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An extended Myc network contributes to glucose homeostasis in cancer and diabetes

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1. ABSTRACT

The Myc network of transcription factors plays pleiotropic roles in normal and pathological cell function. The canonical Myc network controls how the essential nutrients glucose and glutamine are utilized inside cells. The Myc network carries out this function by upregulating glucose and glutamine transporters and key enzymes in the glycolytic or glutaminolytic pathways. The Myc network also coordinates cellular utilization of glucose and glutamine in biosynthetic pathways by directly regulating mitochondrial mass and activity. We present an argument for the existence of an "extended" Myc network comprised of two related transcription factors MondoA and ChREBP. Both MondoA and ChREBP sense glycolytic flux and are the principal regulators of glucose-dependent transcription in their respective tissues, skeletal muscle and liver. MondoA also senses glutaminolytic flux into the tricarboxylic acid cycle and appears to coordinate the utilization of glucose and glutamine by regulating expression of thioredoxin interacting protein. Current data suggest that the extended Myc network regulates the cellular response to changes in nutrient availability and may be altered in cancer and insulin resistance.

2. INTRODUCTION

Alterations in glucose homeostasis are a common feature of both cancer and diabetes. A vast primary and review literature covers the causes and effects of glucose dysregulation in each of these devastating diseases. Here, we focus on how cells sense glucose, how they adapt to changes in intracellular glucose concentration, and how they control glucose flux through various growth-supporting biosynthetic pathways. A growing body of literature suggests a central role for an extended Myc network of transcription factors in each of these processes. We review some of the relevant literature here and focus on how dysregulation of this extended Myc network contributes to cancer and potentially diabetes.

3. AN EXTENDED MYC NETWORK – THE BASICS

The Myc family, including c-Myc, n-Myc and l-Myc, is among the best-studied family of transcriptional regulators in the animal kingdom. In the more than 25 years since its discovery, the Myc family has been implicated as an essential factor in cell growth, proliferation and tumorigenesis, placed atop an ever-

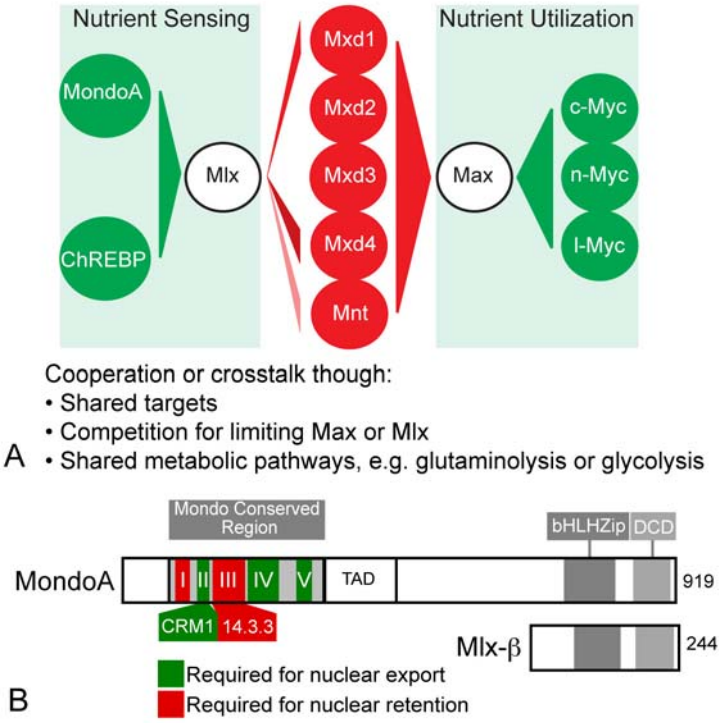

Figure 1. The MondoA family as an extension of the canonical Myc network. A) Schematic of the factors that comprise the extended Myc network with interactions as indicated by triangles. Green denotes primary functions in transcription activation. Red indicates primary function is transcription repression. The Reymond group observed the Mnt and Mlx interaction (5), but we have not observed this interaction in a number of different assays (unpublished). B) Schematic of MondoA and Mlx with domains indicated. Functions of the different MCRs are color-coded. 14.3.3 interacts with MCRIII, but this interaction appears dispensable for MCRIII function in nuclear retention (15).

growing family of structurally related transcription factors (1). The activity of the Myc family is modulated by an immense array of protein-protein interactions. Myc family proteins interact with Max to bind genomic sites with a preference for CACGTG, or closely related sequences known as enhancer boxes, or E-boxes. Myc:Max complexes generally activate transcription – although Myc is known to repress certain targets as well – via the recruitment of a variety of chromatin modifying complexes. Classically, the transcription activation function of Myc:Max complexes is balanced by Mxd:Max or Mnt:Max complexes (Figure 1A), which function as transcription repressors by recruiting the mSin3-histone deacetylase (HDAC) complex (2). The Mxd family, formerly called the Mad family, comprises 4 members, Mxd1 (Mad1), Mxd2 (Mxi1), Mxd3 (Mad3), Mxd4 (Mad4), whereas there is a single Mnt (also known as Mxd6 or Rox) gene. Since their discovery, some refer to this constellation of Myc, Max and Mxd proteins as the Myc network. However, data from our lab and others establishes the existence of an "extended" Myc network (3). MondoA, ChREBP and Mix comprise this extended Myc network and present new opportunities for regulating the functional output of canonical Myc-dependent pathways (Figure 1A). While not yet explored extensively, it appears that the extended Myc network plays a key role in how cells sense, adapt to, and utilize glucose, otherwise known as glucose homeostasis.

The founding member of the extended Myc network is Max-like protein X (Mlx). Mlx was identified first as a binding partner for Mxd1 and subsequently as a Mnt-interactor (4, 5). Like all members of the Myc family, Mlx has a basic helix-loop-helix leucine zipper (bHLHZip) domain required for dimerization with its partners, and for DNA binding by the resulting heterocomplex. Similar to Mxd:Max and Mnt:Max complexes, Mxd1:Mlx complexes function as HDAC-dependent transcriptional repressors on CACGTG-dependent reporter genes (4). Mlx interacts with Mnt, Mxd1, and Mxd4, but not with other members of the Mxd family, Max or any of the Myc family members (4). It is surprising that Mlx has a more limited spectrum of binding partnerships than Max. (Figure 1A). However, interaction studies for Mlx were conducted primarily using a directed two-hybrid approach. Therefore, we cannot formally rule out a more relaxed spectrum of protein partnerships for Mlx in mammalian cells.

We used Mlx as bait in a two-hybrid screen to identify MondoA, a novel bHLHZip protein, as an Mlx-interactor (6). The Reymond group identified a MondoA paralog, WBSCR14 (William-Beuren Syndrome Critical Region 14) by its homology to Mlx (7). The Uyeda group also identified WBSCR14 as a CACGTG-binding protein present in rat liver nuclear extracts prepared from animals that had been starved and then refed a high carbohydrate

diet (8). In this case, the open reading frame was called the Carbohydrate Response Element Binding Protein (ChREBP). For simplicity, the informal naming convention for these paralogs has become MondoA and ChREBP. The official gene names for MondoA and ChREBP are Mlx-interacting protein (MLXIP) and Mlx-interacting protein-like (MLXIPL), respectively. MondoA:Mlx and ChREBP:Mlx complexes were first characterized as transcriptional activators; however, it has recently been shown that MondoA:Mlx complexes act as potent transcriptional repressors at some targets (9). MondoA and ChREBP messenger RNAs appear to be relatively broadly expressed, but their highest expression is in skeletal muscle and liver, respectively (3). The predominant role of these tissues in glucose homeostasis is consonant with the function of MondoA and ChREBP as glucose-dependent transcription factors. Current evidence indicates an important role for MondoA and ChREBP in sensing intracellular glucose-derived metabolites and directing an adaptive transcriptional response when their levels increase. As discussed below, Myc plays a similarly important role in controlling the flux of glucose-derived metabolites into a number of biosynthetic pathways. These findings suggest an important role for this extended Myc network in coordinating the cellular response to glucose.

