Published in final edited form as:

Biochim Biophys Acta. 2015 May ; 1849(5): 484–500. doi:10.1016/j.bbagr.2014.05.016.

Functional Interactions Among Members of the MAX and MLX Transcriptional Network During Oncogenesis

Daniel Diolaiti, Lisa McFerrin, Patrick Carroll, and Robert N. Eisenman*

Division of Basic Sciences, Fred Hutchinson Cancer Research Center

Abstract

The transcription factor MYC and its related family members MYCN and MYCL have been implicated in the etiology of a wide spectrum of human cancers. Compared to other oncoproteins, such as RAS or SRC, MYC is unique because its protein coding region is rarely mutated. Instead, MYC's oncogenic properties are unleashed by regulatory mutations leading to unconstrained high levels of expression. Under both normal and pathological conditions MYC regulates multiple aspects of cellular physiology including proliferation, differentiation, apoptosis, growth and metabolism by controlling the expression of thousands of genes. How a single transcription factor exerts such broad effects remains a fascinating puzzle. Notably, MYC is part of a network of bHLHLZ proteins centered on the MYC heterodimeric partner MAX and its counterpart, the MAX-like protein MLX. This network includes MXD1-4, MNT, MGA, MONDOA and MONDOB proteins. With some exceptions, MXD proteins have been functionally linked to cell cycle arrest and differentiation, while MONDO proteins control cellular metabolism. Although the temporal expression patterns of many of these proteins can differ markedly they are frequently expressed simultaneously in the same cellular context, and potentially bind to the same, or similar DNA consensus sequence. Here we review the activities and interactions among these proteins and propose that the broad spectrum of phenotypes elicited by MYC deregulation is intimately connected to the functions and regulation of the other network members. Furthermore, we provide a meta-analysis of TCGA data suggesting that the coordinate regulation of the network is important in MYC driven tumorigenesis.

1. The MAX/MLX network: identification, evolution and organization of the network members

1.1. From the discovery of v-myc to the MAX/MLX network

Since its identification in the late 1970s the *MYC* gene has been the subject of intense research that has linked it to all major hallmarks of cancer. The ability of *MYC* to influence such a broad spectrum of cellular activities may be explained by the fact that its protein

© 2014 Elsevier B.V. All rights reserved.

*Correspondence: Fred Hutchinson Cancer Research Center MS A2-025, P.O. Box 19024, Seattle WA 98109-1024, Tel: 206 667 4445; eisenman@fhcrc.org.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

product, MYC, belongs to an extended network of transcription factors (referred to here as the MAX/MLX network) with diverse activities, which in turn regulate many biological processes (Fig. 1).

1.1.1. MYC and MAX: enabling MYC activity—The oncogene *v-myc* was among the first retroviral oncogenes described. Initially identified as the common transforming genetic element shared by the retroviruses MC29, CMII, MH2, and OK10, it was later shown to be derived from a cellular oncogene, termed *c-MYC*, (from now on referred to as *MYC*) [1–3]. The prominent role of *MYC* in human cancer etiology was revealed by the findings that *MYC* overexpression, due to chromosomal translocation or gene amplification, was a common event in Burkitt's lymphoma [4, 5] and myeloid leukemia, respectively [6]. Amplification of the subsequently identified *MYC* paralogs *MYCN* and *MYCL* were also reported in neuroblastoma and small cell lung carcinoma, respectively, firmly linking *MYC* family proteins to the etiology of human cancers [7–10].

Initial sequence comparisons of *MYC* family genes revealed they share a predicted basic Helix-Loop-Helix Leucine Zipper (bHLHLZ) domain also found in several transcription factors and known to be involved in dimerization and DNA binding [11], suggesting the possibility that *MYC* proteins may homo or heterodimerize. Initial experiments failed to establish homodimerization of *MYC* protein at physiological expression levels [12–17]. However, the search for a *MYC* heterodimerization partner led to the discovery of a novel bHLHLZ protein, MAX (*MYC* interacting factor X) [18]. Importantly, MAX also recognizes and heterodimerizes with *MYCN* and *MYCL*, implicating MAX in mediating the function of the three *MYC* family proteins, which have distinct and largely mutually exclusive spatiotemporal expression profiles during development [18, 19]. In contrast with *MYC* family members, Max is an abundant and stable protein, expressed in both proliferating and in resting cells, regardless of *MYC* levels [20]. These properties of MAX, together with its ability to bind DNA and repress transcription as a homodimer, led to the first model in which gene expression of *MYC* targets was controlled by the shift between repressive MAX-MAX homodimers and activating *MYC*-MAX heterodimers [21–24].

1.1.2. MXD proteins: MYC antagonists—Because MAX was found to be present under conditions in which *MYC* proteins are not expressed, and because MAX homodimerizes poorly, if at all, *in vivo* [25] it was considered that MAX may bind to other bHLHLZ containing proteins. Indeed, two MAX interacting proteins MXD1 and MXD2 (formerly known as MAD1 and MXI1) were soon identified [26, 27]. MXD1 and MXD2 are structurally similar, sharing 43% of their overall protein sequence, and are evolutionarily related. Moreover, with the exception of the bHLHLZ domain, which mediates their specific interaction with MAX, they are distinct from *MYC*. In vitro DNA binding and protein interaction assays confirmed that both MXD1 and MXD2 are unable to homodimerize, yet efficiently compete with *MYC* for heterodimerization with MAX. More importantly, both MXD1-MAX and MXD2-MAX heterodimers recognize the same E-box containing DNA consensus sequence as *MYC*-MAX heterodimers, but function as transcriptional repressors (MXD1-MAX) [26] or are unable to activate transcription (MXD2-MAX) [27]. The discovery of MXD1 and MXD2 added a new level of complexity to the earlier model by

showing that MAX acts to mediate the activity of both MYC and MXD proteins which appeared to antagonize each other. In addition, the data suggested that it is the relative abundance and regulation of MYC and MXD proteins that define the transcriptional and biological outcome of network activity [26].

Shortly after the discovery of MXD1 and MXD2, four additional MXD-like proteins members were added to the network (MXD3, MXD4, MNT and MGA) [28–30]. While MXD3 and MXD4 are highly homologous with the previously identified MXD1 and MXD2 proteins, MNT and MGA differ significantly. However, apart from MGA, which contains an additional T-box domain and can both activate and repress transcription, the similar functional properties of MXD1–4 and MNT proteins (as all included in the MXD family proteins), which act as transcriptional repressors, further strengthen the model in which MXD proteins antagonize MYC in regulating many aspects of cell biology.

1.1.3 The MLX module: the metabolite sensing arm of the network—The existence of a parallel network which resembles the MYC/MAX/MXD transcriptional network was proposed after the discovery of a MAX-like protein called MLX. Two research groups independently identified MLX as the binding partner of MXD1, MLX4, and MNT proteins, and showed that MXD/MLX complexes mediate transcriptional repression of E-box containing promoters [31, 32]. Subsequently two large bHLHZ proteins were found to heterodimerize with MLX and regulate transcription by binding to E-box containing promoters [33, 34]. MONDOA was identified as an MLX heterodimeric partner by a two hybrid screen, while MONDOB was identified by sequence homology analysis. MONDOB, which had previously been identified as CHREBP (Carbohydrate Response Element Binding Protein) was also known as the *WBSCR14* gene, since it maps to chromosome 7q11.23, a region usually deleted in Williams-Beuren Syndrome (WBS) [33]. CHREBP was subsequently confirmed to be an MLX interacting protein [34]. In contrast with the nuclear localized MYC, MAX and MXD proteins, MLX and MONDO proteins localize principally in the cytoplasm and can shuttle between the two compartments in response to glucose and other metabolic stimuli [35–38]. Because most of the literature refers to MONDOB as CHREBP, we will also refer to it as CHREBP in this review.

1.2. Evolutionary conservation of the MAX/MLX network

Underscoring the fundamental importance of the MAX/MLX transcriptional network in regulating cell biology is the fact that it has been conserved throughout evolution. Orthologs of *human* MAX MYC, MXD, MLX, and MONDO genes have been identified in the Placazoan *Trichoplax adhaerens*, considered the simplest metazoan [39]. Moreover, MYC and MAX orthologs also exist in *Capsaspora owczarzaki*, and in the choanoflagellate *Monosiga brevicollis* [39–41]. The presence of MYC and MAX orthologs in these two unicellular organisms, which are the closest living organisms to metazoa, indicates the emergence of the MAX module of the network even predates the evolution of multicellularity and metazoans.

A comprehensive analysis of the evolutionary conservation of the MAX/MLX network in metazoa identified or predicted the existence of network members in almost all surveyed species (Fig. 2A) [42]. Although the composition and sequence of network members has

changed considerably during evolution, a core network consisting of *MAX*, *MYC*, *MXD*, *MLX*, *MNT*, and *MONDO* orthologs predominates and is the basis from which other networks emerged. The *MAX/MLX* network in vertebrates has acquired additional complexity due to the presence of multiple paralogous genes, which have distinct heterodimerization preference, gene expression profiles and functions. Meanwhile, lower order organisms usually have fewer members and, in the case of nematodes and flies, the network appears to have a distinct, yet balanced structure (Fig. 2B).

The Diptera lineage possesses a minimal network consisting of single orthologs of *human MYC*, *MAX*, *MNT*, *MLX* and *MONDO* genes (Fig. 2b) [36, 43–45]. *Drosophila myc* (*dmyc*), previously known as *diminutive* (*dm*), and *max* (*dmax*) were the first members of the network identified in *Drosophila* [43, 46]. Since then, many laboratories have confirmed the high functional redundancy between human and *Drosophila myc*, *max* and *mnt* orthologs [for review 47, 48]. Moreover a recent report demonstrated *Drosophila mlx* (known as *bigmax*) and its Mondo-like dimerization partner *mio* are involved in sugar sensing and utilization. This suggests the parallel and balancing functions of the *MAX/MLX* network that control cell proliferation and metabolism, have been also maintained during evolution [49, 50].

