisiae*. In *S. cerevisiae*, cell growth is coupled with cell cycle progression, as yeast cells must reach certain size before cell division occurs [22]. Ribosomes, the essential organelle for protein synthesis, constitute the majority of cell mass [23]. Therefore, ribosome biogenesis is the key to cell mass accumulation that ultimately leads to cell growth and division. In yeast, nutrients are the vital external cue for cell growth [24]. Once the nutrients are available, particularly glucose and glutamine, yeast cells initiate ribosome biogenesis thorough protein kinase A and TORC1, which releases transcription of ribosome biogenesis genes (Ribi genes) from the inhibition by Dot6 and Tod6 [25]. Mammalian cells, unlike yeast cells, are constantly exposed to nutrients present in the circulation. To prevent aberrant proliferation, additional cues, such as growth factors, are required for growth [24].

Upon growth factor binding, receptor tyrosine kinases dimerize and autophosphorylate tyrosine residues to relay signals to activate two parallel major signal transduction pathways: PI3K/AKT/mTOR and RAS-RAF-MEK-ERK [26] (Fig. 3). These pathways ultimately result in the regulation of protein synthesis and transcription.

In the PI3K/AKT/mTOR arm of the signaling cascade, phosphorylated tyrosine residues on the receptor tyrosine kinase directly promote phosphoinositide 3-kinase (PI3K) activation. The activated PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate [PI(4,5)P₂] to phosphatidyl-inositol-3,4,5-trisphosphate [PI(3, 4,5)P₃], whereas PTEN mediates the dephosphorylation of PI(3,4,5)P₃ (27). PI(3,4,5)P₃ serves as a docking site to interact with signaling proteins harboring pleckstrin-homology (PH) domain, including protein serine–threonine kinases Akt and phosphoinositide-dependent kinase (PDK1). PDK1 phosphorylates and activates Akt, resulting in a subsequent signaling cascade that leads to cell growth, enhanced metabolic activity and cell survival [27].

An important downstream effector of Akt is mTOR, a serine/threonine kinase that exhibits two functionally distinct complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 is well-characterized as one of the major driver of cell growth by promoting cap-dependent translation through phosphorylation of the ribosomal protein S6 kinases (S6K1 and S6K2) and the eukaryotic initiation factor 4E (eIF4E)-binding proteins (4E-BP1 and 4E-BP2) [28]. mTORC2, on the other hand, is known to activate Akt, which in turn phosphorylates and inhibits mTORC1 repressor tuberous sclerosis complex 2 (TSC2) [29].

In the RAS-RAF-MEK-ERK arm of the signaling cascade, growth factor stimulation initiates a series of phosphorylation events and ultimately leads to the activation of ERK. ERK then translocate to the nucleus to induce the expression of transcription factors such as c-FOS and Myc to promote transcription and translation of genes that involved in growth and proliferation (Fig. 3).

Given the importance of growth factor signaling, one can anticipate that deregulation of the components in the pathway can lead to cancer. Indeed, PI3K and Akt is often upregulated in cancer [30]. Ras is mutated in 30% of human cancer and BRAF is mutated in 7% of human cancer [31]. Cancer cells accumulate genetic and/or epigenetic events that re-wire the existing metabolic network or create new metabolic pathways that endow a capacity to support deregulated cancer cell growth and division.

In addition to stimulating ERK-dependent transcription of Myc, growth factor signaling increases Myc activity in a number of ways. Activated Akt phosphorylates and inhibits glycogen synthase kinase 3 (GSK3), which normally phosphorylates Myc to keep it inactive [27]. A recent study has shown mTORC2 can upregulate Myc expression through forkhead box O (FOXO)-dependent microRNA inhibition [32]. Furthermore, an alternative splicing product Delta Max in response to growth factor signaling has been implicated to increase Myc function [33].

It is not clear though, how mTOR and Myc, two major regulators downstream of growth factor pathway, collaborate to promote cell growth. We surmise that, in response to growth factor and nutrient, mTOR initiates an immediate induction of multiple anabolic pathways through post-transcriptional mechanism. However, a subsequent transcriptional response is essential for cells to sustain growth by producing new mRNAs, ribosomes and other proteins that are involved in translational machinery and various macromolecular biosynthetic pathways. Although mTOR has been implicated in activation of the transcriptional factor SREBP for lipid synthesis and hypoxia inducible factor subunit alpha (HIF1 α) for glycolysis [34], Myc induces a multitude of gene transcription to augment mRNA synthesis of components of the translational machinery as well as key metabolic enzymes to provide both the building blocks and energy that is critical for cell growth [35] (Fig. 4).

4. Transcription factor Myc

Myc is downstream of a number of growth-promoting signaling pathways, including those initiated by growth factor-stimulated receptor tyrosine kinases, T cell receptors and WNT signaling [5]. In non-transformed cells, cell cycle checkpoints protect against aberrant Myc

activity. For example, p53-dependent apoptosis is induced upon acute Myc overexpression in primary mouse embryo fibroblasts [36]. Myc also induces expression of ARF, which directly inhibits Myc-mediated transcription and tumorigenesis in a p53-independent manner [37]. In transformed cells, however, Myc activation selects for surviving cells that have mutation or deletion of p53 or ARF to prevent apoptosis [36].

The indispensable role of Myc in cancer has been observed in both human cell lines and murine models. In human Burkitt's lymphoma cell line, loss of Myc was found to hinder cell proliferation [38]. In a mouse model with liver specific, doxycycline (DOX)-suppressed Myc transgene, Myc activation by withdrawal of DOX gives rise to aggressive liver tumors that resemble human hepatocellular carcinoma [39]. Intriguingly, these invasive tumors regress when the tumor-bearing animals are re-exposed to DOX to inactivate Myc [39]. These findings suggest that Myc is not only essential for tumor initiation, but also critical for maintenance of established tumors, revealing these tumors' addiction to Myc signaling.

It is generally thought that Myc activity is largely through its regulation of transcription. The key mechanism allowing Myc to globally enhance transcription is through augmenting P-TEFb-dependent phosphorylation of RNA polymerase II to release it from the paused state to the elongation state [40]. Studies further demonstrate the presence of Myc at nearly all the open chromatin regions in the genome, eliciting a model that Myc is a universal amplifier and the level of amplification purely depend on the patterns of open chromatin within the genes [20,41,42]. These observations suggest that Myc simply amplifies the existing transcriptional program within the cell but it is not clear how Myc modulates the ratio between growth-promoting genes *versus* growth-arresting genes to promote growth and proliferation. Follow-up studies have revealed that Myc is able to selectively activate expression of certain gene sets while globally enhance transcription [43]. For instance, overexpressed Myc was found to “invade” enhancers and low affinity core promoters that normally bind little Myc at physiological level as it saturates the high affinity promoters, suggesting supraphysiological level of Myc preferentially activate expression of the genes harboring low affinity promoters or enhancers [20,44,45]. Furthermore, high level of Myc recruits Miz1 through protein–protein interaction and enhances Miz1-dependent repression [45]. Together, these findings indicate that while Myc generally augments RNA elongation throughout the genome, it does so in cooperation with other transcription factors that provide for selective gene expression amplification to coordinate metabolism and cell growth [44,46].