3.1. MondoA and ChREBP

At roughly 1000 amino acids in length, MondoA and ChREBP are approximately twice the size of the Myc proteins (Figure 1B). Most of this size difference consists of 5 blocks of sequence encompassing about 250 amino acids in the N-terminus of MondoA and ChREBP (3). These blocks of sequence are highly conserved among metazoan MondoA and ChREBP orthologs, implying a critical regulatory function. We have called these regions the MondoA Conserved Regions (MCRs) as they approximately 50% identical between human MondoA and human ChREBP. Mondo orthologs can be easily identified in all metazoans based on high conservation of the MCRs. The spacing between MCRII, MCRIII and MCRIV is near invariant across species, suggesting that they constitute a structural or functional module. By contrast, the spacing between MCRI and MCRII and between MCRIV and MCRV is more variable, suggesting that MCRI and MCRV may function more independently. However, MCRI and MCRV can only be identified in MondoA/ChREBP orthologs, suggesting that they must function in the context of the MCRII-III-IV module. The transcriptional activation domain (TAD) follows the MCRs; in the case of ChREBP, the MCRs appear to auto regulate the TAD (10). The bHLHZip domain constitutes the C-terminal third of MondoA and ChREBP, and, in MondoA, is followed by a conserved element that we termed the dimerization and cytoplasmic localization domain (DCD). ChREBP has sequences at its C-terminus that resemble a DCD, but this region of ChREBP has not yet been studied. The bHLHZip and the DCD regions share 60% and 70% identity, respectively, between human MondoA and ChREBP paralogs. Interestingly, a similar DCD is also found following the bHLHZip domain of Mlx orthologs. As the nomenclature suggests, the DCD of MondoA is an autonomous dimerization domain that interacts with the DCD of Mlx (11). In the absence of dimerization between MondoA and Mlx, the DCD functions as a cytoplasmic retention module (3, 12). Although the mechanism by which MondoA and/or Mlx monomers are retained in the cytoplasm is unclear, it ensures that only functional heterocomplexes have access to the genome.

One key regulatory difference between MondoA/ChREBP and Myc is how their intranuclear levels are controlled. In growing cells, the steady state nuclear level of Myc protein is primarily controlled by its rapid turnover. By contrast, MondoA and ChREBP are relatively stable proteins, and their intranuclear levels are controlled by highly regulated cytoplasmic-nuclear shuttling mechanisms. In the absence of glucose, both proteins localize primarily to the cytoplasm in a transcriptionally inert form. Following elevations in intracellular glucose, both proteins accumulate in the nucleus and bind promoters of their respective target genes (Figure 2). Although the precise mechanism(s) that control the nuclear accumulation of MondoA and ChREBP have yet to be fully elucidated, the nuclear export factor CRM1, and 14.3.3 proteins, which are also implicated in nuclear export, bind MCRII and MCRIII, respectively (Figure 1B) (11, 13). Elevations in intracellular glucose might abrogate the binding of CRM1 and/or 14.3.3 to the MCRs, or reduce the activity of these cofactors. Lowering the rate of nuclear export would result in a greater number of MondoA:Mlx or ChREBP:Mlx in the nucleus; this mechanism appears to control the nuclear accumulation of MondoA:Mlx complexes (14). By contrast, glucose-dependent nuclear accumulation of ChREBP:Mlx complexes seems to depend on increased nuclear import (15). Whether MondoA:Mlx and ChREBP complexes can be regulated by their reciprocal mechanism has not yet been explored.

Rather than sense intracellular glucose concentration directly, both MondoA and ChREBP sense glucose-derived metabolites. Thus, MondoA and ChREBP sense the flux of glucose into different metabolic pathways, rather than simply sensing increased glucose uptake (Figure 2). Our work indicates that the nuclear accumulation of MondoA is triggered by the accumulation of glucose 6-phosphate (G6P) (16). G6P is formed in the first step of glycolysis, wherein glucose is phosphorylated by hexokinases. We have proposed that G6P binds directly to the MCR region of MondoA and induces an allosteric conformational change that triggers nuclear accumulation of MondoA:Mlx heterodimers (14). Further, we have recently demonstrated that G6P is also required for promoter binding and coactivator recruitment of MondoA:Mlx complexes (14). In addition to sensing G6P, recent studies indicated that MondoA also senses intracellular pH, adenosine containing molecules and activity of the mitochondrial electron transport chain (17-19). Whether all signal to MondoA by modulating glycolytic flux or more indirectly remains to be studied.

Similar to MondoA, ChREBP has been shown to accumulate in the nucleus by sensing G6P (20), as well as the pentose phosphate pathway (PPP) intermediate xylulose 5-phosphate (X5P) (12). Whether ChREBP senses G6P or

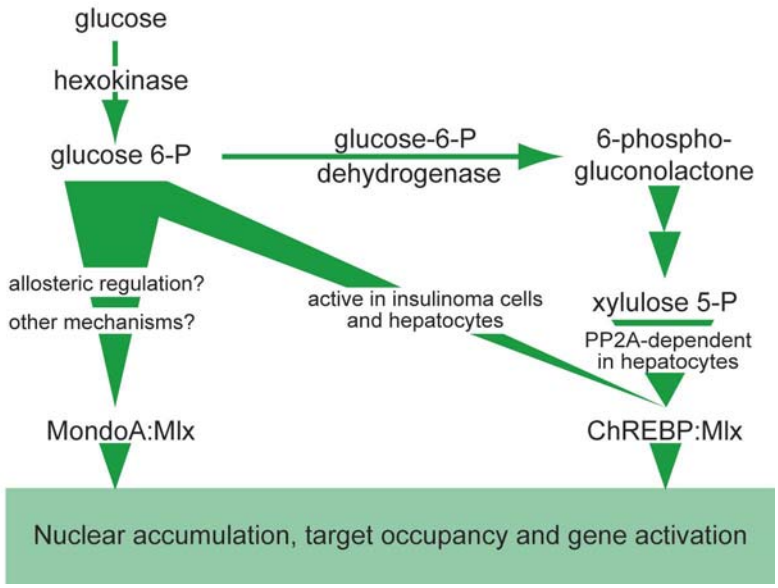

Figure 2. MondoA and ChREBP sense glycolytic flux. MondoA and ChREBP sense glucose 6 phosphate levels, although mechanistic details remain to be determined. ChREBP is regulated by the accumulation of xylulose 5-phosphate via an indirect mechanism that involves activation of PP2A. See text for additional details.