1.3. Organization and Structure of *MAX/MLX* network members

All members of the *MAX/MLX* network are defined by the presence of a bHLHLZ domain which mediates heterodimerization between family members and sequence-specific DNA binding (Fig. 3) [42, 51–53]. *MYC* family members are characterized by having the bHLHLZ domain localized at the C-terminus of the protein, and a transcriptional activation domain (TAD) located near the N-terminus. Within the TAD, *MYC* family members contain two well defined and conserved domains termed *MYC Box I* and *MYC Box II* (*MBI* and *MBII*) both of which are functionally important. While *MBI* serves as phosphodegion and is mainly involved in the regulation of *MYC* protein stability [54]; *MBII* mediates the interaction of *MYC* with multiple proteins such as *TRRAP*, *GCN5*, *TIP60* and *TIP48* [55–58], all of which have been identified as components of histone acetyltransferase complexes [59, 60]. Deletion of *MBII* inhibits most *MYC* functions, consistent with the importance of histone acetyltransferase complexes recruitment in *MYC*'s normal and pathological activities [61]. Moreover, the TAD region of *MYC* also mediates its interaction with other effectors of *MYC* activity such as the bromodomain protein *BRD4* and the *P-TEFb* transcriptional anti-pause complex [62–64]. Recent studies have proposed that *MYC*-mediated augmentation of RNA polymerase II (RNA Pol II) dependent elongation is the principal mechanism through which *MYC* increases expression of its target genes [65–67]. Two other conserved domains *MBIII* [68] and *MBIV* [69] have also been identified within *MYC* proteins. *MYC* mutants lacking these domains are partially defective in their transcriptional activity and have a reduced tumorigenic potential.

While *MYC* proteins possess a TAD which acts as binding sites for factors involved in transcriptional activation, *MXD* proteins possess a repression domain known as the *SIN3* interaction domain (*SID*) [70]. The *SID* domain is localized near the N-termini of *MXD* proteins and mediates interaction with the paired amphipathic helix 2 (*PAH2*) domain of

SIN3 proteins, which in turn recruits histone deacetylase containing complexes [71–73]. The loss of repression activity resulting from the inhibition of MXD-SIN3 interaction suggests that histone deacetylation is the main function contributing to transcriptional repression by MXD proteins [70]. Hence, in addition to competition for MAX and DNA binding, another level of MYC and MXD protein biological antagonism occurs as a consequence of molecular antagonism manifested by histone acetylation and deacetylation.

The antagonistic roles of these proteins also seem to be manifested at the level of histone methylation. High levels of trimethyl Histone H3 lysine 4 (H3K4me3) are associated with active promoters [74, 75]. MXD dependent recruitment of histone demethylase RBP2/KDM5a induces histone H3K4me3 demethylation at the *TERT* promoter and correlates with decreased expression of TERT during DMSO-induced differentiation of HL-60 Human promyelocytic leukemia cells [76]. Interestingly, *Drosophila* Myc interacts directly with the fly ortholog of RBP2, known as Lid, but this binding directly inhibits the demethylase activity of Lid [77, 78]. Moreover, MYC has been reported to interact with MEN1 [79], a subunit of the mixed-lineage leukemia-1 (MLL-1) methylation complex, which mediates H3K4 methylation. Consistent with this, MYC's genomic binding profile strongly correlates with H3K4me3 levels [80, 81]. Several reports have shown that changes in global levels of histone H3K4me3 correspond with increasing or decreasing MYC expression [82, 83]. In the MYC inducible lymphoma cell line p493-6, however, activation of MYC was found to promote H3K4me3 methylation at only a subset of MYC target genes [84]. Overall, these data strongly suggest that the MYC and MXD proteins antagonize each other by promoting and inhibiting, respectively, both histone acetylation and methylation.

MAX and MLX apparently possess neither TAD nor SID domains and therefore are considered transcriptionally inert. However, MLX differs from MAX because, like MONDO proteins, it contains a dimerization and cytoplasmic localization domain (DCD domain) at its C-terminus [33–35]. MONDOA and CHREBP also contain five domains named Mondo Conserved Regions (MCRI-V) within their N-termini, which mediate glucose sensing and transcriptional activity [see reviews 36, 85]. Moreover the existence of a MCR6 domain in MONDOA protein, which represents a potential binding site for glucose-6-phosphate (G6P), has recently been proposed [86]. MONDOA associates to the outer mitochondrial membrane, where it can also potentially sense other metabolites such as glutamine (through α -ketoglutarate), adenine nucleotides and non-glucose sugars [37, 87–91]. Thus, MONDO proteins sense multiple metabolites to directly link MONDO transcriptional activity to the metabolic state of the cell. While the exact mechanism through which MONDO proteins regulates transcription is still not completely clear, it has been shown that ChREBP associates, through its TAD domain, with p300 and mediates histone acetylation [92].

MGA differs from all other members of the network because it contains an additional T-loop DNA binding domain and can either act as activator or repressor depending on its interaction with MAX. Apart from MGA belonging to a repressive complex that binds to MYC and E2F responsive genes in G0, very little is known about its biological functions [30, 93]. Very recently MGA, together with MAX and SMAD4, has been shown to be involved in BMP signaling during gastrulation in *Xenopus* embryonic development [94].

Interestingly *MGA* is recurrently inactivated in high risk chronic lymphocytic leukemia [95] and in lung cancer [96], suggesting that it may function as a tumor suppressor gene.

2. Functional interactions among network members

Many reviews have previously discussed the role of MYC in controlling multiple aspects of cell biology [97–104]. Here we will focus on how the activities of the two arms of the MAX/MLX network are functionally interconnected. In particular, we will discuss how a broad range of common MYC activities (Fig. 4A) are shaped by other network members (Fig. 4B) and the manner in which these biological functions are affected when deregulated MYC drives cell transformation. For example, it is becoming evident that MXD members oppose MYC pro-apoptotic functions, suggesting that some aspects of an intact MAX/MLX network may be required for MYC induced tumorigenesis.

2.1. Proliferation and transformation: in vitro and in vivo experimental models

2.1.1 MXD Proteins: inhibiting MYC induced proliferation and transformation

—MYC proteins promote proliferation by interacting with MAX and altering the expression of thousands of genes. However, all MXD proteins, MXD1–4 and MNT, also heterodimerize with Max, bind to the canonical MYC/MAX E-Box consensus sequence and repress transcription from an E-Box containing promoter [26–29]. Competition between MYC and MXD proteins on each of these levels suggests that these transcriptional regulators act as functional antagonists.

Overexpression of all MXD proteins inhibits MYC/RAS induced co-transformation, confirming that MXD proteins can antagonistically inhibit MYC activities *in vitro* [28, 105–111]. Moreover, overexpression of MXD1 and MXD2 proteins inhibits proliferation and induces accumulation of cells in the G0/G1 cell cycle phase in multiple *in vitro* experimental systems [109, 110, 112–122]. The ability of MXD proteins to block MYC induced transformation requires the HLHLZ domain, the basic region and the SID domain, indicating that dimerizing with MAX, binding DNA, and interacting with the SIN3 containing histone deacetylase complex are required for inhibition of MYC activity [107, 108, 111, 123].

Consistent with MXD1 inhibition of proliferation *in vitro*, transgenic mice expressing MXD1 under the control of a constitutive and ubiquitous β -actin promoter have reduced body size and weight compared to their wild type littermates. Both embryonic fibroblast and hematopoietic progenitor cells derived from *Mxd1* transgenic animals show reduced proliferation [120]. Specifically, mouse embryonic fibroblasts (MEF) overexpressing MXD1 were more sensitive to contact inhibition and underwent proliferative arrest at lower density [120]. However, overexpression of MXD1 under control of the β -actin promoter is not sufficient to completely inhibit proliferation as it does not preclude embryonic development [120]. Similar results were obtained when MXD1 was specifically overexpressed in T and B lymphocytes. Although in both cases mature T and B cells were obtained, neither cell types were able to efficiently proliferate in response to multiple mitogenic stimuli *in vitro* [124, 125].

These results are consistent with the phenotype of *Mxd1*^{-/-} mice where *Mxd1* deletion delays cell cycle exit in granulocytes but does not completely block cell cycle arrest and differentiation [126]. Overall the analysis of *Mxd1* transgenic and *Mxd1*^{-/-} mice suggests that additional pathways, besides those directly affected by *Mxd1* overexpression, are likely required to fully promote cell cycle arrest in collaboration with *Mxd1*. Indeed, *Mxd1/p27Kip1* double knockout mice show a much stronger phenotype than either *Mxd1*^{-/-} or *p27Kip1*^{-/-} mice, with markedly impaired cell cycle arrest and differentiation of double null granulocytes. [127]. Finally, *Mxd1*^{-/-} mice appear normal and are not prone to tumor development, indicating *Mxd1* is involved in, but not absolutely required for cell cycle arrest.

The involvement of MXD proteins in the regulation of cell cycle *in vivo* has been further strengthened by the analysis of *Mxd2* knockout mice. Mice carrying homozygous deletion of *Mxd2* gene show several tissue abnormalities, including prostate and spleen hyperplasia/dysplasia [128]. Moreover, *Mxd2*^{-/-} T lymphocytes show increased proliferation in response to anti-CD3 plus anti-CD28 antibody stimulation and *Mxd2*^{-/-} mice develop B cell lymphoma at low frequency and long latency. These data indicate that *Mxd2* may be a potential tumor suppressor, although additional events may be required for tumorigenesis. A formal proof of this hypothesis came from experiments showing that *Mxd2*^{-/-} mice are more susceptible to developing squamous cell carcinoma of the skin and malignant lymphoma when treated with weekly topical application of the carcinogen 9,10-dimethyl-1,2-benzanthracene (DMBA). Consistently, loss of *Mxd2* also accelerates the development of fibrosarcoma and lymphoma in an *Ink4*^{-/-} background [128].