5. Myc-driven metabolic reprogramming

5.1. Myc regulation of glucose and glutamine metabolism

As discussed earlier, yeast cells sense glucose and glutamine in the environment and activate expression of Ribi genes to initiate ribosome biogenesis [25]. In *Drosophila*, glucose activates insulin signaling, which acts through PI3K/Akt pathway to activate TOR and repress FOXO [47]. *Drosophila* Myc (dMyc) is identified as a convergent node downstream of TOR and FOXO signaling in response to nutrients [48]. When glucose is abundant, activated TOR rapidly increases dMyc protein to drive ribosome biogenesis. Conversely, when fruit flies are under fasting, FOXO is derepressed from Akt and directly inhibits dMyc

expression [48]. In mammals, Myc level was also observed to correlate with nutrient level. For example, in rat liver, Myc expression dramatically decreases under glucose deprivation [49]. Moreover, FOXO3a was found to inhibit Myc activity through induction of Myc antagonist Mxi1 proteins in colon cancer cells [50]. Taken together, the physiological role of Myc as a “nutrient sensor” seems to be well conserved during evolution, suggesting that Myc is critical in the process of utilizing extracellular glucose and glutamine for macromolecule synthesis. The importance of Myc in glucose and glutamine metabolism is further corroborated by heightened glucose and glutamine uptake that observed in neoplastic cells, where unleashed Myc is continuously activated.

Glucose is one of the major nutrients that mammalian cells utilize to synthesize new membranes and organelles as well as to generate high energy molecules, such as ATP, NADH/NADPH and FADH. Glucose is taken up through glucose transporters (GLUTs) and phosphorylated by hexokinase. Glucose-6-phosphate then undergoes a series of reversible and irreversible reactions before conversion to two molecules of pyruvates by pyruvate kinase with a net production of 2 ATPs and 2 NADHs. Pyruvate can be converted to lactate by lactate dehydrogenase (LDH) or to citrate by pyruvate dehydrogenase to enter tricarboxylic acid cycle (TCA cycle).

Many genes involved in glucose metabolism have been documented to be Myc target genes. For example, Myc upregulates genes encoding glucose transporters and hexokinase to increase glucose import [51]. Myc also induces expression of glycolytic genes including phosphoglucose isomerase, phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, and enolase [51]. Furthermore, use of ¹³C-labeled glucose has revealed increased levels of glycolytic intermediates and lactate in Myc-driven Burkitt's lymphoma cells, suggesting Myc overexpression increases overall glycolytic flux [52]. This observation harkens back to findings by Dr. Otto Warburg back in the 1920s indicating high rates of glycolysis and lactate production in cancer cells even in the presence of oxygen [53]. Increased glycolytic flux gives rise to abundant glycolytic intermediates that can be used to generate macromolecules such as nucleotides, amino acids and fatty acids. To maintain high flux of glycolysis, Myc was observed to upregulate lactate dehydrogenase A (LDHA) to generate NAD⁺, which is a co-factor required for the glycolysis particularly by GAPDH [54]. The role of LDHA in Myc-driven cancer cells is underscored by the ability of a specific LDHA inhibitor FX11 to cause cell death [54]. Myc has also been shown to regulate alternative splicing of the rate-limiting enzyme pyruvate kinase by upregulating heterogeneous nuclear ribonucleoproteins (hnRNP) [55]. Pyruvate kinase has two distinct isoforms: PKM1 and PKM2. While the embryonic pyruvate kinase isoform, PKM2, promotes aerobic glycolysis, PKM1 favors oxidative phosphorylation. By upregulating hnRNPA1 and hnRNPA2, Myc maintains high ratio of PKM2/PKM1 to ensure high flux of glycolysis in the presence of oxygen [55].

Glutamine is another major nutrient for tumor cells, as it fuels the TCA cycle and also provides sources of nitrogen. Glutamine is imported into the cells through glutamine transporter ASCT2 and is then converted to glutamate by glutaminase (GLS). Several enzymes can further metabolize glutamate to α -ketoglutarate (α KG) to further enter TCA

cycle including glutamine dehydrogenase (GDH), glutamine pyruvate transaminase (GPT) or glutamine oxaloacetate transaminase (GOT).

Unlike most non-transformed cells that use glucose, Myc-driven cancer cells depend on glutamine metabolism [56]. Myc promotes glutamine import by directly inducing the expression of glutamine transporter ASCT2 [57]. In addition, Myc increases the conversion of glutamine to glutamate for subsequent oxidation in the TCA cycle by upregulating GLS both transcriptionally and post-transcriptionally [57,58]. The reliance on glutamine metabolism of Myc-driven cancers appears especially true under stress conditions, particularly glucose and oxygen deprivation [52]. Specifically, flux analysis using ^{13}C -labeled glutamine in Myc-overexpressing cells has revealed an enrichment of TCA cycle isotopologues under glucose-deprivation, suggesting while these cells favor glucose as the carbon sources for the TCA cycle, glutamine is an alternative carbon source in the absence of glucose [52]. Moreover, Myc overexpression has been shown sufficient to induce glutamine addiction. For example, high levels of Myc can convert fibroblasts to become addicted to glutamine as evidenced by apoptosis after glutamine withdrawal [59]. Intriguingly, TCA cycle intermediates oxaloacetate and pyruvate can rescue cell death, suggesting glutamine is the major carbon source for the TCA cycle in these Myc-overexpressing cells [59]. Furthermore, knockdown Myc in glioma cells can release cells from a glutamine-addicted state [57]. Given the fact that Myc-driven tumor is highly dependent on glutamine, it can be anticipated that Myc-driven tumor might be sensitive to inhibitors targeting glutamine metabolism. Indeed, decreased cell growth is observed in Myc-overexpressing cancer cells that treated with glutaminase inhibitors [52,60]. It further appears that, when glucose is abundant, the requirement for glutamine may stem from a need for glutamate to synthesize glutathione to protect cells against reactive oxygen species [52]. Moreover, it was observed that glutamine-derived glutamate contributes to Myc-dependent proline synthesis [61].

5.2. Myc regulation of protein synthesis and ribosome biogenesis

Like yeast, mammalian cells utilize glucose and glutamine to make protein, which constitutes a significant portion of cell mass. Protein synthesis is carried out by translational machinery, which is comprised of messenger RNA (mRNA), ribosomal RNA (rRNA), transfer RNA (tRNA) and ribosomal proteins. Loss of components in the protein synthesis pathway has been documented to lead to smaller cell size and block cell growth and proliferation [62,63].

The importance of Myc in protein synthesis in physiological conditions is suggested by the small cell and body size observed in flies harboring mutations in the *Drosophila melanogaster* homolog of mammalian Myc, *diminutive* [64–66]. In rapidly proliferating cells, particularly cancer cells, the ability of Myc to directly regulate multiple components of protein synthesis permits efficient response to growth signal. For instance, Myc recruits RNA polymerase I (Pol I) to activate rRNA synthesis, regulates RNA polymerase II (Pol II)-dependent transcription for mRNA and ribosomal protein genes and activates synthesis of 5S rRNA and tRNA through RNA polymerase III (Pol III) [67–69]. Furthermore, a

number of ribosomal proteins, including small ribosomal subunits (RPS) and large ribosomal subunits (RPL) are encoded by direct Myc target genes [70].