X5P seems to depend on cell type; however, it remains to be determined whether these metabolites have additive or cooperative effects on ChREBP nuclear accumulation. X5P appears to function more indirectly, triggering the nuclear accumulation of ChREBP by stimulating the activity of the PP2A phosphatase (21). PP2A is proposed to dephosphorylate two residues that inhibit nuclear entry and DNA binding of ChREBP:Mlx complexes. However, a recent careful study of *in vivo* ChREBP phosphorylation sites revealed that the majority of phosphorylation sites in ChREBP are glucose-independent (22). Furthermore, mutation of the putative glucose-regulated phosphorylation sites does not cancel the glucose responsiveness of ChREBP (23). These two findings bring into question some of the main tenets of the predominant regulatory model for ChREBP. We have not formally tested whether MondoA can sense X5P as does ChREBP. The MCRs in MondoA are required for its response to G6P (14, 16), and the high degree of conservation of MCRs between MondoA and ChREBP suggests that both proteins use similar mechanistic strategies to sense and respond to G6P. However, the presumptive PP2A-targeted regulatory phosphorylation sites in ChREBP are not conserved in MondoA, suggesting that different mechanisms control the nuclear accumulation of each protein. Additional studies are required to determine the precise circumstances and mechanisms by which these transcription factors respond to X5P or G6P.

Regardless of the exact glucose-derived metabolites sensed by MondoA and/or ChREBP, and the mechanisms that trigger their nuclear accumulation and activity, it is clear that these proteins are prominent regulators of glucose-induced transcription. One study using a dominant negative Mlx showed that ChREBP is required for approximately 60% of glucose-induced transcription in primary hepatocytes (24). Our study using control and MondoA knockdown epithelial cells similarly demonstrated that the majority of glucose-induced transcription, about 75% in this case, required MondoA (16). Thus MondoA and ChREBP are prominent regulators of glucose-induced transcription and sense glucose metabolism rather that glucose *per se*. These conclusions are consistent with the higher expression of MondoA and ChREBP in glucose-responsive post-mitotic tissues such as skeletal muscle and liver. More recent studies have examined the function MondoA and ChREBP in mitotic cells (see below). These early findings suggest that MondoA and ChREBP may have largely non-overlapping roles in cell division: ChREBP seems to be required for cellular proliferation (25), whereas MondoA seems to restrict proliferation (9).

4. METABOLIC REPROGRAMMING AND CELL PROLIFERATION

Myc plays many roles in cell growth, including reprogramming metabolism to support cell growth and division. To understand this latter facet of Myc in cell growth and division control, it is first worth considering how growing cells reprogram their metabolism (26-28). In the quiescent or post-mitotic/differentiated state, cells rely on oxidative metabolism – glucose, amino acids and fatty acids being the primary substrates – to generate sufficient ATP to meet their modest bioenergetic demands. By contrast, rapidly proliferating normal cells and most tumor cells switch their metabolism to favor aerobic glycolysis. Aerobic glycolysis is inherently less efficient at generating ATP than oxidative phosphorylation (OXPHOS), so there must be clear advantages in order for proliferating normal cells or tumor cells to undergo this dramatic reprogramming of cell metabolism. From the perspective

of ATP generation, glycolysis is kinetically faster than OXPHOS. Hence, as long as glucose is in excess, glycolysis can produce sufficient ATP to support the increased bioenergetic demands of high cell division rates, and the inefficiency of glycolysis in ATP production does not present a limitation for cell growth.

High rates of glycolysis also supply the carbon skeletons required for various biosynthetic processes that are essential for cell growth. G6P can enter PPP to generate the pentose sugars required for nucleotide synthesis. G6P entry into the PPP also generates NADPH, which is required for other biosynthetic reactions. Interestingly, growth promoting tyrosine kinase receptors can control the flux of glucose-derived carbons into biosynthetic pathways by binding and regulating the function of the glycolytic enzyme pyruvate kinase M2 (29, 30) . Glucose-derived pyruvate can be converted to lactate, which is secreted from the cell and may provide tumor cells certain advantages in the surrounding microenvironment (31, 32). Pyruvate may also enter mitochondria, where it is converted to acetyl-CoA. The entry of pyruvate into mitochondrial appears to be a central point of regulation controlling growth as this transition is controlled by both oncogenes, tumor suppressors and antiproliferative compounds (33-35).

Once in the mitochondria, pyruvate is converted to acetyl-CoA that fills the tricarboxylic acid (TCA) cycle and its attendant biosynthetic reactions. In rapidly growing cells however, acetyl-CoA-derived citrate can be shunted from the mitochondria to support lipid biosynthesis required for cell division. This efflux short-circuits the TCA cycle, thereby limiting its biosynthetic potential. To address this problem, cells use glutaminolysis to take up glutamine, convert it to glutamate, and ultimately to alpha-ketoglutarate (alpha-KG). alpha-KG in turn refills the TCA cycle by a process called anapleurosis. Therefore, glutaminolysis restores the downstream biosynthetic reactions of the short-circuited TCA cycle (27, 36). A significant portion of the glutamine is converted to lactate via the malate shunt, producing additional NADPH to support reductive biosynthetic reactions. Together these data suggest that one component of metabolic reprogramming is the coordinated upregulation of both glycolysis and glutaminolysis to support biosynthetic reactions (Figure 3). We suggest below that the extended Myc network plays a central role in coordinating the utilization of glucose and glutamine.

4.1. Myc and metabolic reprogramming

It has been known for more than 80 years that tumor cells consume more glucose than their normal counterparts, yet the molecular mechanisms that drive metabolic reprogramming are only now coming into focus. In general, oncogenes such as Myc, HIF1alpha, Ras and PI3K appear to play direct roles in driving glucose uptake and utilization, and suppressing OXPHOS. By contrast, tumor suppressors such as p53 and PTEN appear to stimulate OXPHOS and suppress glucose uptake and utilization (28, 37). In most cases, these oncogenes and tumor suppressors affect metabolic pathways directly, suggesting that metabolic reprogramming isn't simply a downstream effect of increased or decreased proliferation rates. Rather, it has been suggested that metabolic reprogramming is a proximal effect of both oncogenes and tumor suppressors and may be considered one of the "hallmarks" of cancer. We restrict our discussion here to the involvement of the extended Myc network in metabolic reprogramming. The contribution of other oncogenes/tumor suppressors has recently been reviewed (28, 37).

Myc has a remarkably diverse impact on cell growth, which may explain why Myc is dysregulated in a large percentage of human cancers (1). Myc directly regulates a large fraction of the RNA polymerase II-dependent transcriptome, as well as RNA polymerase I and RNA polymerase III-dependent transcripts including ribosomal RNA and tRNAs (38, 39). Myc's chief role in cell growth likely stems from regulating transcription of these polymerases, by directing 5' capping of some mRNA species, and by directly regulating DNA replication (40, 41). Furthermore, Myc also affects global chromatin architecture, which implies that it may be broadly permissive for the activities of other transcription factors (42). At present it is unclear how these less canonical functions of Myc impact metabolic reprogramming; thus, we restrict our discussion to its direct regulation of metabolic targets.

4.2. Myc and glucose utilization

Myc activation leads to sweeping changes in a number of anabolic processes. For example, a recent study following the metabolic fate of labeled glucose showed that Myc drove glucose-derived carbons into ribose sugars, purines and amino acids (43). Furthermore, Myc drove the production of acetyl-CoA, which is required for de novo lipid biosynthesis. Acetyl-CoA is also required for protein acetylation, most notably, the acetylation of N-terminal histone tails typically associated with gene activation (44). Thus, the Myc-dependent production of acetyl-CoA may be one mechanism by which Myc controls global chromatin structure. In support of this notion, a high glycolytic rate can lead to histone tail hyperacetylation (45). Many non-histone proteins are also acetylated (46), e.g. p53, raising the possibility that their activity may also be tied to Myc-driven production of acetyl-CoA.