2.1.2. MNT: the major MYC antagonist—In contrast with *Mxd1*, overexpression or deletion of *Mnt* in mice results in very strong phenotypes. *Mnt* transgenic mice die during embryonic development and are significantly smaller than their wildtype littermates [108]. Preliminary analysis of the nervous system indicated defective proliferation and differentiation. *Mnt* deficient mice develop normally, yet are subviable, as more than 95% of the *Mnt*^{-/-} animals die within a few days of birth [129, 130]. These broad organismal effects most likely reflect the ubiquitous expression of *Mnt* during development. [108, 129, 131–134].

Several lines of evidence point to MNT as a major MYC antagonist. First, primary MEFs lacking *Mnt* phenocopy *Myc* overexpressing cells. *Mnt*^{-/-} MEFs proliferate at an accelerated rate, grow to high density and prematurely enter S phase [129, 131]. Analogous results were obtained when *Mnt* expression was silenced by the use of a *Mnt*-specific siRNA [131]. Interestingly, the expression cyclin E1 (*Ccne1*) and cyclin-dependent kinase 4 (*Cdk4*), both previously identified as *Myc* responsive genes [129, 135–137], are elevated in *Mnt*^{-/-} cells. Hence, increased activity of CCNE1/CDK2 and CCND1/CDK4 complexes probably drives the hyper-proliferative phenotype of *Mnt* null cells. Moreover, *Mnt*^{-/-} MEFs are more sensitive to apoptosis, display reduced senescence, and are more susceptible to RAS induced transformation both *in vitro* and *in vivo* [129, 131].

The close functional antagonism between MYC and MNT is further underscored by the observation that, in *Mnt* deficient fibroblasts, MYC expression is drastically reduced,

suggesting that cells compensate for the lack of MNT by decreasing the levels of MYC. Nevertheless, such cells are still capable of proliferation [129]. Notably, silencing of *Mnt* in rat *Myc*^{-/-} fibroblasts promotes both proliferation and apoptosis under conditions of serum starvation. These results suggest a model in which MYC, in response to mitogenic stimuli competes for MAX binding and alleviates MNT-mediated repression. The demonstration that MNT/MAX heterodimeric complexes are converted to MYC/MAX complexes when cells enter the cell cycle supports this model [138]. In addition *Mnt* deficiency rescues the proliferation defect caused by *Myc* deletion in primary MEFs [138]. This is consistent with data showing that in *Drosophila* while complete inactivation of *dmyc* is lethal due to arrested development at the early larval stage, double mutants flies lacking both *dmyc* and *dmnt* grow larger and advance further in development [139, 140]. Overall, the data obtained in both mammalian and *Drosophila* experimental systems provide a strong argument for considering MNT as the principal MYC antagonist.

In this regard it is not surprising that conditional disruption of *Mnt* in mouse mammary epithelium and T cells promotes the development of mammary adenocarcinoma and T cell lymphomas, respectively [129, 132, 141]. However, in both cases, the latency of tumor formation, ranging from 6 to 22 months, indicates that secondary mutations are likely to be cooperating with loss of *Mnt* in tumorigenesis, as proposed for *Mxd2* knockout mice. In the case of Eε-Myc induced B-cell lymphomas such secondary mutations generally act to disable the pro-apoptotic machinery and are required for the development of the B-cell lymphoma in this mouse model [142, 143]. However, recent reports demonstrate that loss of *Mnt* inhibits *Myc* driven tumorigenesis by promoting apoptosis. Therefore MNT may promote or inhibit tumorigenesis depending on the context [144] (see apoptosis section).

2.1.3. The MLX module: an underappreciated player in tumorigenesis—While much research on the functions of the MYC/MAX/MXD module has been carried out, and, as described above, the metabolic functions of MLX and MONDO proteins have been well defined [36, 145–148], comparatively little is known concerning the role of MONDO proteins in tumorigenesis and the possible interactions between the MAX and the MLX branches of the network, specifically in relationship to MYC activity.

It is important to note that, in addition to MONDOA and CHREBP, MXD1, MXD4, and MNT are also able to form heterodimers with MLX [31, 32]. *In vitro* assays show that both MXD1 and MNT maintain their function as transcriptional repressors when bound to either MAX or MLX. [31, 32]. Therefore, some aspects of the phenotypes observed when expression of MXD1 and MNT are altered, may be mediated by the heterodimerization with MLX. MONDO/MLX heterodimers have been considered the positive transcriptional arm of the MONDO/MLX/MXD module. Interestingly, initial experiments investigating the transcriptional capacity of MONDOA or CHREBP suggested they may possess both transcriptional activation and repression functions. MONDOA/MLX heterodimers activated an E-box driven artificial luciferase reporter, while CHREBP repressed the same promoter either when expressed alone or in combination with MLX [33, 34]. However, both MONDOA and CHREBP transcriptionally activate many genes involved in glucose and lipid metabolism [85]. Moreover, MONDOA may act either as an activator or as a repressor

depending on nutrient availability [87]. Therefore, the ability of MONDO proteins to activate or repress gene expression may be context specific.

Considerable experimental evidence indicates that modulation of MONDO protein expression can affect tumor cell growth. MONDOA knockdown in HA1ER transformed embryonic human kidney epithelial cells increases both proliferation and soft agar colony formation compared to control cells [87]. CHREBP overexpression has also been shown to reduce the ability of U2OS cells to form colonies [34], suggesting that loss of MONDO proteins may promote tumorigenesis. However, silencing of MONDOA in Acute Lymphoblastic Leukemia (AML) and CHREBP in pancreatic beta cells and human colon carcinoma HCT116 or HepG2 hepatoblastoma cells, decreases cell proliferation and promotes apoptosis [149–151]. These contradictory results may simply be due to the use of overexpression versus silencing experimental strategies to investigate the role of the MONDO proteins. Alternatively, they may reflect cell type intrinsic functions of MONDO proteins. A recent review discussing *MONDOA* and *MLX* expression, as derived from available databases, showed that high *MONDOA* expression can correlate with either good or poor cancer prognosis [147]. It is important to note that because *MONDOA* transcriptional function depends on availability of metabolites and heterodimerization with *MLX*, the mRNA levels of *MONDOA* alone may not reflect its activity. Moreover, it is possible that *MONDOA* may act either as an oncogene or a tumor suppressor depending on the cellular context.

To date, the ability of MONDO/*MLX* heterodimers to promote or antagonize MYC dependent transformation in a defined mammalian system has not been fully investigated. However, *dmv* and *mio* genetically interact in *Drosophila*, where double homozygosity for a hypomorphic *dmv* diminutive allele (*dm*) and a *mio* mutant (*dmon1*) resulted in a synthetic lethal interaction [36]. This result implies that the two modules of the MAX/*MLX* network functionally interact in invertebrates. Overall these results point toward functional cross-talk between the two modules of the network and suggest a collaborative relationship between MYC/MAX and MONDO/*MLX* heterodimers.

2.2. Role of the network in differentiation

The expression patterns of individual network proteins are distinct and cell context dependent. In general, MAX and MNT are mainly unchanged during the cell cycle and are broadly expressed throughout development and adulthood. In contrast, MYC expression peaks upon cell cycle entry at the G0-G1 transition and is expressed at a constant level during the proliferating cell cycle [152]. Conversely, MXD1, 2 and 4 expression is increased during cell cycle exit and differentiation [153]. MXD3 differs from the other MXD proteins because it is expressed during S phase in proliferating cells [154, 155]. While CHREBP and *MLX* have both been shown to be induced after growth factor or serum stimulation [32, 150], *MONDOA* expression profiles have not been extensively studied during cell cycle, development or differentiation.

High levels of expression of MYC inhibit differentiation of many cell types and promote the reprogramming of somatic cells to induced pluripotent stem cells (iPS cells) [156–158]. In hematopoietic cells induced to differentiate, MYC levels decrease while MXD1 expression

levels increase [159–162]. Analysis of hematopoiesis in *Mxd1*^{-/-} mice revealed a delay in granulocyte differentiation [126] and overexpression of MXD1 has been shown to be sufficient to induce erythroid differentiation of murine and human erythroleukemia cells [163, 164]. This suggests MXD1 plays an active role during differentiation. There is evidence indicating that the contribution of MXD1 to the differentiation process may primarily be due to its role as a MYC antagonist. Indeed, expression of a chimeric protein containing the MYC bHLHLZ domain fused to the MXD1 transcriptional repression domain represses MYC target genes and efficiently induces differentiation of the erythroleukemia cell line K562 [163].

Furthermore, *Mxd1* knockout mice, as well as *Mxd2*, *Mxd3* null mice develop properly and do not show any major differentiation defect [28, 126, 128]. While compensation among MXD proteins may partly explain the lack of a developmental defect in *Mxd* deficient mice, these results strongly suggest that, while MXD proteins may participate in the differentiation process, they are not individually necessary.

Interestingly, rat PC12 pheochromocytoma cells, which lack the *Max* gene [165] and therefore would be expected to be lacking any MYC or MXD dependent activities, are capable of differentiating in response to nerve growth factor (NGF) and RAS [166–168]. In addition, *Max*-null murine embryonic stem cells expressing an inducible *Max* gene cease proliferating, differentiate, and eventually undergo apoptosis when *Max* expression is repressed [169]. However, cell viability and proliferation can be rescued by treating these cells with MAPK and GSK3 β inhibitors (2i condition) or overexpressing OCT4 [169]. The fact that both PC12 and *max* null mECS can undergo differentiation, strongly suggests that MXD proteins are not directly responsible for induction of differentiation, at least as heterodimers with MAX. Instead, these results imply that activation of MAPK and GSK3 β signaling pathways are required for mESC differentiation. Interestingly in the 2i condition, MYC expression is strongly diminished; suggesting that in absence of cell cycle arrest or differentiation signals MYC expression can be reduced without impacting proliferation. Genetic studies in *Drosophila* have demonstrated that *dmax* loss of function mutations are not as severe as *dmyc* deletion, suggesting that *dmyc* possesses functions that are independent of *dmax* [170, 171]. The finding that loss of human *MAX* gene is strongly associated with heritable pheochromocytomas is consistent with MAX independent function of Myc in mammalian cells [172, 173]. Interestingly, MYC overexpression in PC12 cells inhibits Ras-mediated differentiation in the absence of MAX [174].