Myc-induced protein synthesis has been shown to be directly linked to its ability to promote tumorigenesis. Specifically, it was found that crossing E μ -Myc transgenic mice (Myc-induced lymphoma model) with mice compound heterozygous knockouts of ribosomal protein L24 and L38 (RPL24 and RPL38), which exhibit small overall body size and decreased protein synthesis rate, can restore protein synthesis rate to normal and thereby abrogate Myc-dependent tumor growth [70,71].

In addition to activating components of the ribosome, Myc regulates translational initiation factors, such as eIF4E, eIF4A and eIF4G [70,72]. Myc also induces mTOR-dependent phosphorylation and inhibition of eIF4E binding protein (4E-BP), a repressor of eIF4E, to facilitate cap-dependent mRNA translation [73]. In addition, simply blocking mTOR mediated 4E-BP phosphorylation in E μ -Myc mice dramatically decreases lymphoma occurrences in these animals. Furthermore, Myc was indicated to promote mRNA cap methylation, leading to faster translation initiation [74]. Perturbations of mRNA cap methylation by knocking down S-adenosyl-L-homocysteine hydrolase (SAHH) hinder cell growth and transformation [74]. Altogether, increased protein synthesis is not just a consequence of Myc hyperactivation; instead, it contributes to Myc-induced tumorigenesis [70]. Interestingly, eIF4E has been shown to directly induce expression of nucleotide synthesis enzyme phosphoribosyl pyrophosphate synthetase 2 (PRPS2), suggesting a synchrony between Myc-induced protein synthesis and nucleotide synthesis [75].

5.3. Myc regulation of nucleotide biosynthesis

Nucleotide synthesis is essential for highly proliferative cancer cells and glucose is the major source for making new DNA and RNA. Glucose can enter pentose phosphate pathway to produce ribose 5-phosphate (R5P), the precursor for DNA and RNA synthesis, and NADPH. R5P can further be converted by PRPS2 to 5-phosphoribosyl-PP (PRPP), which is a substrate for the enzymes of the nucleotide salvage pathway as well as *de novo* purine and pyrimidine synthesis pathways. Glucose can also contribute to nucleotide synthesis through incorporation of glyceraldehyde-3-phosphate (G3P) into serine and glycine. G3P is converted to 3-phospho-hydroxy-pyruvate by phosphoglycerate dehydrogenase (PHGDH), the rate-limiting enzyme in the serine synthesis pathway. A series of reactions that catalyzed by phosphoserine aminotransferase 1 (PSAT1) and phosphoserine phosphatase (PSPH) convert 3-phospho-hydroxy-pyruvate to serine. Serine can further be metabolized to glycine by serine hydroxymethyltransferase (SHMT).

Several genes that are involved in *de novo* purine and pyrimidine synthesis pathways have shown to be direct Myc targets [76]. For example, Myc directly regulates PRPS2, inosine monophosphate dehydrogenase (IMPDH 1/2) and thymidylate synthase (TS) [77,78]. Hence, inhibition of IMPDH using its inhibitor mycophenolic acid (MPA) leads to apoptosis in Myc-driven lymphoma cells [78].

Beyond directly regulating enzymes that are involved in *de novo* nucleotide synthesis pathway, Myc also promotes serine and glycine synthesis by channeling glycolytic

intermediates [79]. Interestingly, the expression of PHGDH, PSAT1 and PSPH is upregulated in Myc-induced liver tumors [79]. In addition, both cytoplasmic and mitochondrial SHMTs, SHMT1 and SHMT2, respectively, were documented to be direct Myc targets [80]. Specifically, SHMT2, but not SHMT1, is overexpressed in a variety of human cancers and coexpressed with PHGDH in neuroblastoma and breast cancer [81]. SHMT mediates the conversion of tetrahydrofolate (THF) into 5,10-methylenetetrahydrofolate (5,10-methylene THF), which indirectly contributes to NADPH production. Myc has been shown to induce SHMT2 under hypoxia in a HIF1 α -dependent manner to protect cells from the toxicity of ROS through NADPH production [81]. Accordingly, knockdown of SHMT2 gives rise to high level of ROS, which in turn impair cell survival both *in vitro* and *in vivo* [81].

Serine and glycine are two major inputs for one-carbon metabolism. One-carbon metabolism is comprised of the folate cycle and the methionine cycle and is the nexus of nucleotide synthesis, redox balance and methylation processes [82]. For example, in the folate cycle, THF can be converted to 5,10-methylene THF for pyrimidine synthesis or further converted to 10-formyl THF for purine synthesis. As discussed previously, the conversion of folate can concurrently generate NADPH for redox reactions. The methylene cycle, which is tightly linked to the folate cycle, starts when homocysteine accepts a carbon from 5-methyltetrahydrofolate (mTHF) to generate methionine. Methionine is further converted to S-adenyl methionine (SAM), the universal methyl group donor for DNA, RNA, histone and protein methylation events. Donation of a methyl group by SAM produces S-adenosylhomocysteine (SAH) that can be further hydrolyzed to homocysteine by S-adenosylhomocysteine hydrolase (SAHH).

SAHH is the link between one carbon metabolism and Myc-induced protein synthesis. Myc has been found to increase protein synthesis by promoting cap methylation of mRNA, a SAHH-dependent process [74,83]. For methylation to occur, an appropriate ratio of SAM to SAH is critical, since high levels of SAH will inhibit the demethylation reaction from SAM to SAH. SAHH neutralizes SAH thus maintains high SAM/SAH ratio. SAHH is a direct Myc target and loss of SAHH significantly decreases the cap methylation of mRNA without changing the total mRNA level [74]. Interestingly, knockdown of SAHH decreases Myc-induced cell proliferation and cell transformation, suggesting SAHH mediated mRNA cap methylation is required [74].

A large body of evidence has uncovered the roles of Myc in regulating nearly all components of the metabolic pathway. A key question begins to emerge: how does Myc coordinate multiple macromolecular biosynthesis pathways? As discussed earlier, SAHH is one example that couples one carbon metabolism with Myc-driven protein synthesis. Another example is phosphoribosyl pyrophosphate synthetase 2 (PRPS2), a rate-limiting enzyme that converts R5P to PRPP, which serves as a link between Myc-dependent protein translations and nucleotide synthesis [75]. PRPS2, but not PRPS1, exhibits a pyrimidine-rich translational element (PRTE) that interacts with the major translation initiation factor, eIF4E, which is also a direct Myc target [75,84,85]. Myc-induced eIF4E expression elevates translation of PRPS2 through PRTE, thereby stimulating nucleotide synthesis [75]. Deletion or knockdown of PRPS2 *in vitro* and *in vivo* increases apoptosis of B lymphocytes in the

context of Myc overexpression but has no effect in normal physiology, suggesting the junction controlled by PRPS2 between protein translation and nucleotide synthesis is particularly crucial for cancer cells compared to normal B cells [75].