Myc likely controls anabolic processes in two ways. First, Myc directly activates the expression of the glucose transporters GLUT-1, GLUT-2 and GLUT-4, allowing for increased glucose uptake (Figure 3). A seminal paper from the Dang lab showed that Myc could regulate glycolysis through its regulation of lactate dehydrogenase A (LDH-A) (47). It is now known that Myc activates the expression of virtually every gene that encodes a glycolytic enzyme (48). Thus Myc can control the rate of glucose influx, the intracellular availability of glucose-derived carbon backbones, and the rate of glycolysis. Second, Myc dictates the flux of glucose-derived carbons into different biosynthetic pathways. For example, carbamoyl phosphate synthatase, aspartate transcarbamylase, dihydroorotase (CAD) and ornithine

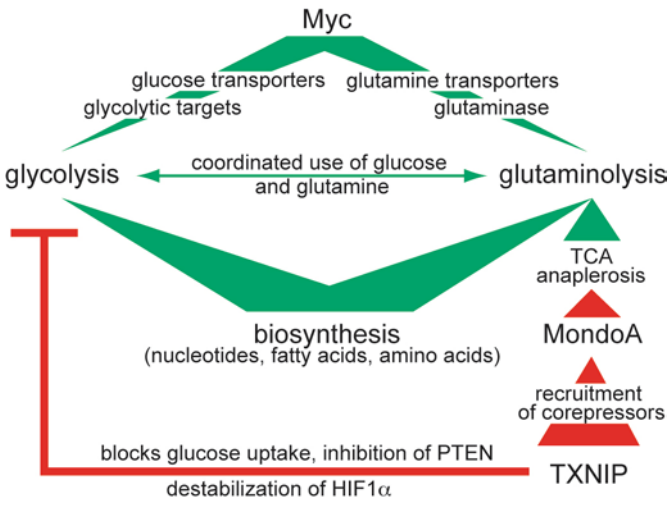

Figure 3. The extended Myc network has a broad command over nutrient utilization. Myc controls flux of glucose and glutamine into many biosynthetic pathways, whereas MondoA senses the TCA cycle and functions as a repressor of TXNIP when the TCA cycle is filled by glutamine-dependent anapleurosis. TNXIP down regulation is permissive for cell growth. See text for additional details.

decarboxylase (ODC) (49, 50), each required for nucleotide synthesis, were among the first well-characterized direct Myc targets. Extending this theme, a recent review indicated that about 15% of a sampling of 1,561 Myc targets could be classified as being involved in either metabolism or carbohydrate metabolism (51), suggesting a broad role in anabolic biosynthesis. These results make clear that Myc is a powerful regulator of glucose availability and utilization by biosynthetic pathways.

4.3. Myc and glutaminolysis

Glutamine is synthesized by most cell types and thus is considered a non-essential amino acid; however, the demand for glutamine in cell growth cannot typically be satisfied by de novo synthesis. The high demand for exogenous glutamine stems from its requirement for the synthesis of several biomolecules required for cell growth. For example, in addition to being required for protein synthesis, glutamine is required to provide nitrogen for synthesis of purines and pyrimidines necessary for DNA synthesis (52). Glutamine enters the TCA cycle by being metabolized to glutamate and subsequently to the TCA intermediate alpha-KG where it can supply carbons to support TCA-mediated biosynthesis and to generate NADPH. Myc also regulates metabolic reprogramming by controlling the rate of glutaminolysis (Figure 3). Myc-overexpressing normal human diploid fibroblasts undergo apoptosis when glutamine is removed from the medium, demonstrating that Myc expression can induce "addiction" to glutamine (53). Glutamine removal caused depletion of TCA cycle intermediates and addition of the TCA cycle intermediate oxaloacetate blocked apoptosis, suggesting that cell death was driven by defects in the TCA. Thus, like glucose, glutamine is required for cell growth and viability, although whether apoptosis was also caused by defects in other biosynthetic pathways or by other mechanisms was not investigated. Given the pleiotropic role of glutamine in different biosynthetic reactions, it is not surprising that glutamine uptake and utilization is increased in a variety of tumor types (54).

Two mechanisms have recently been proposed by which Myc overexpression can drive glutaminolysis (Figure 3). First, Myc can increase glutamine uptake into cells by directly regulating the expression of the glutamine transporters, SLC38A5 and SLC1A5 (55). Further, Myc also activates the expression of the glutaminase GLS1, which deamidates glutamine to produce glutamate. Flux through glutaminolysis may also be increased by the Myc-dependent activation of LDH-A, which converts glutamine-derived pyruvate to lactate (55). In the second study, Myc was also shown to regulate GLS1, but by a completely different mechanism. In this case, Myc overexpression caused repression of the microRNAs miR-23a and miR-23b, which target the 3' untranslated region of the GLS1 message to block its translation (56). Thus, Myc overexpression lifts the microRNA-dependent translational repression of GLS1 and increases mitochondrial glutaminase activity. Collectively, Myc can regulate GLS1 expression by transcriptional and translational mechanisms, providing the mechanistic framework for understanding how Myc controls glutamine utilization.

4.4. Myc and mitochondrial function

Another way that Myc regulates metabolism is by controlling mitochondrial function. Cells that lack mitochondrial DNA, which encodes key components of the electron transport machinery, are viable, indicating that OXPHOS *per se* is dispensable for cell growth. Yet, given their central role in housing the TCA cycle and its attendant biosynthetic reactions, mitochondria are essential organelles (27). With the increased biosynthetic demands of rapidly growing cells, it makes intuitive sense that mitochondrial function would be increased in tumorigenesis. Accordingly, Myc overexpression is sufficient to increase mitochondrial mass and activity by

activating expression of several nuclear-encoded genes that are required for mitochondrial function (57). Further, Myc can directly activate the mitochondrial transcription factor TFAM. TFAM is required for transcription of the genes encoded by the mitochondrial genome and mitochondrial DNA replication. This mechanism enables Myc to broadly dictate expression of the mitochondrial genome and its copy number. Thus, in addition to its role in driving aerobic glycolysis, Myc can also dictate the utilization of glucose-derived carbons for biosynthetic reactions by controlling mitochondrial mass and function.

Together, these data suggest that along with its myriad other functions in controlling cell physiology, Myc plays a central role in regulating cell growth by controlling glucose uptake and its utilization by mitochondrial and non-mitochondrial biosynthetic pathways. While difficult to test definitively, it seems likely that Myc's influence over macromolecular biosynthetic reactions is a critical facet of its function as a transforming oncogene.

5. MYC AND INSULIN RESISTANCE

Alteration of glucose homeostasis is a central feature of insulin resistance, wherein the peripheral tissues fail to respond appropriately to increases in circulating insulin levels following a meal. Insulin resistance is primarily manifest in skeletal muscle and adipose tissues that fail to take up adequate glucose in response to insulin, and in liver where glucose production is normally suppressed by insulin (58). These effects combine to drive hyperglycemia, which in turn stimulates pancreatic beta-cells to secrete more insulin as a compensatory mechanism, resulting in hyperinsulinemia. The mechanisms that mediate glucose uptake into muscle, or adipose and liver glucose production are fairly well understood; how these processes are altered in insulin resistance is also becoming more clear (59).

A series of papers from the Bosch lab show that transgenic overexpression of Myc in liver can counteract obesity and insulin resistance. In these studies, hepatic overexpression of Myc lead to increases in expression of the glucose transporter GLUT2 (60). In addition, lactate production, as well as a number of glycolytic target genes, including L-type pyruvate kinase (Pklr) were also upregulated by Myc overexpression (60). Transgenic expression of Myc in mouse liver resulted in reductions in the levels of both blood glucose and insulin. Importantly, liver overexpression of Myc largely suppressed the diabetic effects of streptozotocin (61), which kills insulin-producing beta-cells in the pancreas, and suppressed the insulin resistance and obesity induced by a high fat diet (62). Together, these data suggest that simply increasing hepatic glucose uptake and utilization is sufficient to counteract a number of phenotypes associated with diabetes.