Both MLX and MONDO protein expression are developmentally regulated but their specific involvement in cellular differentiation has not been fully evaluated. However, *Mlx* (Carroll unpublished), *MondoA* (Don Ayer, personal communication) and *Chrebp* [175] knockout mice develop to adulthood without major developmental defects, indicating that the MLX module is not required for either cell viability or differentiation during embryonic development.

2.3. Apoptosis: a matter of balance

The induction of apoptosis by the deregulated expression of MYC has been well documented [for reviews see 97, 98, 176–179]. Deregulated expression of MYC elicits the

activation of an intrinsic tumor suppressor program, which induces apoptosis. Therefore, the establishment of MYC driven tumors requires the inactivation of the apoptotic machinery, which can result from the loss of tumor suppressor genes such as TP53, or gain of function of anti-apoptotic genes [142, 143, 180, 181]. Moreover, high levels of MYC also sensitize cells to many different extrinsic apoptotic stimuli such as UV, ionizing irradiation, pro-apoptotic signals and nutrient or serum withdrawal [182–187].

Among the network members, MXD1, MXD4 and MNT have been shown to inhibit apoptosis in several different experimental systems. The first observation that MXD proteins are involved in apoptosis came from the analysis of *Mxd1*^{-/-} mice. *Mxd1*^{-/-} granulocytes are more sensitive to cytokine withdrawal and other apoptotic stimuli such as γ -irradiation and doxorubicin [126]. Conversely, overexpression of *Mxd1* protects against apoptosis induced by tumor necrosis factor-related apoptosis inducing ligand (TRAIL), Fas ligand, serum withdrawal, UV and cisplatin treatments [113, 117]. Moreover, MXD1 is induced by hypoxia and mediates hypoxia-induced resistance to doxorubicin [188]. Importantly, MXD1 overexpression also specifically inhibits MYC dependent apoptosis upon serum withdrawal [113]. The mechanistic basis for this may be at least in part due to MXD1 mediated repression of PTEN expression and promotion of the PI3K/AKT pathway. Both MYC and MXD1 bind to the PTEN promoter, indicating that MXD may block MYC induced apoptosis at least in part by repression of a subset of MYC target genes [189]. However, in a set of experiments designed to evaluate the functional equivalence of MYC and MXD1 bHLHLZ domains it was shown that a chimeric protein containing the N-terminal domain of MYC and the bHLHLZ of MXD1 was unable to induce apoptosis upon serum withdrawal [190]. An interpretation of this result is that the bHLHLZ of MYC and MXD1 differ in their ability to interact with additional factors involved in apoptosis. Notably the MYC, but not the MXD1 bHLHLZ domain mediates the interaction with the transcription factor MIZ1. Binding of MYC to MIZ1 inhibits expression of the MIZ1 targets p21^{Cip1} and p15^{ink4b}. [191–193] and is required for MYC induced apoptosis of rat cells upon serum withdrawal [194].

Similarly, overexpression of MNT inhibits MYC transcriptional activity and oncogenic properties, while loss of MNT promotes proliferation but also sensitizes cells to apoptosis [108, 129, 131]. Surprisingly, RNAi mediated silencing of MNT effectively sensitizes primary MEFs, 3T3 immortalized mouse fibroblasts, and Rat *Myc*^{-/-} fibroblasts to serum withdrawal induced apoptosis. This indicates that loss of MNT mediated apoptosis is not solely MYC dependent [131]. In agreement with this interpretation, MYC inactivation only partially rescues MNT dependent apoptosis in MEFs [138]; hence inactivation of MNT promotes apoptosis, which is further exacerbated by activation of MYC.

Analysis of developing T-cells in a mouse model harboring a conditional deletion allele of *Mnt* confirmed that loss of *Mnt* promotes apoptosis in single (CD4+ or CD8+) and double positive (CD4+/CD8+) T-cells and also when MYC is ectopically expressed either in T cells or in primary and immortalized MEFs, suggesting that MNT provides a pro-survival signal and protects cells against MYC mediated apoptosis [144].

The precise molecular mechanisms through which loss of MNT sensitizes cells to apoptosis remain unclear. Nonetheless there are some clues. Inappropriate and increased levels of TP53 and ARF19 have been reported in primary *Mnt*^{-/-} MEFs and *Mnt* KD cells compared to control cells [129, 131]. However, acute CRE-mediated deletion of *Mnt* in primary MEFs failed to confirm these results [138]. Moreover, *Mnt*-deficient thymocytes do not show abnormal TP53 expression levels. Notably, while BCL2 can rescue cells from MYC induced apoptosis, overexpression of BCL2 in either the presence or absence of MYC fails to rescue the *Mnt*^{-/-} apoptotic phenotype, suggesting that MYC overexpression and loss of MNT promote apoptosis, at least in part, through two different pathways [144].

Another possible mechanism related to loss of MNT induced apoptosis comes from a recent study reporting a high level of reactive oxygen species (ROS) in cells overexpressing MYC and lacking MNT. Furthermore treatment of *Mnt* null cells with antioxidants rescues cell viability, indicating that increased levels of ROS might be responsible for the reduced viability of *Mnt* null cells [144]. The fact that *Mnt*^{-/-} cells also have reduced oxygen consumption indicates that such cells have impaired mitochondrial metabolism. Activation of MYC has been shown to promote mitochondria activity and ROS production [144]. Consistent with this, MYC overexpressing *Mnt*^{-/-} cells have higher ROS than either *Mnt*^{-/-} or MYC overexpressing wild type cells. Similar to MNT, MXD4 has also been shown to protect cells from apoptosis during OX40 mediated activation of T cells [195].

Underscoring the close connection between oxidative metabolism and the MAX/MXD network, MXD2 has been reported to be a target of Hypoxia Inducible Factor-1 (HIF-1) [196]. HIF-1 is a heterodimer comprised of the constitutively expressed HIF1b subunit (also known as ARNT) and an oxygen regulated HIF-1a or HIF2a (commonly known as EPAS1) subunit. HIF1 regulates glucose metabolism and has been linked to tumor growth, metastasis and angiogenesis. In renal carcinoma cells, HIF1 is hyper-activated because the tumor suppressor Von Hippel-Lindau (VHL), which mediates its degradation, is lost [197]. In both renal cell cancer cell lines and primary tumors, MXD is highly expressed and antagonizes MYC transcriptional activity [198]. Surprisingly cells with reduced expression of MXD2 proliferate faster in vitro, yet require MXD2 to efficiently form tumors *in vivo* [198]. A possible explanation is that MXD2 expression partially antagonizes MYC activity and prevents MYC induced apoptosis under conditions of limited oxygen availability. Moreover, MXD2-mediated, HIF-dependent downregulation of mitochondrial metabolism may provide a survival advantage by reducing ROS levels [196, 198]. Indeed, MXD1 is also induced by hypoxia in colon cancer cells and mediates resistance to doxorubicin under hypoxia [188].

Overall MXD proteins can be considered to promote survival under stress conditions particularly when MYC is highly expressed. Therefore, although decreased MXD protein expression may be beneficial for tumor growth when expression of MYC is not deregulated, low levels or complete loss of MXD proteins becomes counterproductive and may promote apoptosis when MYC is overexpressed.

Very little is known about the role of MONDOA and CHREBP in apoptosis and more specifically in MYC mediated apoptosis. Silencing of MONDOA in acute lymphoblastic leukemia and MYCN amplified neuroblastoma cells promotes apoptosis suggesting that

MONDOA may cooperate with MYC in tumorigenesis [151](Carroll, Diolaiti, McFerrin and Eisenman, unpublished).

CHREBP by contrast has been shown to mediate pancreatic beta cell sensitivity to high glucose (glucotoxicity). In response to sustained high levels of glucose, CHREBP induces oxidative stress and apoptosis through its target gene TXNIP which promotes oxidative stress by inhibition of thioredoxin [199]. CHREBP is also required for HCT116 colon cancer and HepG2 liver cancer cells survival [150], however how CHREBP activity affects MYC dependent apoptosis remains to be understood.

2.4. Growth and metabolism: does the MLX module enable nutrient addiction in Myc transformed cells?

Early studies defining MYC transcriptional targets provided a list of genes involved in several metabolic processes, indicating that MYC not only drives proliferation but also promotes the activity of both catabolic and anabolic metabolism [200–204]. Recently the role of MYC as a master regulator of growth and metabolism has become widely accepted [102, 104, 205]. MYC not only promotes the activity of all three RNA polymerases [206–208], but also directly promotes the expression of many genes involved in glucose and glutamine metabolism [209–216] as well as lipid and nucleotide biosynthesis and mitochondrial activity [104, 217–219]. Moreover MYC regulates ribosome biogenesis and protein synthesis [220]. Overall, MYC activation promotes an extensive reprogramming of cellular metabolism in order to fuel anabolic pathways and provide the building blocks for cellular growth and thus, proliferation.