5.4. Myc regulation of fatty acid and cholesterol metabolism

Cell growth and division rely on new membrane synthesis and require energy. Fatty acids are main components of phospholipids, which make up cell membrane, and triglyceride, the major form of energy storage in the cell. Cholesterol, another type of lipids, maintains membrane integrity and regulates its fluidity. Given the importance of lipid in cell growth, fatty acid and cholesterol metabolism is strictly regulated in normal cells and often upregulated in rapidly growing cancer cells [86–90]. In addition, blocking key step in fatty acid synthesis can lead to cell death [91].

Fatty acids can be acquired from the extracellular environment or through *de novo* fatty-acid synthesis [86]. The major sources of free fatty acids in the circulation are from diet or mobilized from adipose tissue [86]. Alternatively, in some tissues, such as liver or adipose tissue, lipid can be generated from glucose and glutamine by *de novo* fatty-acid synthesis. Acetyl-CoAs, converted from citrate by ATP-citrate lyase (ACLY), provides the two-carbon units that required in fatty acid synthesis. The first, rate-limiting step for *de novo* fatty-acid synthesis is regulated by acetyl-CoA carboxylase (ACC), which converts acetyl-CoA to malonyl-CoA. Fatty acid synthase (FASN) then couples malonyl-CoA to multiple two-carbon units derived from acetyl-CoAs in a series of reactions that ultimately result in a 16-carbon saturated fatty acid, palmitic acid. Palmitic acids then can be further elongated, desaturated by stearoyl-CoA desaturase (SCD), or incorporated into triglycerides or phospholipids.

Early global mapping of Myc target genes has revealed that several genes involved in fatty acid metabolism are regulated by Myc, including ACC, FASN and SCD [20,76]. Overexpression of Myc in human prostate epithelial cells upregulates FASN transcriptionally and translationally [92]. Metabolomic analysis also shows enrichment of phospholipid and lipid precursor citrate in the Myc overexpressing cells, suggesting high levels of Myc are linked to elevated fatty acid synthesis [92]. Direct evidence that c-Myc drives *de novo* fatty-acid biosynthesis includes use of ¹³C-labeled glucose, which reveals a near doubling in the fraction of palmitate isotopomers in Myc-expressing rat fibroblasts (11%) compared to Myc-null cells (6%) [93]. Recently, MondoA, a member of Myc extended network, appears to be crucial for N-Myc-induced, Srebp1-dependent *de novo* fatty-acid biosynthesis [94]. Importantly, synthetic lethality induced by loss of MondoA in N-Myc overexpressing cells can be rescued by 18-carbon fatty acid oleate (C18:1), suggesting lipogenesis is critical for viability in cells with high levels of N-Myc [94].

In addition to membrane synthesis, fatty acids can also be used as an efficient energy source. Mitochondrial fatty acid β -oxidation (FAO) is the main reaction that breaks down fatty acids to acetyl-CoAs. FAO generates multiple reduced dinucleotides (FADH₂, NADH) which can donate electrons to the mitochondrial electron transport for ATP synthesis. In addition, the acetyl-CoA end-product of FAO can enter the TCA cycle and likewise contribute to ATP production through the formation of reduced dinucleotides and triphosphorylated guanosine

(GTP). In contrast to Myc-driven fatty acid synthesis, loss of Myc directs cell to use fat as an energy source. It was found that Myc null rat fibroblasts have higher rate of fatty acid uptake and FAO compared to wild-type cells, which instead incorporate fatty acids into both neutral lipids and phospholipids [95]. Despite of high rate of FAO, neutral lipid accumulation is observed in the Myc-null cells, reflecting a consequence of mitochondrial dysfunction due to Myc depletion [95,96]. Similarly, using inhibitor 10058-F4 that disrupts N-Myc/Max interaction, lipid droplets are observed to accumulate intracellularly [97]. Proteomic analysis reveals that mitochondrial enzymes involved in oxidative phosphorylation and fatty acid oxidation are down-regulated upon treatment of 10058-F4 or short hairpin RNA targeting *MYCN*, suggesting that N-Myc inhibition impairs β -oxidation, which in turn results in lipid droplet accumulation [97]. Taken together, while Myc generally upregulates fatty acid synthesis and represses FAO, mitochondrial dysfunction caused by loss of Myc may attenuate lipid usage in the cells.

As discussed, cholesterol is an important component of cell membrane. Cholesterol is synthesized from acetyl-CoA, which is condensed to form 3-hydroxy-3 methylglutaryl (HMG)-CoA and then reduced to mavalonate by HMG-CoA reductase (HMGCR). HMGCR is rate-limiting in the cholesterol synthesis pathway; hence, it is an important therapeutic target for treating hypercholesterolemia. HMGCR has been documented to be essential for Myc-driven liver cancer as it mediates the phosphorylation and transactivation of Myc [98]. Recent study also showed c-Myc upregulates HMGCR in esophageal cancer cells [99].

6. Myc and the PI3K-Akt-mTOR pathway

Myc and the PI3K-Akt-mTOR pathway both respond to growth factor signaling and drive growth and metabolism in normal and neoplastic cells. While glycolysis is upregulated downstream of both, there are distinct metabolic programs associated with each oncoprotein. By studying dual-regulatable pre-B cell lines in which either Akt or Myc is overexpressed, it was observed that Myc drives both glycolysis and mitochondrial function, whereas Akt drives glycolysis alone [100]. Interestingly, these differences in metabolic pathway preference determine sensitivity to metabolic perturbation. For example, Akt-overexpressing cells are more sensitive to anti-glycolytic molecule 2-deoxyglucose (2-DG). In contrast, Mycoverexpressing cells are effectively targeted with mitochondrial ATP synthase inhibitor oligomycin [100]. Notably, by activating multiple metabolic pathways, Myc-overexpressing cells are more resistant to individual metabolic perturbations [100]. Furthermore, metabolomic analyses in mouse and human prostate cancer tissue expressing high levels of Akt or Myc have shown that, while high level of Akt is associated with accumulation of glycolytic metabolites, Myc overexpression prompts accumulation of metabolites downstream of glutamine and lipid metabolism [92]. Although further work is necessary to address the effectiveness of anti-metabolic therapy for cancer cells, differentially targeting according to the particular metabolic dependencies arising from the genetic of each cancer appears important.

Despite the distinct role of Myc and PI3K-Akt-mTOR in cancer, recent evidence has elucidated the crosstalk between these two pathways. In Burkitt's lymphoma, it was found

Myc and PI3K cooperate to promote tumorigenesis [101]. Given the fact that both Myc and mTOR are master regulators of protein synthesis, it is expected that Myc coordinates with mTOR in cancer cells. In fact, Myc inhibits mTOR repressor TSC2, thus increasing mTOR activity to facilitate cap-dependent translation through S6K and 4E-BP phosphorylation [102]. In addition, expressing a 4E-BP1 mutant rendered resistance to mTOR phosphorylation (4E-BP^m) in the B cell compartment of Eμ-Myc mice leads to 80% reduction in pretumor cells [73]. Conversely, normal B cells are not affected by 4E-BP^m expression, indicating Myc-induced, mTOR-dependent protein synthesis is uniquely vital in Myc-driven lymphoma cells [73]. Recently, mTORC1 has been shown to enhance glutamine flux through S6K1-dependent c-Myc upregulation, which in turn increases GLS activity [103]. Interestingly, work that has been carried out in glioblastoma cells also shows that mTORC2 releases Myc inhibition from miR-34C through deacetylation of FOXO1 and FOXO3 [32]. Taken together, it becomes clear that Myc and mTOR cooperate to promote tumor survival in many different ways, making the case for combination therapy especially persuasive.