There is no evidence that Myc participates directly in the development of insulin resistance, and there is hence limited utility in using transgenic Myc expression as a therapeutic in insulin resistance/diabetes. Therefore, a clear next step will be to determine direct and/or indirect Myc targets in the liver that mediate its protective effect. If this can be accomplished, these targets may have greater utility for therapeutic intervention in insulin resistance/diabetes. Current data suggests that Myc overexpression can regulate a number of metabolic enzymes such as glucokinase, Pklr and 6-phosphofructo-2-kinase (PFK2) , and transcription factors involved in metabolic pathways, e.g. sterol regulatory element binding protein 1c (SREBP1c) (63). Therefore, it is likely that both direct and indirect Myc targets are relevant. Hepatic overexpression of PFK2 suppresses insulin-resistance phenotypes similarly to Myc (64), suggesting that it may be a particularly important Myc effector in terms of hepatic glucose metabolism. Furthermore, several PFK2 paralogs are overexpressed in tumors, suggesting that they may also be relevant Myc targets in tumorigenesis (65) .

6. TXNIP AND ARRDC4 ARE KEY MONDOA AND CHREBP EFFECTORS

The Myc transcriptome is vast, and its characterization has revealed how Myc directly regulates cell metabolism and macromolecular biosynthetic reactions. By comparison, our understanding of the direct transcriptional targets of MondoA and ChREBP is in its infancy. Two members of the arrestin superfamily, which govern a diverse array of receptor-dependent biological processes, appear to be critical MondoA and/or ChREBP effectors in both growth control and insulin resistance. Arrestin proteins can be grouped into one of three major classes. The visual arrestins work prototypically in rhodopsin signaling and light sensitivity (66). The β-arrestins demonstrate function in ubiquitin-dependent endocytotic signaling, cardiac function, and metabolism, among many roles (67). The third and emerging class of arrestins, the α-arrestins, comprise 6 proteins in humans: thioredoxin interacting protein (TXNIP), and arrestin domain-containing (ARRDC) 1-5 (68, 69). Two members of this family, TXNIP and ARRDC4, are glucose-induced at the transcriptional level. TXNIP is a direct target of both MondoA and ChREBP, and MondoA also binds the promoter of the ARRDC4 gene in a glucose-inducible manner (14). Whether ARRDC4 is also a target for ChREBP has not been reported.

Several studies from our lab and others establish that TXNIP is a direct and glucose-induced transcriptional target of both MondoA and ChREBP in multiple cell types (9, 14, 16, 18, 70). In the presence of elevated glucose, MondoA or ChREBP enter the nucleus, bind the carbohydrate response element (ChoRE) in the TXNIP promoter and activate its expression (Figure 4). One function of TXNIP is to negatively regulate glucose uptake (71). Thus, the glucose-dependent regulation of TXNIP by MondoA and ChREBP triggers a negative feedback circuit that restricts further glucose uptake. Importantly, not all TXNIP function acts through thioredoxin-dependent pathways, because TXNIP's blockade of glucose uptake is independent of thioredoxin binding (71). Since the TXNIP paralog, ARRDC4, is a glucose-dependent direct target of MondoA (9, 14, 17, 71), and is also a negative regulator of glucose uptake, we have speculated that TXNIP and

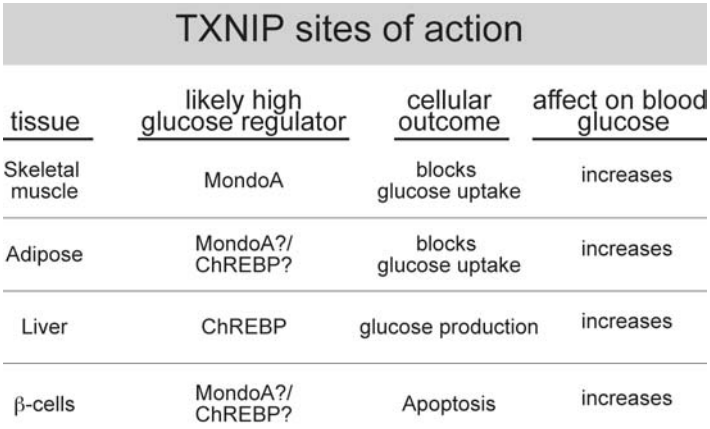

Figure 4. TXNIP is a regulator of blood glucose levels. See text for details.

ARRDC4 function redundantly or, perhaps, cooperatively downstream of MondoA (32, 72).

TXNIP is also known as VDUP-1 (vitamin D_3 up-regulated protein 1), reflecting that its expression is upregulated by a wide array of proliferative and stress response stimuli, including glucose, irradiation, hydrogen peroxide, TGF-β, HDAC inhibitors, low pH and many other stressors (73). In essence, TXNIP appears to be a prominent integrator of cellular stress signals. TXNIP interacts with thioredoxin and this interaction negatively regulates thioredoxin activity (73). Consequently, it is well established that TXNIP over expression leads to increased intracellular reactive oxygen species (ROS). Given the pleiotropic functions of ROS, it is not surprising that TXNIP upregulation has been observed in several pathophysiological settings. For example, TXNIP upregulation leads to activation of ASK1-mediated apoptosis, and down-regulation of pathogen-induced, thioredoxin-mediated inflammation (74, 75). TXNIP also likely plays a broad role in cell growth by regulating the stability of additional protein partners. For example, TXNIP expression stabilizes the cyclin-dependent kinase inhibitor, $p27^{kip1}$ (76) and destabilizes HIF1alpha (77). Whether ARRDC4 also responds similarly to a broad range of stresses is not yet known.

How TXNIP blocks glucose uptake is not known, but several possibilities are suggested in the literature (Figure 3). First, TXNIP overexpression leads to destabilization of HIF1alpha (77). HIF1alpha has a well-established role in activating transcription of most glycolytic targets (78). Thus, high TXNIP levels might lead to a wholesale downregulation of glycolysis and glucose uptake. Second, TXNIP loss leads to the inactivation of the lipid phosphatase PTEN (79), and the subsequent activation of the PI3K-AKT signaling pathway, which is known to stimulate glucose uptake (80). In this scenario, TXNIP overexpression would lead to PI3K-AKT inhibition, which would in turn block glucose uptake. Third, TXNIP may function as a classical beta-arrestin, scaffolding the signaling pathways that control glucose uptake; overexpression of TXNIP would function as a dominant negative in this scenario. Finally, there is some evidence that TXNIP may function in the nucleus as a component of transcriptional corepressor complexes (81, 82), perhaps repressing metabolic target genes directly (83). Further experiments are needed to differentiate between these models.

6.1. TXNIP function in metabolism

Given the importance of glucose homeostasis, it is not surprising that TXNIP appears to play physiological roles in multiple tissues. For instance, TXNIP is upregulated in skeletal muscle samples from prediabetics and diabetics (84). This finding suggests that TXNIP also controls postprandial glucose uptake in muscle (Figure 5). Consistent with this model, mice with whole body deletion TXNIP are hypoglycemic and have high triglyceride levels that seem to stem from a failure in beta-oxidation of fatty acids (85-87). Importantly, skeletal muscle-specific deletion of TXNIP recapitulates these phenotypes, pointing to skeletal muscle as the key site of action (79).