In contrast to the large amount of data available on MYC, the role of MXD proteins in regulating metabolism has not been systematically addressed. Interestingly MNT, as well as MXD1 and MXD4, can bind not only to MAX but also to MLX. Therefore, MXD proteins can potentially control cellular metabolism by influencing the transcriptional activity of both MAX and MLX modules. However, to date only MNT has been linked, albeit indirectly, to metabolism. CRE-mediated deletion of *Mnt* in T cells results in diminished oxygen consumption rate (OCR) and increased ROS production, implying an imbalance in cellular oxidative metabolism [144]. Moreover, miR-210, a hypoxia inducible miRNA, targets MNT to promote cellular proliferation under hypoxic conditions. Since both miR-210 overexpression and MNT silencing promote expression of many metabolic genes previously identified as MYC targets, it is possible that this effect is mediated indirectly by increased MYC transcriptional activity [221].

Both MONDOA and CHREBP play a crucial role in metabolism [36, 85, 222]. Both the subcellular localization and transcriptional activity of MONDO proteins are regulated by glucose. In general, upon glucose stimulation, MONDO/MLX heterodimers accumulate in the nucleus and activate transcription of their target genes. However, additional mechanisms control MONDO expression and transcriptional activity [223–225]. CHREBP and MONDOA are expressed in most tissues, but while CHREBP is enriched in liver [175], MONDOA is highly expressed in skeletal muscle [33]. This differential expression profile also appears to reflect the major biological functions of these proteins. Glucose induced CHREBP activation in the liver promotes the conversion of glucose to lipid. Indeed

Chrebp^{-/-} mice exhibit hyperglycemia and diminished serum triglycerides [175]. Gene expression analysis and chromatin immunoprecipitation experiments have shown that CHREBP activates and directly binds to many glycolytic genes and to key regulators of lipogenesis such as Acetyl coA carboxylase (*ACC*), fatty acid synthase (*FAS*) and sterol-coA desaturase (*SCD*) [226–230]. MONDOA instead has been shown to regulate mainly glycolytic genes [37, 91]. Moreover both CHREBP and MONDOA regulate the common target gene *TXNIP* implying that they can mediate, at least in part, similar biological processes [37, 92].

As discussed in the next section, both MYC and MONDO proteins have been detected bound to the promoters of specific metabolic genes indicating that these proteins functionally cooperate to orchestrate cellular metabolism. [91, 231–233]. While MYC has been shown to be required for CHREBP and glucose-dependent activation of glycolytic genes [231, 232], it is not known whether MYC requires MONDO activity to properly reprogram metabolic pathways and drive proliferation. It is possible that MYC and MONDO may transcriptionally regulate common target genes; although it cannot be excluded that MONDO proteins may affect MYC biological activity by regulating a complementary subset of genes. MYC driven tumor cells have been shown to be dependent on high level of either glutamine or glucose [185, 186, 234] and inactivation of metabolic genes such as *LDH-A* (a MYC and MONDOA common transcriptional target) inhibits MYC dependent tumorigenesis [204, 235]. Interestingly MONDOA responds to both glucose and glutamine and has been proposed to coordinate glycolysis with glutaminolysis by directly sensing TCA cycle function to regulate *TXNIP* expression and modulate glucose uptake and glycolytic rate [87, 146]. Therefore, it is possible that altering MONDO function would negatively impact MYC oncogenic activity.

2.5. The target gene enigma

Based on the biochemical, transcriptional, and functional studies described above, it has been suggested that MAX/MLX network members may bind to common target genes. This model is also supported by the fact that MXD1 and MXD2 specifically inhibit MYC transforming activity but are less effective in blocking E1A mediated transformation. [105, 109]. Hence MXD proteins do not behave as general inhibitors of transformation, but specifically antagonize MYC activity. Indeed, there is compelling evidence that MXD1 and MNT bind to MYC target genes *in vivo*. However, very little genomic binding data are available for the other network members. MYC and MXD1 have been shown to directly bind and respectively activate and repress relevant genes such as *TERT*, *CCND2* and *PTEN* [189, 236–243]. In each of these cases MYC/MAX heterodimers could be replaced by MXD1/MAX heterodimers, resulting in histone deacetylation and transcriptional repression through recruitment of an HDAC containing complex. In addition, MXD1 recruits RBP2 to the *TERT* promoter, which mediates histone H3k4me3 demethylation [76]. Other studies have reported an antagonistic role for MYC and MXD1 in controlling the expression of other important genes such as *CDC25A* [244, 245] and the three EIF4F complex subunits *EIF4EF*, *EIF4EAI*, *EIF4EGI* [246, 247]. However the direct binding of MXD1 to the promoters of these genes has not been examined.

Interestingly, the most comprehensive analysis of MYC and MXD target gene overlap has been performed *in vivo*. Overexpression of MXD1 in lymphocytes inhibits expansion, maturation, and growth of T cells. Gene expression analysis showed that over 80% of the genes repressed by MXD1 have previously been found to be induced by MYC [124]. These results strongly support the hypothesis that both MYC and MXD1 control the expression of a common set of target genes. Similar results were obtained by comparing the expression profiles of mammary adenocarcinomas resulting from either the overexpression of MYC or the conditional deletion of *Mnt* in murine mammary gland epithelial cells [132]. In this case the tumor gene expression profiles were basically indistinguishable and MYC and MNT genomic binding profiles were highly overlapping, strongly arguing that MYC and MNT regulate the same target genes *in vivo*.

While there is evidence showing that both MYC and MXD proteins reciprocally control the expression of a large set of common genes, it is not known whether MXD/MLX heterodimers share target genes with MXD/MAX or MONDO/MLX heterodimers. So far, the specific biological functions of MXD/MLX heterodimers, if any, are completely unknown. By contrast, the role of MONDOA and CHREBP in the regulation of glycolysis and lipogenesis has been well established [49, 85, 248, 249]. Surprisingly, MONDOA and CHREBP seem to bind to a much smaller number of genes than MYC. The fact that CHREBP binds to a tandem E-box spaced by 6 nucleotides (Carbohydrate response element or ChoRE) may in part explain this difference [250–252]. Moreover, while MYC target genes regulate many biological processes, most of the MONDO/MLX target genes are involved in metabolism. Specifically, when a constitutively transcriptionally active version of MONDOA defined N237NLS-MondoA was overexpressed in C2C12 myoblasts, only 81 genes were activated more than 1.8 fold. Among these, 53% and 29% of the MONDOA targets genes were respectively involved in metabolism and cell growth [91]. Among these genes, MONDOA was shown to directly bind and regulate the expression of *LDHA* and *HKII*, two previously reported MYC target genes, and *PFK3FB* [204, 213, 253]. Importantly, both MYC and MONDOA bind to the promoter of all three genes in C2C12 cells [91]. Although these results suggest a coordinate regulation of metabolic genes by MYC and MONDOA, a detailed analysis of the specific and relative contribution of MYC and MONDOA to the expression of these genes has not been evaluated.

MYC and CHREBP are both required for the glucose dependent activation of the Liver-type pyruvate kinase gene (*PKLR*). MYC and CHREBP bind to the *PKLR* promoter in presence of glucose. Silencing of MYC expression or inhibition of MYC/MAX heterodimerization decreases the binding of CHREBP/MLX to the *PKLR* gene promoter and its expression [233]. These data suggest that MYC, through an unknown mechanism, facilitates the recruitment of CHREBP to the *PKLR* promoter. However the reciprocal analysis was not performed leaving it an open question as to whether CHREBP affects MYC transcriptional activity or how general this cooperation may be.

Overall, these data strongly argue for a crosstalk between MAX and MLX modules; however the full extent of the transcriptional and functional overlap between them has not been rigorously established. This will require detailed analyses of genomic binding,

epigenetic changes, and gene expression for all network members in specific biological contexts.

3. The balance among network components determines distinct cellular states: a comprehensive model

Overall the results discussed so far can be summarized by the model shown in figure 5, where external signals define the balance between MYC, MXD and MONDO proteins and promote distinct cellular fates. High levels of MYC drive growth and proliferation while the switch from MYC to MXD proteins promotes cell cycle arrest and differentiation. However MXD family expression alone is not sufficient to completely arrest proliferation. Therefore, additional signals are necessary to inhibit MYC expression or promote MYC degradation in order to allow MXD proteins to efficiently counteract MYC activity. An apoptotic response occurs in situations with extremely high levels of MYC activity. MYC driven apoptosis may be further facilitated by loss of MXD, MNT or MONDO activities or under stress conditions such as fluctuation in nutrient availability, hypoxia or chemotherapy.

In order to support proliferation, MYC promotes the metabolic reprogramming of the cell. Therefore coordinate regulation of metabolic genes by MYC and MONDO proteins may be required to sustain tumor cell viability. In support of this model, MYC binds to the promoter and appears to regulate the expression of both CHREBP and MONDOA, and inhibiting MONDO protein expression promotes apoptosis in several experimental system [83, 146, 150, 151].

The prevailing view of MYC function has been that MYC/MAX heterodimers bind genomic DNA and recruit different transcriptional co-regulator complexes to modulate gene expression – in essence considering MYC as a standard transcription factor albeit with a very large number of gene targets [103]. However two recent reports have taken another view, namely that MYC acts as a universal amplifier of gene expression [66, 67]. The authors proposed a new model in which MYC, instead of acting as a molecular ON/OFF switch for a specific subset of target genes, globally amplifies the pre-existing gene expression program already in place in a given cell. This general augmentation of gene expression occurs through MYC recruitment of P-TEFb, a complex that alleviates transcriptional pausing by RNA Pol II, and therefore promotes transcript elongation. While the “universal amplifier” model accounts for some properties of MYC, such as the wide variability of gene targets identified in different studies, its general validity is being hotly debated [254–257]. Moreover, whether and how the activities of the other members of the MAX/MLX network relate to this model remains an open question. Nonetheless, it is possible to imagine that, by competing with MYC for its genomic targets, MXD proteins may also globally counteract MYC’s anti-pausing activity. Alternatively MXD proteins may recruit pausing factors and inhibit RNA Pol II elongation. Thus, in the context of the “universal amplifier” model MXD proteins may be required to attenuate MYC pro-proliferative transcriptional activity, thereby weakening the ongoing transcriptional program and allowing other factors to promote cell cycle arrest or differentiation.