7. Conclusion and future perspectives

Compelling evidence has underscored the multifaceted role of Myc in controlling cancer metabolism. Myc persistently emerges as a global growth regulator that drives glucose metabolism, glutamine metabolism, fatty acid synthesis, oxidative phosphorylation, nucleotide synthesis and ribosomal biogenesis (Fig. 5). Given the fact that Myc globally regulates multiple components of cellular processes, an outstanding question remains how Myc modulates pathways to ensure proper cellular function while preventing the occurrence of futile cycle. Recent studies have discovered individual nexuses that link protein synthesis with nucleotide synthesis or one-carbon metabolism [74,75]. However, future studies are required to characterize these “nodes”, particularly those occurring in cancer cells specifically as a result of the increased complexity of active metabolic pathways. In addition, whether Myc selectively activates pathways at different times would be an important area to explore. Lastly, high throughput screening has revealed that genetic aberration in a given human cancer usually involve multiple oncogenic proteins, thus it can be anticipated that there will be more crosstalk between different growth regulators. Dissecting the relationship between Myc and other oncogenes or tumor suppressors will augment the discovery of synergistic targets for new therapeutic approaches.

Acknowledgements

We thank Dr. Hong Kai Ji and Jason Ji for analyzing c-Myc-induced mouse liver model gene expression profiling (Fig. 1). The laboratory of C.V.D. is partially supported by the National Cancer Institute of the National Institutes of Health #R01CA057341, The Leukemia and Lymphoma Society #LLS 6106-14, and the Abramson Family Cancer Research Institute.

Abbreviations

2-DG	2-deoxyglucose
4E-BP	eIF4E-binding protein

5,10-methylene THF	5,10-methylenetetrahydrofolate
ACC	acetyl-CoA carboxylase
ACLY	ATP-citrate lyase
AMPK	AMP-activated protein kinase
ATP	adenosine triphosphate
ChIP-seq	chromatin immunoprecipitation sequencing
ChREBP	carbohydrate response element binding protein
CRY	cryptochrome
DOX	doxycycline
eIF4E	eukaryotic initiation factor 4E
FADH	flavin adenine dinucleotide
FAO	fatty acid β -oxidation
FASN	fatty acid synthase
FOXO	forkhead box O
G3P	glyceradehyde-3-phosphate
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GDH	glutamine dehydrogenase
GLS	glutaminase
GLUT	glucose transporter
GOT	glutamine oxaloacetate transaminase
GPT	glutamine pyruvate transaminase
GSK3	glycogen synthase kinase 3
GTP	triphosphorylated guanosine
HIF1α	hypoxia inducible factor subunit alpha
HMG	3-hydroxy-3 methylglutaryl
HMGCR	HMG-CoA reductase
hnRNP	heterogeneous nuclear ribonucleoproteins
IMDPH	inosine monophosphate dehydrogenase
LDH	lactate dehydrogenase
Max	MYC associated factor X
Mga	MAX gene associated
MITF	microphthalmia-associated transcription factor

Miz1	Myc-interacting zinc finger protein 1
Mnt	MAX network transcriptional repressor
MPA	mycophenolic acid
mRNA	messenger RNA
mTHF	5-methyltetrahydrofolate
mTOR	mammalian target of rapamycin
mTORC1	mTOR complex 1
mTORC2	mTOR complex 2
Mxd	MAX dimerization protein
Mxi1	MAX-interacting protein 1
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NAMPT	nicotinamide phosphoribosyltransferase
PKD1	phosphoinositide-dependent kinase
PER	period
PFK	phosphofructokinase
PH	pleckstrin-homology
PHGDH	phosphoglycerate dehydrogenase
PI(3,4,5)P3	phosphatidyl-inositol-3,4,5-trisphosphate
PI(4,5)P2	phosphatidylinositol-4,5-bisphosphate
PI3K	phosphoinositide 3-kinase
PKM	pyruvate kinase
Pol I	RNA polymerase I
Pol II	RNA polymerase II
Pol III	RNA polymerase III
PRPP	5-phosphoribosyl-PP
PRPS1	phosphoribosyl pyrophosphate synthetase 1
PRPS2	phosphoribosyl pyrophosphate synthetase 2
PRTE	pyrimidine-rich translational element
PSAT1	phosphoserine aminotransferase 1
PSPH	phosphoserine phosphatase
R5P	ribose 5-phosphate

ROS	reactive oxygen species
RPL	large ribosomal subunit
RPS	small ribosomal subunit
rRNA	ribosomal RNA
SAH	S-adenosylhomocysteine
SAHH	S-adenosyl-L-homocysteine hydrolase
SAM	S-adenyl methionine
SCD	stearoyl-CoA desaturase
SHMT	serine hydroxymethyltransferase
SREBP	sterol regulatory element-binding protein
TCA cycle	tricarboxylic acid cycle
TFE3	transcription factor E3
TFEB	transcription factor EB
THF	tetrahydrofolate
tRNA	transfer RNA
TS	thymidylate synthase
TSC2	tuberous sclerosis complex 2
αKG	α -ketoglutarate

References

1. Duesberg PH, Vogt PK. Avian acute leukemia viruses MC29 and MH2 share specific RNA sequences: evidence for a second class of transforming genes. *Proc Natl Acad Sci U S A*. 1979; 76:1633. [PubMed: 221900]
2. Vennstrom B, Sheiness D, Zabielski J, Bishop JM. Isolation and characterization of c-myc, a cellular homolog of the oncogene (v-myc) of avian myelocytomatosis virus strain 29. *J Virol*. 1982; 42:773. [PubMed: 6284994]
3. Dalla-Favera R, et al. Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. *Proc Natl Acad Sci U S A*. 1982; 79:7824. [PubMed: 6961453]
4. Taub R, et al. Translocation of the c-myc gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. *Proc Natl Acad Sci U S A*. 1982; 79:7837. [PubMed: 6818551]
5. Dang CV. MYC on the path to cancer. *Cell*. 2012; 149:22. [PubMed: 22464321]
6. Brodeur GM, Seeger RC, Schwab M, Varmus HE, Bishop JM. Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. *Science*. 1984; 224:1121. [PubMed: 6719137]
7. Nau MM, et al. L-myc, a new myc-related gene amplified and expressed in human small cell lung cancer. *Nature*. 1985; 318:69. [PubMed: 2997622]
8. He TC, et al. Identification of c-MYC as a target of the APC pathway. *Science*. 1998; 281:1509. [PubMed: 9727977]