A recent report examined the function of TXNIP deletion on insulin resistance following 4 weeks of high-fat feeding (83). Compared to wildtype littermate controls, TXNIP null animals gained significantly more adipose mass. This increase in mass was tracked to increased caloric consumption and increased adipogenesis. *In vitro* experiments confirmed that TXNIP negatively regulated adipogenesis, likely by modulating the activity of the nuclear hormone receptor PPAR-gamma. The mechanism behind this unexpected finding was not explored, but presents an exciting avenue for future investigation. Interestingly, in spite of the increased mass in the high-fat fed TXNIP knockout animals, they remained largely insulin sensitive compared to wild type controls. This phenotype was ascribed to increased glucose uptake into both skeletal muscle and adipose tissue. Together, these studies further highlighted TXNIP's function as a negative regulator of glucose uptake in muscle, extended this activity to adipose tissue, and uncovered an unexpected role for TXNIP in adipogenesis (Figure 4).

TXNIP also plays important roles in glucose homeostasis in the liver and in insulin-secreting beta-cells. In the liver, TXNIP is an important regulator of glucose

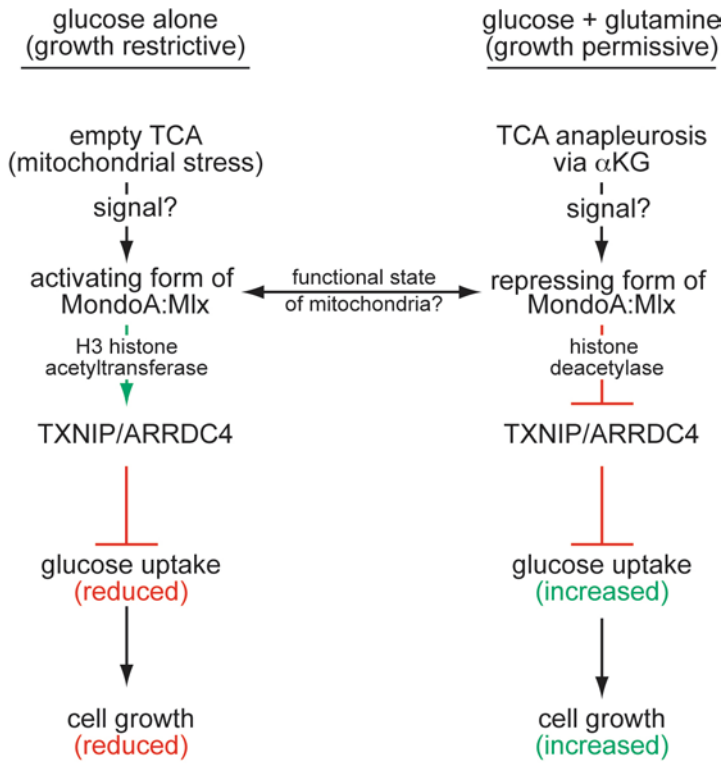

Figure 5. MondoA's transcriptional function is correlated with TCA cycle status. In media with glucose but lacking glutamine, TCA intermediates are reduced. We propose this causes mitochondrial stress that drives the transcription activation function of MondoA at the TXNIP promoter. The resulting blockade of glucose uptake likely inhibits cell growth in the case of cancer cell proliferation and may contribute to insulin resistance in muscle. In the presence of both glucose and glutamine, the TCA cycle is refilled by anapleurosis and mitochondrial stress is relieved. This drives the transcription repression function of MondoA at the TXNIP promoter. The increase in glucose uptake is predicted to fuel cancer cell growth and counteract insulin resistance. See text for additional details.

production, and this function requires interaction with thioredoxin (88) (Figure 4). This finding is in contrast to TXNIP's blockade of glucose uptake, which is independent of thioredoxin binding (69). Finally, TXNIP is required for hyperglycemia-induced apoptosis of beta-cells (89). Given that both MondoA and ChREBP can activate TXNIP in other cell types in response to hyperglycemia, it seems likely that one or both of these transcription factors will be required for hyperglycemia-induced beta-cell death. Collectively, these data suggest that TXNIP plays multiple roles in organismal glucose homeostasis, with key target tissues being skeletal muscle, liver, adipose and beta-cells (Figure 4). The MondoA- and ChREBP-dependent regulation of TXNIP suggests a similar broad role for the extended Myc network in regulating glucose homeostasis at the organismal level. Clearly, further experimentation in this area is warranted.

6.2 TXNIP function in cell growth

Because TXNIP regulates glucose uptake, $p27^{kip1}$, and HIF1alpha, it is not surprising that it is a potent negative regulator of cell growth. TXNIP overexpression leads to growth arrest or senescence, and loss of function experiments show that TXNIP is required for quiesence of hematopoetic stem cells (90), and for growth arrest following serum removal (our unpublished data). In a number of cell types, deletion or knockdown of TXNIP is sufficient to increase glucose uptake, glycolysis, and lactate production (9, 79, 84), all of which are hallmarks of aerobic glycolysis, i.e. the Warburg effect. Collectively these findings suggest a tumor suppressor function for TXNIP. It is still unclear which of TXNIP's effectors – inhibition of glucose uptake, $p27^{kip1}$ stabilization or HIF1alpha destabilization - is most important for these different phenotypes, or whether all contribute. TXNIP knockout mice develop hepatocellular carcinoma at about 8 months of age (91), and high TXNIP expression portends a good prognosis in both gastric and breast cancer (17, 77, 92), supporting a tumor suppressive function. Furthermore, growth factor signaling pathways, which are often dysregulated in cancer, negatively regulate TXNIP transcription and translation (our unpublished data), further linking TXNIP levels to tumorigenesis. Related to this, MondoA mRNA levels have recently been correlated with a good prognosis in ovarian cancer (93).

7. MONDOA AND GLUTAMINOLYSIS

Both glucose and glutamine are required for cell growth: tumor cells fail to divide in medium that contains glucose but lacks glutamine (94). MondoA seems to play an important role in coordinating glycolysis and

glutaminolysis by activating or repressing transcription of the TXNIP promoter depending on the status of the TCA cycle (Figure 3). For example, in medium that contains glucose but lacks glutamine, MondoA is a potent activator of TXNIP expression, which restricts glucose uptake and cell proliferation. Addition of glutamine to glucose-containing medium stimulates growth as expected. Surprisingly, MondoA represses TXNIP expression in this condition, resulting in a stimulation of glucose uptake and activation of cellular metabolic/growth programs. Thus, glutamine effectively represses glucose-dependent and MondoA-dependent transcriptional activation of TXNIP (Figure 5). How glutamine converts MondoA to a transcriptional repressor is not yet understood. However, our data suggests that glutamine triggers displacement of coactivators from promoter-bound MondoA:Mlx complexes and/or recruits corepressors to promoter-bound MondoA complexes. These findings suggest that MondoA coordinates glycolysis and glutaminolysis via its regulation of TXNIP. Under such a metabolic checkpoint model, TXNIP would restrict cell growth until levels of glucose and glutamine are sufficiently high to support growth and division.