Interestingly, the amplifier model can also be considered in the context of cooperative regulation of genes involved in metabolism. MYC has been shown to regulate the expression of many metabolic genes, a subset of which are also targets of MONDOA and CHREBP. Perhaps MONDO proteins act as specific selectors of critical genes, with their expression mainly dependent on MONDO activity, while MYC binding would further amplify their expression through its RNA Pol II anti-pausing activity. This is consistent with the need to coordinate nutrient availability with mitogenic signals and with the ability of MYC to promote proliferation and cellular metabolic reprogramming. In this model, activation of MYC in the presence of nutrients would promote cellular metabolic reprogramming by co-opting and enhancing the MONDO-dependent transcriptional program. On the other hand, the absence of nutrients such as glucose would turn off MONDO transcriptional activity and prevent MYC driven metabolic reprogramming and proliferation, thus triggering apoptosis in a deregulated MYC state.

4. The MAX/MLX network in human cancer

Accumulating evidence indicates that the biological outcome of MYC deregulation is dependent on the expression and function of the other members of the network, which either antagonize or collaborate with MYC. Based on the organization of the network, it is reasonable to consider that changes in the expression of one network member may have a significant biological impact by modifying the relative abundance and activities of other functionally distinct network members. This notion is based on several considerations. First, MYC, MXD, or MONDO family proteins are expected to compete for binding to limiting amounts of MAX or MLX. Second, alternative heterodimers will bind the same or related DNA sequences and thus compete for binding sites, and third, network members impose different consequences in terms of gene target expression.

The importance of maintaining a homeostatic balance among network components is also suggested by their extremely tight regulation of expression. For example, MYC represses itself in a negative feedback loop which is often inactivated in tumor cells [258]. Also, MYC family members exhibit transcriptional crosstalk. For example overexpression of MYCN leads to downregulation of MYC in neuroblastoma and B cell lymphoma [259, 260]. In addition, loss of MNT is balanced by a decrease in MYC and MYCN expression [129, 130]. Moreover, MYC represses MXD4 expression by its interaction with MIZ1 [261, 262]. By repressing MXD4 expression MYC may not only promote its own association with MAX, but potentially influence the balance between MXD/MLX and MONDO/MLX heterodimers. Finally, MYC can also influence expression of the MONDO factors. For example MYC binds to the *CHREBP* gene promoter [ENCODE data and 83] and both MYC and MYCN overexpression enhances MONDOA expression and nuclear localization in several tumor cell lines (Carroll, Diolaiti, McFerrin and Eisenman, unpublished).

Interestingly, although MXD proteins antagonize MYC activity in many experimental systems, there are contradictory evidence that MXD family members are lost in human cancers [172, 173, 263–275]. As discussed above, it is generally accepted that MXD proteins are not frequently lost during oncogenesis because they are mainly expressed in differentiated tissues where MYC expression is low or absent. However, another explanation

may be that MXD and MONDO proteins may be required to suppress MYC pro-apoptotic activity [276]. If correct it would be expected that MXD proteins should be widely expressed in tumor cells. Consistent with this hypothesis, MXD genes expression has been detected in several hematopoietic and solid tumors [263, 264, 266, 274, 277, 278]. However, a comprehensive analysis of MXD expression in human tumors has not been reported.

Even less is known about MONDO protein expression in human cancers. By sensing nutrient availability and regulating metabolic processes such as glycolysis and lipogenesis, Mondo proteins may profoundly impact tumor growth. MONDOA is overexpressed in acute lymphoblastic leukemia and its silencing promoted differentiation and apoptosis in leukemic cells [151]. Recent data from our laboratory show that MONDOA silencing promotes apoptosis in cells expressing high level of either MYC or MYCN, suggesting that MONDOA may be required by MYC for properly reprogram cellular metabolism and for tumorigenesis (Carroll, Diolaiti, McFerrin, and Eisenman, unpublished).

4.1. The MAX/MLX Network as viewed from the TCGA

To evaluate the profile of the MAX/MLX network in human tumors, we analyzed the relative expression levels of the network members using the Pan-Cancer data from The Cancer Genome Atlas (TCGA) database (<http://cancergenome.nih.gov/>). This publically available dataset is mean normalized to reflect the variability of gene expression across 6609 samples representing twenty-four diverse tumor types, including both primary tumor and normal tissue where available. Using spearman correlation as a distance measure during hierarchical clustering [279], samples were largely clustered according to tissue and tumor type, with clusters of normal tissue being grouped with the corresponding primary tumor (Fig. 6A). Interestingly, it appears that most tumor types can be distinguished according to a specific expression pattern of MAX/MLX network members suggesting that each tissue has a distinct balance among members of the network. Lung and breast cancers are the exception, as the expression pattern of network members within these tissues is highly heterogeneous causing them to be broadly interspersed with the other tumor types and not distinctly clustered. For this reason, lung and breast samples were excluded from Figure 6A to avoid obfuscating the other tumor type clusters. The reason for such difference is not known, but it may in part reflect the great heterogeneity of lung and breast cancers.

In general, *MAX*, *MLX* and *MNT* genes expression show minimal variability among tissues including either normal or tumor samples, as indicated by the almost homogeneous black color. This is consistent with the fact that they are expressed both widely and constitutively. In contrast, *MYC* family members show a variable expression profile being enriched or depleted dependent upon the tumor type. In particular, while *MYC* is expressed at high levels in a broad spectrum of cancers, *MYCN* and *MYCL* are highly expressed in a smaller number of tumors consistent with their more restricted tissue expression profile. Surprisingly, many cancers express high levels of multiple *MYC* family members (e.g. Ovarian cancer, Acute Myeloid Leukemia and Lower Grade Glioma).

MXD genes also show variable expression profiles. *MXD2* has higher relative expression in kidney where it has been shown to be required for tumor growth [196, 198]. Interestingly *MXD3* seems to be more highly expressed in the same samples as *MYC* family members,

consistent with the reported connection between *MXD3* expression, proliferation and protection from DNA damage induced apoptosis [154]. Unexpectedly *MXD1* is present at higher levels in a subset of tissues where *MYC* is also more highly expressed. Finally, both *MONDOA* and *CHREBP* show a tissue specific profile perhaps reflecting alternative metabolic functions and requirements of different tissues and related tumors. The observation that the network expression profile is tissue specific is important because it implies different tissues may have diverse thresholds of susceptibility to *MYC* deregulation and variable biological outcomes depending on the specific network structure.

Moreover, because it is known that the expression levels of the network members influence each other's expression profile, we asked if there is any relationship between the expression levels of each network member by calculating the Pearson correlation between network genes using all available samples, including those from lung and breast cancer (Fig 6B). Intriguingly, five of the core network members (*MAX*, *MYC*, *MXD1*, *MNT*, *MONDOA*) as well as *MGA* are all positively correlated with each other and form a distinct clade (within dashed line). This again suggests their expression patterns are potentially linked and supports a model in which MXD proteins may be required to balance MYC pro-apoptotic activity, while MONDOA would collaborate with MYC to sustain the increased metabolic demand of cancer cells.

Interestingly, MYC drives the formation of tumors possessing different metabolic profiles when expressed in cells of different origins [280]. Conceivably, MYC driven metabolic reprogramming can vary depending on the cell type specific expression profile of MONDOA, CHREBP and other metabolic genes. Moreover, although MONDO proteins seem to mediate at least partially overlapping metabolic pathways, it is possible that they possess specific functions. As shown by figure 6A–C their expression, especially *CHREBP*, is highly variable among samples, therefore potentially influencing MYC-induced metabolic reprogramming in a tissue specific manner.

Overall, the analysis of TCGA data represented in fig. 6A,B suggests that *MXD* genes expression is not globally downregulated during oncogenesis. However, while the analysis highlights the tissue-specific distribution of the network members across tumor types, it does not provide comparative information regarding the relative expression level among network members within tumor types.

Figure 6C shows the relative log (base 2) gene expression profile of all network members within each tumor type. Although the median expression level is a simplified statistic and does not reflect the heterogeneity associated with each tumor type, the data shows that most network members have comparable ranges of expression and that their expression is, in general, greater than the overall median gene expression. The exceptions being *MYCN*, *MYCL* and *ChREBP*, which have more tissue specific expression, and *MXD3*, which is expressed at a lower level in all tissues. These data, together with the fact that loss of *MXD* or *MONDO* genes promotes MYC driven apoptosis supports a model where extreme network imbalance, as a result of the loss of a network member, may not be beneficial for tumor cells and suggests an intact and functional MAX/MLX network may be required for tumorigenesis.

5. Concluding remarks

In conclusion, while MYC undoubtedly is the major factor in driving tumorigenesis, the presence and activities of the other members of the network is likely to modulate the biological consequences of MYC deregulation. Understanding the specific biological functions of each network member in both normal and pathological conditions will lead to a deeper understanding of how deregulated MYC alters cellular behavior and potentially provide new opportunities for inhibiting tumorigenesis.

Acknowledgments

We are thankful to Don Ayer for sharing unpublished data and Alexandre Neves for a critical reading of the manuscript. We apologize to colleagues whose work was not cited because of limited space. Work from the laboratory of R.N.E. described in this review was supported by grants RO1-CA20525 and R37-CA57138 from NIH/NCI.