9. Beroukhi R, et al. The landscape of somatic copy-number alteration across human cancers. *Nature*. 2010; 463:899. [PubMed: 20164920]
10. Carroll PA, et al. Deregulated Myc requires MondoA/Mlx for metabolic reprogramming and tumorigenesis. *Cancer Cell*. 2015; 27:271. [PubMed: 25640402]
11. Efeyan A, Zoncu R, Sabatini DM. Amino acids and mTORC1: from lysosomes to disease. *Trends Mol Med*. 2012; 18:524. [PubMed: 22749019]
12. Hardie DG, Alessi DR. LKB1 and AMPK and the cancer-metabolism link – ten years after. *BMC Biol*. 2013; 11:36. [PubMed: 23587167]
13. Green CB, Takahashi JS, Bass J. The meter of metabolism. *Cell*. 2008; 134:728. [PubMed: 18775307]
14. Bass J. Circadian topology of metabolism. *Nature*. 2012; 491:348. [PubMed: 23151577]
15. Yasumoto K, Yokoyama K, Shibata K, Tomita Y, Shibahara S. Microphthalmia-associated transcription factor as a regulator for melanocyte-specific transcription of the human tyrosinase gene. *Mol Cell Biol*. 1994; 14:8058. [PubMed: 7969144]
16. Sardiello M, et al. A gene network regulating lysosomal biogenesis and function. *Science*. 2009; 325:473. [PubMed: 19556463]
17. Giangrande PH, Hallstrom TC, Tunyaplin C, Calame K, Nevins JR. Identification of E-box factor TFE3 as a functional partner for the E2F3 transcription factor. *Mol Cell Biol*. 2003; 23:3707. [PubMed: 12748276]
18. Fernandez PC, et al. Genomic targets of the human c-Myc protein. *Genes Dev*. 2003; 17:1115. [PubMed: 12695333]
19. Koike N, et al. Transcriptional architecture and chromatin landscape of the core circadian clock in mammals. *Science*. 2012; 338:349. [PubMed: 22936566]
20. Lin CY, et al. Transcriptional amplification in tumor cells with elevated c-Myc. *Cell*. 2012; 151:56. [PubMed: 23021215]
21. Hu S, et al. ¹³C-pyruvate imaging reveals alterations in glycolysis that precede c-Myc-induced tumor formation and regression. *Cell Metab*. 2011; 14:131. [PubMed: 21723511]
22. Jorgensen P, Tyers M. How cells coordinate growth and division. *Curr Biol*. 2004; 14:R1014. [PubMed: 15589139]
23. Rudra D, Warner JR. What better measure than ribosome synthesis? *Genes Dev*. 2004; 18:2431. [PubMed: 15489289]
24. Dang CV. Links between metabolism and cancer. *Genes Dev*. 2012; 26:877. [PubMed: 22549953]
25. Lippman SI, Broach JR. Protein kinase A and TORC1 activate genes for ribosomal biogenesis by inactivating repressors encoded by Dot6 and its homolog Tod6. *Proc Natl Acad Sci U S A*. 2009; 106:19928. [PubMed: 19901341]
26. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol*. 2001; 2:127. [PubMed: 11252954]
27. Cantley LC. The phosphoinositide 3-kinase pathway. *Science*. 2002; 296:1655. [PubMed: 12040186]
28. Ma XM, Blenis J. Molecular mechanisms of mTOR-mediated translational control. *Nat Rev Mol Cell Biol*. 2009; 10:307. [PubMed: 19339977]
29. Inoki K, Li Y, Zhu T, Wu J, Guan KL. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol*. 2002; 4:648. [PubMed: 12172553]
30. Fruman DA, Rommel C. PI3K and cancer: lessons, challenges and opportunities. *Nat Rev Drug Discov*. 2014; 13:140. [PubMed: 24481312]
31. McCubrey JA, et al. Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. *Biochim Biophys Acta*. 2007; 1773:1263. [PubMed: 17126425]
32. Masui K, et al. mTOR complex 2 controls glycolytic metabolism in glioblastoma through FoxO acetylation and upregulation of c-Myc. *Cell Metab*. 2013; 18:726. [PubMed: 24140020]
33. Babic I, et al. EGFR mutation-induced alternative splicing of Max contributes to growth of glycolytic tumors in brain cancer. *Cell Metab*. 2013; 17:1000. [PubMed: 23707073]
34. Duvel K, et al. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Mol Cell*. 2010; 39:171. [PubMed: 20670887]

35. Dang CV. MYC, metabolism, cell growth, and tumorigenesis. *Cold Spring Harb Perspect Med.* 2013; 3:a014217. [PubMed: 23906881]
36. Eischen CM, Weber JD, Roussel MF, Sherr CJ, Cleveland JL. Disruption of the ARF-Mdm2-p53 tumor suppressor pathway in Myc-induced lymphomagenesis. *Genes Dev.* 1999; 13:2658. [PubMed: 10541552]
37. Qi Y, et al. p19ARF directly and differentially controls the functions of c-Myc independently of p53. *Nature.* 2004; 431:712. [PubMed: 15361884]
38. Yustein JT, et al. Induction of ectopic Myc target gene JAG2 augments hypoxic growth and tumorigenesis in a human B-cell model. *Proc Natl Acad Sci U S A.* 2010; 107:3534. [PubMed: 20133585]
39. Shachaf CM, et al. MYC inactivation uncovers pluripotent differentiation and tumour dormancy in hepatocellular cancer. *Nature.* 2004; 431:1112. [PubMed: 15475948]
40. Rahl PB, et al. c-Myc regulates transcriptional pause release. *Cell.* 2010; 141:432. [PubMed: 20434984]
41. Chen X, et al. Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. *Cell.* 2008; 133:1106. [PubMed: 18555785]
42. Nie Z, et al. c-Myc is a universal amplifier of expressed genes in lymphocytes and embryonic stem cells. *Cell.* 2012; 151:68. [PubMed: 23021216]
43. Sabo A, et al. Selective transcriptional regulation by Myc in cellular growth control and lymphomagenesis. *Nature.* 2014; 551
44. Wolf E, Lin CY, Eilers M, Levens DL. Taming of the beast: shaping Myc-dependent amplification. *Trends Cell Biol.* 2014; 25
45. Walz S, et al. Activation and repression by oncogenic MYC shape tumour-specific gene expression profiles. *Nature.* 2014; 551
46. Dang CV. Gene regulation: fine-tuned amplification in cells. *Nature.* 2014; 511:417. [PubMed: 25043013]
47. Wullschlegel S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell.* 2006; 124:471. [PubMed: 16469695]
48. Teleman AA, Hietakangas V, Sayadian AC, Cohen SM. Nutritional control of protein biosynthetic capacity by insulin via Myc in *Drosophila*. *Cell Metab.* 2008; 7:21. [PubMed: 18177722]
49. Corcos D, et al. Expression of c-myc is under dietary control in rat liver. *Oncogene Res.* 1987; 1:193. [PubMed: 3329714]
50. Delpuech O, et al. Induction of Mxi1-SR alpha by FOXO3a contributes to repression of Myc-dependent gene expression. *Mol Cell Biol.* 2007; 27:4917. [PubMed: 17452451]
51. Osthus RC, et al. Deregulation of glucose transporter 1 and glycolytic gene expression by c-Myc. *J Biol Chem.* 2000; 275:21797. [PubMed: 10823814]
52. Le A, et al. Glucose-independent glutamine metabolism via TCA cycling for proliferation and survival in B cells. *Cell Metab.* 2012; 15:110. [PubMed: 22225880]
53. Racker E. Bioenergetics and the problem of tumor growth. *Am Sci.* 1972; 60:56. [PubMed: 4332766]
54. Le A, et al. Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. *Proc Natl Acad Sci U S A.* 2010; 107:2037. [PubMed: 20133848]
55. David CJ, Chen M, Assanah M, Canoll P, Manley JL. HnRNP proteins controlled by c-Myc deregulate pyruvate kinase mRNA splicing in cancer. *Nature.* 2010; 463:364. [PubMed: 20010808]
56. Reitzer LJ, Wice BM, Kennell D. Evidence that glutamine, not sugar, is the major energy source for cultured HeLa cells. *J Biol Chem.* 1979; 254:2669. [PubMed: 429309]
57. Wise DR, et al. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. *Proc Natl Acad Sci U S A.* 2008; 105:18782. [PubMed: 19033189]
58. Gao P, et al. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. *Nature.* 2009; 458:762. [PubMed: 19219026]