Glutamine is metabolized to glutamate and subsequently to alpha-KG, and alpha-KG refills the TCA cycle by anapleurosis. It is difficult to rule out the involvement of other glutamine-dependent pathways in this process; however, blocking the conversion of glutamine to glutamate blocks glutamine-dependent repression of TXNIP. This blockade can be bypassed by cell-permeable analogs of alpha-KG to phenocopy the effects of glutamine (94). alpha-KG-dependent anapleurosis refills the TCA cycle and drives transcriptional repression of TXNIP (Figure 5). Together, these data suggest that MondoA senses low flux through the TCA cycle, or a TCA intermediate *per se*, and activates TXNIP expression. By contrast, when the TCA cycle is refilled by glutaminolysis, TCA-derived signals convert MondoA from an activator to a repressor of TXNIP transcription. In this regard, MondoA function is conceptually similar to several nuclear hormone receptors, in that it represses specific targets under one nutrient condition (+glutamine) and activates those same targets under a different nutrient condition (-glutamine). At this time, how MondoA senses a functional TCA cycle is not known. However, the localization of MondoA:Mlx complexes at the outer mitochondrial membrane places them perfectly to sense TCA cycle status (95). A final interesting implication of these findings is that glutaminolysis, via MondoA-dependent repression of TXNIP, functions upstream of glucose uptake and glycolysis.

8. MITOCHONDRIAL DYSFUNCTION CONTRIBUTES TO SKELETAL MUSCLE INSULIN RESISTANCE

Given the roles of Myc in mitochondrial function, MondoA in sensing TCA cycle activity, and TXNIP in fuel selection (i.e. glucose vs. fatty acids) it is worth considering how mitochondrial dysfunction may contribute to insulin resistance. Elevated fatty acid levels contribute to skeletal muscle insulin resistance (59). One mechanism that drives this effect is the cytoplasmic accumulation of triglycerides, diacylglycerol, and ceramide, leading to the activation of stress-activated protein serine kinases. This kinase activation blocks insulin signaling and GLUT4 translocation to the plasma membrane (59). A second and emerging mechanism connecting excess fatty acid and insulin resistance is an imbalance between excess fatty acid beta-oxidation and deficiencies in TCA intermediates (59). This imbalance may cause mitochondrial stress that contributes to insulin resistance by a number of mechanisms. Support for this model includes: 1) imbalances in skeletal muscle acylcarnitines and a dearth of TCA intermediates in several models of insulin resistance (96) 2) overexpression of PPARalpha in skeletal muscle drives beta-oxidation, glucose intolerance, insulin resistance and GLUT4 downregulation, which is in turn blocked by inhibitors of beta-oxidation or inhibitors of electron transport (97), 3) genetic deletion of malonyl-CoA decarboxylase, required for fatty acid import into mitochondria, or pharmacologic inhibition of carnitine palmitoyl transferase 1 (CPT1), blocks fatty acid induced insulin resistance *in vivo* and *in vitro*, respectively (96), 5) blockade of oxidative phosphorylation by skeletal muscle-specific deletion of apoptosis inducing factor (AIF), a key component of the electron transport chain, leads to improved glucose tolerance and insulin sensitivity and reduced diet-induced obesity and diabetes (98). Together, these data strongly suggest that the rate of beta-oxidation must be appropriately balanced with TCA intermediates and/or electron transport to achieve glucose tolerance and insulin sensitivity. Conversely, an imbalance between beta-oxidation and the availability of TCA intermediates leads to mitochondrial stress and subsequent insulin resistance. At present it is unclear how this imbalance causes insulin resistance. However, our data show that MondoA senses a depleted TCA cycle (Figure 5), which we suggest is also a form of mitochondrial stress, to activate TXNIP gene expression, triggering a negative feedback loop to restrict glucose uptake. Fatty acids and other models of insulin resistance also lead to a depletion of TCA cycle intermediates (96), thus it conceivable that MondoA activates TXNIP under these mitochondrial stress conditions and contributes to insulin resistance.

9. CHREBP AND METABOLISM

ChREBP is most highly expressed in liver where it has been shown to regulate many of the genes involved in lipogenesis and is required for the majority of glucose-dependent transcription. Consistent with these findings, ChREBP null mice are deficient in both glycolytic and lipogenic readouts (99, 100). Consistent with a key role in lipogenesis, ChREBP whole-body knockout mice suppressed the marked obesity phenotype observed in leptin-deficient ob/ob mice (101, 102). The authors postulated that the effects in these experiments were due to loss of ChREBP in the liver. However, CHREBP deletion was not conditional in this study; thus, a liver-specific knockout of ChREBP is needed to test this hypothesis definitively. In humans, a number of Genome Wide Association Studies (GWAS) have identified single

nucleotide polymorphisms (SNPs) associated with triglyceride levels near or within the ChREBP open reading frame (103-105). Collectively, these studies suggest that ChREBP is a lipogenic transcription factor, whose activity is upregulated when high levels of intracellular glucose require conversion to lipid, and repressed when high levels of lipid necessitate down-regulation of further lipogenesis.

Interestingly, ChREBP phenotypically overlaps with the best-known transcriptional regulator of lipid synthesis, SREBP (106). In an intriguing parallel, SREBP proteins translocate to the nucleus from the endoplasmic reticulum in response to low sterol levels, whereas the model of Uyeda and colleagues proposed an analogous cytoplasm-to-nucleus route for ChREBP. Additionally, SREBP-1c and ChREBP have been shown to act coordinately at several lipid response gene promoters (107). Further work has demonstrated that this cooperativity is specific: ChREBP and SREBP only co-occupy a subset of target promoters (108, 109), and other transcription factors with similar functional profiles, like LXR, do not show coordinate action with ChREBP (110).

10. MONDOA AND METABOLISM

We have discussed multiple findings demonstrating that MondoA is important for proper glucose homeostasis in cell culture models. Several groups are now using animal models to further investigate how MondoA, ChREBP, and TXNIP contribute to proper glucose homeostasis. Given their largely non-overlapping expression patterns, we presume that MondoA is the primary regulator of TXNIP in skeletal muscle whereas ChREBP is the primary regulator of TXNIP in liver (Figure 4). This hypothesis has not yet been rigorously tested. Further, MondoA and ChREBP are both expressed in pancreatic beta-cells, yet whether one or both are the primary drivers of TXNIP expression in these cells is unclear. ChREBP can regulate TXNIP in a number of cell lines, including insulinomas; however, the contribution of MondoA has not been investigated in beta-cells or beta-cell lines. One study compared the activity of MondoA and ChREBP at the TXNIP promoter using a luciferase-based reporter system in Hela cells and showed that MondoA was about 10 times more active than ChREBP in regulating TXNIP in this head-to-head comparison (19). More detailed studies are required to determine whether MondoA is more active than ChREBP at the TXNIP promoter *per se*, or whether cell context also contributes.

Although little information is available regarding the *in vivo* functions of MondoA, many *in vivo* activities of TXNIP have been described. Because TXNIP is strictly dependent on MondoA for its expression, one can speculate about MondoA's TXNIP-dependent functions *in vivo*. TXNIP expression is elevated in skeletal muscle isolated from prediabetics and diabetics (84), suggesting a role for MondoA in control of peripheral glucose uptake and insulin resistance. No alterations were identified in the TXNIP gene or regulatory sequences in this study, suggesting that the function of its upstream regulators, i.e. MondoA, may be altered in these patients. Related to this is the finding that high expression of MondoA mRNA in skeletal muscle correlated with response of insulin resistance patients to thiazolidinediones (111). These preliminary findings validate the need to directly assess the role of MondoA in glucose metabolism. We are currently addressing MondoA function in skeletal muscle using a conditional null allele of murine MondoA, and plan to compare metabolic phenotypes of tissue-specific deletion of MondoA to those of TXNIP and ChREBP knockout animals (83, 99).