References

1. Lee CM, Reddy EP. The v-myc oncogene. *Oncogene*. 1999; 18:2997–3003. [PubMed: 10378695]
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; 42:773–779. [PubMed: 6284994]
3. Sheiness D, Bishop JM. DNA and RNA from uninfected vertebrate cells contain nucleotide sequences related to the putative transforming gene of avian myelocytomatosis virus. *J Virol*. 1979; 31:514–521. [PubMed: 225569]
4. Taub R, Kirsch I, Morton C, Lenoir G, Swan D, Tronick S, Aaronson S, Leder P. 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; 79:7837–7841. [PubMed: 6818551]
5. Dalla-Favera R, Bregni M, Erikson J, Patterson D, Gallo RC, Croce CM. 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; 79:7824–7827. [PubMed: 6961453]
6. Collins S, Groudine M. Amplification of endogenous myc-related DNA sequences in a human myeloid leukaemia cell line. *Nature*. 1982; 298:679–681. [PubMed: 6285209]
7. Schwab M, Alitalo K, Klempnauer KH, Varmus HE, Bishop JM, Gilbert F, Brodeur G, Goldstein M, Trent J. Amplified DNA with limited homology to myc cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. *Nature*. 1983; 305:245–248. [PubMed: 6888561]
8. Kohl NE, Kanda N, Schreck RR, Bruns G, Latt SA, Gilbert F, Alt FW. Transposition and amplification of oncogene-related sequences in human neuroblastomas. *Cell*. 1983; 35:359–367. [PubMed: 6197179]
9. 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; 224:1121–1124. [PubMed: 6719137]
10. Nau MM, Brooks BJ, Battey J, Sausville E, Gazdar AF, Kirsch IR, McBride OW, Bertness V, Hollis GF, Minna JD. L-myc, a new myc-related gene amplified and expressed in human small cell lung cancer. *Nature*. 1985; 318:69–73. [PubMed: 2997622]
11. Murre C, McCaw PS, Baltimore D. A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and myc proteins. *Cell*. 1989; 56:777–783. [PubMed: 2493990]
12. Dang CV, Barrett J, Villa-Garcia M, Resar LM, Kato GJ, Fearon ER. Intracellular leucine zipper interactions suggest c-Myc hetero-oligomerization. *Mol Cell Biol*. 1991; 11:954–962. [PubMed: 1990293]

13. Blackwell TK, Kretzner L, Blackwood EM, Eisenman RN, Weintraub H. Sequence-specific DNA binding by the c-Myc protein. *Science*. 1990; 250:1149–1151. [PubMed: 2251503]
14. Penn LJ, Laufer EM, Land H. C-MYC: evidence for multiple regulatory functions. *Semin Cancer Biol*. 1990; 1:69–80. [PubMed: 2133113]
15. Luscher B, Eisenman RN. New light on Myc and Myb. Part I. *Myc. Genes Dev*. 1990; 4:2025–2035. [PubMed: 2269425]
16. Luscher B, Eisenman RN. New light on Myc and Myb. Part II. *Myb. Genes Dev*. 1990; 4:2235–2241. [PubMed: 2279697]
17. Prendergast GC, Ziff EB. Methylation-sensitive sequence-specific DNA binding by the c-Myc basic region. *Science*. 1991; 251:186–189. [PubMed: 1987636]
18. Blackwood EM, Eisenman RN. Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. *Science*. 1991; 251:1211–1217. [PubMed: 2006410]
19. Pirity M, Blanck JK, Schreiber-Agus N. Lessons learned from Myc/Max/Mad knockout mice. *Curr Top Microbiol Immunol*. 2006; 302:205–234. [PubMed: 16620030]
20. Blackwood EM, Luscher B, Eisenman RN. Myc and Max associate in vivo. *Genes Dev*. 1992; 6:71–80. [PubMed: 1730411]
21. Blackwood EM, Eisenman RN. Regulation of Myc: Max complex formation and its potential role in cell proliferation. *Tohoku J Exp Med*. 1992; 168:195–202. [PubMed: 1306304]
22. Kretzner L, Blackwood EM, Eisenman RN. Myc and Max proteins possess distinct transcriptional activities. *Nature*. 1992; 359:426–429. [PubMed: 1406956]
23. Blackwood EM, Kretzner L, Eisenman RN. Myc and Max function as a nucleoprotein complex. *Curr Opin Genet Dev*. 1992; 2:227–235. [PubMed: 1638116]
24. Amati B, Brooks MW, Levy N, Littlewood TD, Evan GI, Land H. Oncogenic activity of the c-Myc protein requires dimerization with Max. *Cell*. 1993; 72:233–245. [PubMed: 8425220]
25. Berberich SJ, Cole MD. Casein kinase II inhibits the DNA-binding activity of Max homodimers but not Myc/Max heterodimers. *Genes Dev*. 1992; 6:166–176. [PubMed: 1737614]
26. Ayer DE, Kretzner L, Eisenman RN. Mad: a heterodimeric partner for Max that antagonizes Myc transcriptional activity. *Cell*. 1993; 72:211–222. [PubMed: 8425218]
27. Zervos AS, Gyuris J, Brent R. Mxi1, a protein that specifically interacts with Max to bind Myc-Max recognition sites. *Cell*. 1993; 72:223–232. [PubMed: 8425219]
28. Hurlin PJ, Queva C, Koskinen PJ, Steingrimsson E, Ayer DE, Copeland NG, Jenkins NA, Eisenman RN. Mad3 and Mad4: novel Max-interacting transcriptional repressors that suppress c-myc dependent transformation and are expressed during neural and epidermal differentiation. *EMBO J*. 1995; 14:5646–5659. [PubMed: 8521822]
29. Hurlin PJ, Queva C, Eisenman RN. Mnt: a novel Max-interacting protein and Myc antagonist. *Curr Top Microbiol Immunol*. 1997; 224:115–121. [PubMed: 9308234]
30. Hurlin PJ, Steingrimsson E, Copeland NG, Jenkins NA, Eisenman RN. Mga, a dual-specificity transcription factor that interacts with Max and contains a T-domain DNA-binding motif. *EMBO J*. 1999; 18:7019–7028. [PubMed: 10601024]
31. Billin AN, Eilers AL, Queva C, Ayer DE. Mlx, a novel Max-like BHLHZip protein that interacts with the Max network of transcription factors. *J Biol Chem*. 1999; 274:36344–36350. [PubMed: 10593926]
32. Meroni G, Cairo S, Merla G, Messali S, Brent R, Ballabio A, Reymond A. Mlx, a new Max-like bHLHZip family member: the center stage of a novel transcription factors regulatory pathway? *Oncogene*. 2000; 19:3266–3277. [PubMed: 10918583]
33. Billin AN, Eilers AL, Coulter KL, Logan JS, Ayer DE. MondoA, a novel basic helix-loop-helix-leucine zipper transcriptional activator that constitutes a positive branch of a max-like network. *Mol Cell Biol*. 2000; 20:8845–8854. [PubMed: 11073985]
34. Cairo S, Merla G, Urbinati F, Ballabio A, Reymond A. WBSR14, a gene mapping to the Williams--Beuren syndrome deleted region, is a new member of the Mlx transcription factor network. *Hum Mol Genet*. 2001; 10:617–627. [PubMed: 11230181]

35. Eilers AL, Sundwall E, Lin M, Sullivan AA, Ayer DE. A novel heterodimerization domain, CRM1, and 14-3-3 control subcellular localization of the MondoA-Mlx heterocomplex. *Mol Cell Biol.* 2002; 22:8514–8526. [PubMed: 12446771]
36. Billin AN, Ayer DE. The Mlx network: evidence for a parallel Max-like transcriptional network that regulates energy metabolism. *Curr Top Microbiol Immunol.* 2006; 302:255–278. [PubMed: 16620032]
37. Stoltzman CA, Peterson CW, Breen KT, Muoio DM, Billin AN, Ayer DE. Glucose sensing by MondoA:Mlx complexes: a role for hexokinases and direct regulation of thioredoxin-interacting protein expression. *Proc Natl Acad Sci U S A.* 2008; 105:6912–6917. [PubMed: 18458340]
38. Kawaguchi T, Takenoshita M, Kabashima T, Uyeda K. Glucose and cAMP regulate the L-type pyruvate kinase gene by phosphorylation/dephosphorylation of the carbohydrate response element binding protein. *Proc Natl Acad Sci U S A.* 2001; 98:13710–13715. [PubMed: 11698644]
39. Young SL, Diolaiti D, Conacci-Sorrell M, Ruiz-Trillo I, Eisenman RN, King N. Premetazoan ancestry of the Myc-Max network. *Mol Biol Evol.* 2011; 28:2961–2971. [PubMed: 21571926]
40. King N, Westbrook MJ, Young SL, Kuo A, Abedin M, Chapman J, Fairclough S, Hellsten U, Isogai Y, Letunic I, Marr M, Pincus D, Putnam N, Rokas A, Wright KJ, Zuzow R, Dirks W, Good M, Goodstein D, Lemons D, Li W, Lyons JB, Morris A, Nichols S, Richter DJ, Salamov A, Sequencing JG, Bork P, Lim WA, Manning G, Miller WT, McGinnis W, Shapiro H, Tjian R, Grigoriev IV, Rokhsar D. The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. *Nature.* 2008; 451:783–788. [PubMed: 18273011]
41. Ruiz-Trillo I, Roger AJ, Burger G, Gray MW, Lang BF. A phylogenomic investigation into the origin of metazoa. *Mol Biol Evol.* 2008; 25:664–672. [PubMed: 18184723]
42. McFerrin LG, Atchley WR. Evolution of the Max and Mlx networks in animals. *Genome Biol Evol.* 2011; 3:915–937. [PubMed: 21859806]
43. Gallant P, Shiiro Y, Cheng PF, Parkhurst SM, Eisenman RN. Myc and Max homologs in *Drosophila*. *Science.* 1996; 274:1523–1527. [PubMed: 8929412]
44. Peyrefitte S, Kahn D, Haenlin M. New members of the *Drosophila* Myc transcription factor subfamily revealed by a genome-wide examination for basic helix-loop-helix genes. *Mech Dev.* 2001; 104:99–104. [PubMed: 11404084]
45. Loo LW, Secombe J, Little JT, Carlos LS, Yost C, Cheng PF, Flynn EM, Edgar BA, Eisenman RN. The transcriptional repressor dMnt is a regulator of growth in *Drosophila melanogaster*. *Mol Cell Biol.* 2005; 25:7078–7091. [PubMed: 16055719]
46. Schreiber-Agus N, Stein D, Chen K, Goltz JS, Stevens L, DePinho RA. *Drosophila* Myc is oncogenic in mammalian cells and plays a role in the diminutive phenotype. *Proc Natl Acad Sci U S A.* 1997; 94:1235–1240. [PubMed: 9037036]
47. Gallant P. Myc/Max/Mad in invertebrates: the evolution of the Max network. *Curr Top Microbiol Immunol.* 2006; 302:235–253. [PubMed: 16620031]
48. Gallant P. Myc function in *Drosophila*. *Cold Spring Harb Perspect Med.* 2013; 3
49. Havula E, Teesalu M, Hyotylainen T, Seppala H, Hasygar K, Auvinen P, Oresic M, Sandmann T, Hietakangas V. Mondo/ChREBP-Mlx-regulated transcriptional network is essential for dietary sugar tolerance in *Drosophila*. *PLoS Genet.* 2013; 9:e1003438. [PubMed: 23593032]
50. Musselman LP, Fink JL, Ramachandran PV, Patterson BW, Okunade AL, Maier E, Brent MR, Turk J, Baranski TJ. Role of fat body lipogenesis in protection against the effects of caloric overload in *Drosophila*. *J Biol Chem.* 2013; 288:8028–8042. [PubMed: 23355467]
51. Nair SK, Burley SK. X-ray structures of Myc-Max and Mad-Max recognizing DNA. Molecular bases of regulation by proto-oncogenic transcription factors. *Cell.* 2003; 112:193–205. [PubMed: 12553908]
52. Montagne M, Naud JF, McDuff FO, Lavigne P. Toward the elucidation of the structural determinants responsible for the molecular recognition between Mad1 and Max. *Biochemistry.* 2005; 44:12860–12869. [PubMed: 16171401]
53. Ferre-D'Amare AR, Prendergast GC, Ziff EB, Burley SK. Recognition by Max of its cognate DNA through a dimeric b/HLH/Z domain. *Nature.* 1993; 363:38–45. [PubMed: 8479534]
54. Hann SR. Role of post-translational modifications in regulating c-Myc proteolysis, transcriptional activity and biological function. *Semin Cancer Biol.* 2006; 16:288–302. [PubMed: 16938463]