59. Yuneva M, Zamboni N, Oefner P, Sachidanandam R, Lazebnik Y. Deficiency in glutamine but not glucose induces MYC-dependent apoptosis in human cells. *J Cell Biol.* 2007; 178:93. [PubMed: 17606868]
60. Wang JB, et al. Targeting mitochondrial glutaminase activity inhibits oncogenic transformation. *Cancer cell.* 2010; 18:207. [PubMed: 20832749]
61. Liu W, et al. Reprogramming of proline and glutamine metabolism contributes to the proliferative and metabolic responses regulated by oncogenic transcription factor c-MYC. *Proc Natl Acad Sci U S A.* 2012; 109:8983. [PubMed: 22615405]
62. Kirn-Safran CB, et al. Global growth deficiencies in mice lacking the ribosomal protein HIP/RPL29. *Dev Dyn.* 2007; 236:447. [PubMed: 17195189]
63. Anderson SJ, et al. Ablation of ribosomal protein L22 selectively impairs alphabeta T cell development by activation of a p53-dependent checkpoint. *Immunity.* 2007; 26:759. [PubMed: 17555992]
64. Gallant P, Shiiro Y, Cheng PF, Parkhurst SM, Eisenman RN. Myc and Max homologs in *Drosophila*. *Science.* 1996; 274:1523. [PubMed: 8929412]
65. Schreiber-Agus N, et al. *Drosophila* Myc is oncogenic in mammalian cells and plays a role in the diminutive phenotype. *Proc Natl Acad Sci U S A.* 1997; 94:1235. [PubMed: 9037036]
66. Johnston LA, Prober DA, Edgar BA, Eisenman RN, Gallant P. *Drosophila* Myc regulates cellular growth during development. *Cell.* 1999; 98:779. [PubMed: 10499795]
67. Grandori C, et al. c-Myc binds to human ribosomal DNA and stimulates transcription of rRNA genes by RNA polymerase I. *Nat Cell Biol.* 2005; 7:311. [PubMed: 15723054]
68. van Riggelen J, Yetil A, Felsher DW. MYC as a regulator of ribosome biogenesis and protein synthesis. *Nat Rev Cancer.* 2010; 10:301. [PubMed: 20332779]
69. Gomez-Roman N, Grandori C, Eisenman RN, White RJ. Direct activation of RNA polymerase III transcription by c-Myc. *Nature.* 2003; 421:290. [PubMed: 12529648]
70. Ruggero D. The role of Myc-induced protein synthesis in cancer. *Cancer Res.* 2009; 69:8839. [PubMed: 19934336]
71. Barna M, et al. Suppression of Myc oncogenic activity by ribosomal protein haploinsufficiency. *Nature.* 2008; 456:971. [PubMed: 19011615]
72. Schmidt EV. The role of c-myc in regulation of translation initiation. *Oncogene.* 2004; 23:3217. [PubMed: 15094771]
73. Pourdehnad M, et al. Myc and mTOR converge on a common node in protein synthesis control that confers synthetic lethality in Myc-driven cancers. *Proc Natl Acad Sci U S A.* 2013; 110:11988. [PubMed: 23803853]
74. Fernandez-Sanchez ME, Gonatopoulos-Pournatzis T, Preston G, Lawlor MA, Cowling VH. S-adenosyl homocysteine hydrolase is required for Myc-induced mRNA cap methylation, protein synthesis, and cell proliferation. *Molecular and cellular biology.* 2009; 29:6182. [PubMed: 19805518]
75. Cunningham JT, Moreno MV, Lodi A, Ronen SM, Ruggero D. Protein and nucleotide biosynthesis are coupled by a single rate-limiting enzyme, PRPS2, to drive cancer. *Cell.* 2014; 157:1088. [PubMed: 24855946]
76. Zeller KI, et al. Global mapping of c-Myc binding sites and target gene networks in human B cells. *Proc Natl Acad Sci U S A.* 2006; 103:17834. [PubMed: 17093053]
77. Mannava S, et al. Direct role of nucleotide metabolism in C-MYC-dependent proliferation of melanoma cells. *Cell Cycle.* 2008; 7:2392. [PubMed: 18677108]
78. Liu YC, et al. Global regulation of nucleotide biosynthetic genes by c-Myc. *PLoS ONE.* 2008; 3:e2722. [PubMed: 18628958]
79. Vazquez A, Markert EK, Oltvai ZN. Serine biosynthesis with one carbon catabolism and the glycine cleavage system represents a novel pathway for ATP generation. *PLoS ONE.* 2011; 6:e25881. [PubMed: 22073143]
80. Nikiforov MA, et al. A functional screen for Myc-responsive genes reveals serine hydroxymethyltransferase, a major source of the one-carbon unit for cell metabolism. *Mol Cell Biol.* 2002; 22:5793. [PubMed: 12138190]