11. MONDOA AND CHREBP IN CELL PROLIFERATION

Although studies examining MondoA and ChREBP function in cell growth are currently limited, those that have been published suggest that they may have largely opposite functions, which can likely be traced to the different targets regulated by each transcription factor. For example, ChREBP regulates expression of lipogenic target genes in liver cells. Given that de novo lipid synthesis is required for cell division (112), it is not surprising that ChREBP knockdown in HCT116 cells resulted in a reduction in lipid synthesis and other anabolic processes, and a reduced growth rate (25). Its seems likely that a fraction of this growth defect can be attributed to a reduction in anabolic reactions, yet ChREBP knockdown also triggers a p53-dependent stress response that may also contribute to the slow growth phenotype. At present it is unclear how ChREBP loss signals to p53, but this finding is consistent with a role for p53 in sensing metabolic stress (113). Determining the effect of ChREBP loss in p53 null cells will address this unanswered question.

MondoA appears to play a negative role in regulating cell proliferation, due at least in part to its direct regulation of TXNIP. We have proposed that the glucose-induced regulation of TXNIP by MondoA constitutes a potent negative feedback mechanism to further restrict glucose uptake. MondoA or TXNIP loss of function in several different cell types results in increased glucose uptake and increased division rates. Conversely, overexpression of a dominant active form of MondoA restricts glucose uptake and cell growth in wild type murine embryonic fibroblasts (MEFs), but not TXNIP knockout MEFs (our unpublished data) (9, 14, 16, 79). These findings suggest that TXNIP is a key MondoA effector in restricting cell growth, and are in stark contrast to the reduced growth rate observed in ChREBP loss of function experiments. Future work must address whether the differing roles of MondoA and ChREBP in cell growth stem from their tissue-specific expression patterns, or if these two transcription factors may act in the same cells, competing for the promoters of cell growth-regulating genes.

12. POTENTIAL COOPERATION BETWEEN MEMBERS OF THE EXTENDED MYC NETWORK

Myc and Mondo family members have similar bHLHZip domains, DNA binding specificities and similar overall domain structure (3). Therefore, they may cooperate in specific biological processes by coregulating

target genes. We observed synthetic lethal interactions after loss of the Drosophila orthologs of Myc and Mondo (3), implying that Myc and Mondo proteins are required for at least one essential function in the development of the fruit fly. Although this type of analysis has yet to be conducted in higher eukaryotes, cooperation between the Myc and Mondo families might manifest in several ways (Figure 1). We outline some of these below:

Myc proteins have several highly conserved and defining sequences in their N-termini critical for function. Myc Box II recruits different transcriptional coregulatory complexes and plays an essential role in cell transformation (1). MondoA and ChREBP each have a sequence in their N-termini with some sequence homology to Myc box II (3). Thus, it is possible that MondoA or ChREBP might cooperate with Myc in transformation, or, under some circumstances, be able to drive transformation in isolation. Although this hypothesis remains largely unexplored, ChREBP knockdown reduces cell proliferation in culture and reduces tumorigenesis in xenograft models (25). Thus, ChREBP may be necessary for transformation. It remains to be formally tested whether MondoA and/or ChREBP are sufficient for transformation.

There may be functional cooperation between Myc and MondoA or Myc and ChREBP at specific target genes (114, 115). Myc and ChREBP are both required for the direct and glucose-dependent activation of liver-type pyruvate kinase (Pklr) transcription. Furthermore, both Myc and ChREBP bind the Pklr promoter under hyperglycemic growth conditions, suggesting that promoter occupancy by each of these factors is glucose-dependent. Interestingly, Myc knockdown or small molecule inhibition of Myc:Max complex formation reduced the glucose-induced binding of ChREBP to the Pklr promoter, suggesting a key role for Myc:Max complexes in the recruitment of ChREBP:Mlx complexes. Importantly, transgenic overexpression of Myc in mouse liver also drove Pklr (60), suggesting that coregulation of targets by Myc and ChREBP may also extend *in vivo*. Myc inactivation also blocked ChREBP recruitment to the promoters of several other glucose-dependent target genes and blocked their induction. This finding suggests that there may be a general requirement for Myc:Max complexes at ChREBP-dependent, and presumably MondoA-dependent, glucose-induced target genes. Since Myc expression depends on the presence of growth factors, it is possible that glucose-dependent target genes regulated by MondoA and/or ChREBP can only be activated in the presence of growth factors. Such a model represents a novel means of coordinating the availability of growth factors and glucose. Finally, Myc overexpression in liver can upregulate SREBP1c (63), and SREBP1c and ChREBP co-occupy and cooperate to activate several lipogenic targets (23). Therefore, cooperation between Myc and ChREBP may also occur by this more indirect mechanism. These previous studies did not determine whether ChREBP and/or MondoA were required for induction of Myc targets. Although it is of great interest to determine how widespread the co-dependence between Myc and MondoA or ChREBP may be, no genome-wide occupancy studies have been published for MondoA or ChREBP. Determining the promoterome of MondoA and ChREBP will facilitate important comparisons to the broad promoterome of Myc.

Myc can drive glutaminolysis, and MondoA senses glutamine levels in the TCA cycle via alpha-KG (55, 56, 94). Thus, a functional TCA cycle may also contribute to Myc-MondoA cooperation. While not tested in the original publications, it seems likely that Myc-dependent glutaminolysis drives TCA anapleurosis and a functional TCA cycle converts MondoA to a transcriptional repressor of TXNIP (94). Thus in Myc-dependent tumor cells, high glutamine-derived TCA intermediates may increase the relative transcriptional repression activity of MondoA at the TXNIP promoter, resulting in elevated glucose uptake. Supporting this model, simply blocking glutaminolysis results in the upregulation of TXNIP and decreases in glucose uptake (9). Thus, in addition to its well-established role in the direct regulation of glycolysis and target genes encoding glycolytic enzymes, Myc may also stimulate glucose uptake by a glutaminolysis-dependent mechanism.

13. SUMMARY AND PERSPECTIVE

Glucose is a fundamental nutrient required to support growth and division of all organisms, yet elevated uptake and utilization of glucose drives aerobic glycolysis, i.e. the Warburg effect, a common and apparently required feature of tumorigenesis. Similarly, alterations in glucose homeostasis in several tissues – liver, skeletal muscle, adipose and pancreatic beta-cells – contribute to insulin resistance and diabetes. We propose that the bHLHZip transcription factors MondoA and ChREBP constitute an arm of an extended Myc network, as they are related to Myc not only in domain structure but also in their broad command of glucose homeostasis. Understanding the regulation of MondoA and ChREBP, and their direct transcriptional targets in different cell and tissue contexts, will likely provide novel insights into how cells sense and respond to changing extra- and intracellular nutrient levels. Myc directly regulates key transcriptional targets encoding metabolic enzymes, allowing it to command glucose uptake and shunting of glucose-derived carbons into key biosynthetic pathways. It is likely that MondoA and/or ChREBP cooperate with Myc at several levels. Synergy between these structurally related transcription factors almost certainly underlie the coordination of glucose sensing and utilization pathways in the cell.

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Abbreviations: alpha-KG: alpha-ketoglutarate, TXNIP: thioredoxin interacting protein, ChREBP; carbohydrate response element binding protein, OXPHOS; oxidative phosphorylation, TCA; tricarboxylic acid, G6P: glucose 6-phosphate, X5P; xylulose 5-phosphate; MLXIP; mlx-interacting protein, MLXIPL; mlx-interacting protein like, ChoRE; carbohydrate response element, SNPs; single nucleotide polymorphism, GWAS; genome wide association study, MCR: Mondo conserved region, PPP: pentose phosphate pathway, WBSCR14; William-Beuren Syndrome Critical Region 14

Key Words: Myc, MondoA, ChREBP, Mlx, Metabolism, Glucose, Glutamine, Glycolysis, Glutaminolysis, Mitochondria, Transcription, Review

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