81. Ye J, et al. Serine catabolism regulates mitochondrial redox control during hypoxia. *Cancer Discov.* 2014; 4
82. Locasale JW. Serine, glycine and one-carbon units: cancer metabolism in full circle. *Nat Rev Cancer.* 2013; 13:572. [PubMed: 23822983]
83. Cole MD, Cowling VH. Specific regulation of mRNA cap methylation by the c-Myc and E2F1 transcription factors. *Oncogene.* 2009; 28:1169. [PubMed: 19137018]
84. Jones RM, et al. An essential E box in the promoter of the gene encoding the mRNA cap-binding protein (eukaryotic initiation factor 4E) is a target for activation by c-myc. *Mol Cell Biol.* 1996; 16:4754. [PubMed: 8756633]
85. Hsieh AC, Ruggero D. Targeting eukaryotic translation initiation factor 4E (eIF4E) in cancer. *Clin Cancer Res.* 2010; 16:4914. [PubMed: 20702611]
86. Santos CR, Schulze A. Lipid metabolism in cancer. *FEBS J.* 2012; 279:2610. [PubMed: 22621751]
87. Kuhajda FP, et al. Fatty acid synthesis: a potential selective target for antineoplastic therapy. *Proc Natl Acad Sci U S A.* 1994; 91:6379. [PubMed: 8022791]
88. Yoon S, et al. Up-regulation of acetyl-CoA carboxylase alpha and fatty acid synthase by human epidermal growth factor receptor 2 at the translational level in breast cancer cells. *J Biol Chem.* 2007; 282:26122. [PubMed: 17631500]
89. Swinnen JV, et al. Selective activation of the fatty acid synthesis pathway in human prostate cancer. *Int J Cancer.* 2000; 88:176. [PubMed: 11004665]
90. Clendening JW, et al. Dysregulation of the mevalonate pathway promotes transformation. *Proc Natl Acad Sci U S A.* 2010; 107:15051. [PubMed: 20696928]
91. Beckers A, et al. Chemical inhibition of acetyl-CoA carboxylase induces growth arrest and cytotoxicity selectively in cancer cells. *Cancer Res.* 2007; 67:8180. [PubMed: 17804731]
92. Priolo C, et al. AKT1 and MYC induce distinctive metabolic fingerprints in human prostate cancer. *Cancer Res.* 2014; 74:7198. [PubMed: 25322691]
93. Morrish F, et al. Myc-dependent mitochondrial generation of acetyl-CoA contributes to fatty acid biosynthesis and histone acetylation during cell cycle entry. *J Biol Chem.* 2010; 285:36267. [PubMed: 20813845]
94. Carroll PA, et al. Dereglated Myc requires MondoA/Mlx for metabolic reprogramming and tumorigenesis. *Cancer Cell.* 2015; 27
95. Edmunds LR, et al. c-Myc programs fatty acid metabolism and dictates acetyl-CoA abundance and fate. *J Biol Chem.* 2014; 289:25382. [PubMed: 25053415]
96. Li F, et al. Myc stimulates nuclearly encoded mitochondrial genes and mitochondrial biogenesis. *Mol Cell Biol.* 2005; 25:6225. [PubMed: 15988031]
97. Zirath H, et al. MYC inhibition induces metabolic changes leading to accumulation of lipid droplets in tumor cells. *Proc Natl Acad Sci U S A.* 2013; 110
98. Cao Z, et al. MYC phosphorylation, activation, and tumorigenic potential in hepatocellular carcinoma are regulated by HMG-CoA reductase. *Cancer Res.* 2011; 71:2286. [PubMed: 21262914]
99. Zhong C, et al. HMGCR is necessary for the tumorigenicity of esophageal squamous cell carcinoma and is regulated by Myc. *Tumour Biol.* 2014; 35:4123. [PubMed: 24390662]
100. Fan Y, Dickman KG, Zong WX. Akt and c-Myc differentially activate cellular metabolic programs and prime cells to bioenergetic inhibition. *J Biol Chem.* 2010; 285:7324. [PubMed: 20018866]
101. Sander S, et al. Synergy between PI3K signaling and MYC in Burkitt lymphomagenesis. *Cancer Cell.* 2012; 22:167. [PubMed: 22897848]
102. Ravitz MJ, Chen L, Lynch M, Schmidt EV. c-myc Repression of TSC2 contributes to control of translation initiation and Myc-induced transformation. *Cancer Res.* 2007; 67:11209. [PubMed: 18056446]
103. Csibi A, et al. The mTORC1/S6K1 pathway regulates glutamine metabolism through the eIF4B-dependent control of c-Myc translation. *Curr Biol.* 2014; 24:2274. [PubMed: 25220053]

Fig. 1. Expression of metabolic genes bound by both Bmal1 and Myc in different stages of Myc-driven liver tumor in mice. Gene set enrichment analysis of a subset of metabolic genes bound by both Bmal1 and Myc (shown in Table 1) in four stages of liver tumors in liver specific, doxycycline-suppressed, Myc transgenic mice: control (+DOX), pre-tumor (-DOX), tumor (-DOX), tumor regression (+DOX). DOX, doxycycline; red, upregulation; blue, downregulation.

Fig. 2.

Hypothesis of Myc-disrupted circadian homeostasis. A schematic representation of rhythmic control of “E-box” containing metabolic genes in normal cells and Myc-dependent, sustained control of “E-box” containing metabolic genes in cancer cells.

Fig. 3.

Growth factor signaling pathway. Upon growth factor engaging to growth factor receptor, receptor tyrosine kinase (RTK) activates both PI3K-AKT-mTOR pathway and RAS-RAF-MEK-ERK pathway. Myc is activated downstream of ERK. Oncogenes are framed with green burst and tumor suppressor genes are framed with red octagon. Both pathways collectively drive synthesis of macromolecules and bioenergetics molecules to promote cell growth and proliferation.

Fig. 4.

Schematic of divergent effects of mTOR and Myc on growing cells. In a hypothetical cell containing only mTOR downstream of growth factor signaling (upper row), mTOR enhances the rate of translation (ribosomes labeled in red representing they are undergoing faster translation process, so called “speedy” ribosomes) through posttranslational regulation. However, since there is no new components of translational machinery were made, the growth promoting effect is short-lived. In most mammalian cells (lower row), growth factor signaling activates both mTOR and Myc, in addition to elevated mTOR-dependent protein translation, Myc induces transcriptions of components of translational machinery to further enhance and sustain growth.

Fig. 5.

Myc-driven metabolic network. A schematic representation of the metabolic pathways regulated by Myc. Metabolic enzymes (except for eIF4E, which is eukaryotic translation initiation factor) labeled in red representing enzymes encoded by Myc direct target genes. Myc drives glucose metabolism by upregulating glucose transporter (GLUT1), glycolysis genes hexokinase (HK), phosphofructokinase (PFK3), pyruvate kinase (PKM) and lactate dehydrogenase (LDH). Myc also upregulates pyruvate dehydrogenase kinase (PDK) to inhibit pyruvate dehydrogenase, which further enhances the conversion of pyruvate to lactate. Myc upregulates phosphoglycerate dehydrogenase (PHGDH) and serine hydroxymethyltransferase (SHMT) to increase serine and glycine metabolism. Myc promotes glutamine metabolism by upregulating glutamine transporter (ASCT2) and glutaminase (GLS). Fatty acid synthesis is often prominent in Myc-driven cancer cells. Global mapping of Myc target genes shows acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN) and stearoyl-CoA desaturase (SCD1) are directly regulated by Myc [76].

Table 1

Potential shared target genes of Bmal1 and Myc.

Cellular processes or metabolic pathways	Genes bound by both Bmal1 [19] and Myc [20]
Glycolysis	Pfkfb3
	Pdk1
	Pdhb
Oxidative phosphorylation	Ndufa4
Ribosome biogenesis	Rplp2
Fatty acids and cholesterol synthesis	Srebf1
	Insig1
	Hmgcr
NAD ⁺ salvage pathway	NAMPT
Autophagy	Bnip3