55. McMahon SB, Van Buskirk HA, Dugan KA, Copeland TD, Cole MD. The novel ATM-related protein TRRAP is an essential cofactor for the c-Myc and E2F oncoproteins. *Cell*. 1998; 94:363–374. [PubMed: 9708738]
56. McMahon SB, Wood MA, Cole MD. The essential cofactor TRRAP recruits the histone acetyltransferase hGCN5 to c-Myc. *Mol Cell Biol*. 2000; 20:556–562. [PubMed: 10611234]
57. Frank SR, Parisi T, Taubert S, Fernandez P, Fuchs M, Chan HM, Livingston DM, Amati B. MYC recruits the TIP60 histone acetyltransferase complex to chromatin. *EMBO Rep*. 2003; 4:575–580. [PubMed: 12776177]
58. Wood MA, McMahon SB, Cole MD. An ATPase/helicase complex is an essential cofactor for oncogenic transformation by c-Myc. *Mol Cell*. 2000; 5:321–330. [PubMed: 10882073]
59. Cole MD, McMahon SB. The Myc oncoprotein: a critical evaluation of transactivation and target gene regulation. *Oncogene*. 1999; 18:2916–2924. [PubMed: 10378688]
60. Liu X, Tesfai J, Evrard YA, Dent SY, Martinez E. c-Myc transformation domain recruits the human STAGA complex and requires TRRAP and GCN5 acetylase activity for transcription activation. *J Biol Chem*. 2003; 278:20405–20412. [PubMed: 12660246]
61. Cole MD, Nikiforov MA. Transcriptional activation by the Myc oncoprotein. *Curr Top Microbiol Immunol*. 2006; 302:33–50. [PubMed: 16620024]
62. Eberhardy SR, Farnham PJ. Myc recruits P-TEFb to mediate the final step in the transcriptional activation of the cad promoter. *J Biol Chem*. 2002; 277:40156–40162. [PubMed: 12177005]
63. Gargano B, Amente S, Majello B, Lania L. P-TEFb is a crucial co-factor for Myc transactivation. *Cell Cycle*. 2007; 6:2031–2037. [PubMed: 17700062]
64. Zuber J, Shi J, Wang E, Rappaport AR, Herrmann H, Sison EA, Magoon D, Qi J, Blatt K, Wunderlich M, Taylor MJ, Johns C, Chicas A, Mulloy JC, Kogan SC, Brown P, Valent P, Bradner JE, Lowe SW, Vakoc CR. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. *Nature*. 2011; 478:524–528. [PubMed: 21814200]
65. Rahl PB, Lin CY, Seila AC, Flynn RA, McCuine S, Burge CB, Sharp PA, Young RA. c-Myc regulates transcriptional pause release. *Cell*. 2010; 141:432–445. [PubMed: 20434984]
66. Lin CY, Loven J, Rahl PB, Paranal RM, Burge CB, Bradner JE, Lee TI, Young RA. Transcriptional amplification in tumor cells with elevated c-Myc. *Cell*. 2012; 151:56–67. [PubMed: 23021215]
67. Nie Z, Hu G, Wei G, Cui K, Yamane A, Resch W, Wang R, Green DR, Tessarollo L, Casellas R, Zhao K, Levens D. c-Myc is a universal amplifier of expressed genes in lymphocytes and embryonic stem cells. *Cell*. 2012; 151:68–79. [PubMed: 23021216]
68. Herbst A, Hemann MT, Tworowski KA, Salghetti SE, Lowe SW, Tansey WP. A conserved element in Myc that negatively regulates its proapoptotic activity. *EMBO Rep*. 2005; 6:177–183. [PubMed: 15678160]
69. Cowling VH, Chandriani S, Whitfield ML, Cole MD. A conserved Myc protein domain, MBIV, regulates DNA binding, apoptosis, transformation, and G2 arrest. *Mol Cell Biol*. 2006; 26:4226–4239. [PubMed: 16705173]
70. Ayer DE, Laherty CD, Lawrence QA, Armstrong AP, Eisenman RN. Mad proteins contain a dominant transcription repression domain. *Mol Cell Biol*. 1996; 16:5772–5781. [PubMed: 8816491]
71. Eilers AL, Billin AN, Liu J, Ayer DE. A 13-amino acid amphipathic alpha-helix is required for the functional interaction between the transcriptional repressor Mad1 and mSin3A. *J Biol Chem*. 1999; 274:32750–32756. [PubMed: 10551834]
72. Laherty CD, Yang WM, Sun JM, Davie JR, Seto E, Eisenman RN. Histone deacetylases associated with the mSin3 corepressor mediate mad transcriptional repression. *Cell*. 1997; 89:349–356. [PubMed: 9150134]
73. Brubaker K, Cowley SM, Huang K, Loo L, Yochum GS, Ayer DE, Eisenman RN, Radhakrishnan I. Solution structure of the interacting domains of the Mad-Sin3 complex: implications for recruitment of a chromatin-modifying complex. *Cell*. 2000; 103:655–665. [PubMed: 11106735]
74. Bernstein BE, Humphrey EL, Erlich RL, Schneider R, Bouman P, Liu JS, Kouzarides T, Schreiber SL. Methylation of histone H3 Lys 4 in coding regions of active genes. *Proc Natl Acad Sci U S A*. 2002; 99:8695–8700. [PubMed: 12060701]

75. Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NC, Schreiber SL, Mellor J, Kouzarides T. Active genes are tri-methylated at K4 of histone H3. *Nature*. 2002; 419:407–411. [PubMed: 12353038]
76. Ge Z, Li W, Wang N, Liu C, Zhu Q, Bjorkholm M, Gruber A, Xu D. Chromatin remodeling: recruitment of histone demethylase RBP2 by Mad1 for transcriptional repression of a Myc target gene, telomerase reverse transcriptase. *FASEB J*. 2010; 24:579–586. [PubMed: 19762557]
77. Secombe J, Eisenman RN. The function and regulation of the JARID1 family of histone H3 lysine 4 demethylases: the Myc connection. *Cell Cycle*. 2007; 6:1324–1328. [PubMed: 17568193]
78. Secombe J, Li L, Carlos L, Eisenman RN. The Trithorax group protein Lid is a trimethyl histone H3K4 demethylase required for dMyc-induced cell growth. *Genes Dev*. 2007; 21:537–551. [PubMed: 17311883]
79. Bres V, Yoshida T, Pickle L, Jones KA. SKIP interacts with c-Myc and Menin to promote HIV-1 Tat transactivation. *Mol Cell*. 2009; 36:75–87. [PubMed: 19818711]
80. Guccione E, Martinato F, Finocchiaro G, Luzi L, Tizzoni L, Dall' Olio V, Zardo G, Nervi C, Bernard L, Amati B. Myc-binding-site recognition in the human genome is determined by chromatin context. *Nat Cell Biol*. 2006; 8:764–770. [PubMed: 16767079]
81. Kim J, Woo AJ, Chu J, Snow JW, Fujiwara Y, Kim CG, Cantor AB, Orkin SH. A Myc network accounts for similarities between embryonic stem and cancer cell transcription programs. *Cell*. 2010; 143:313–324. [PubMed: 20946988]
82. Knoepfler PS, Zhang XY, Cheng PF, Gafken PR, McMahon SB, Eisenman RN. Myc influences global chromatin structure. *EMBO J*. 2006; 25:2723–2734. [PubMed: 16724113]
83. Lin CH, Lin C, Tanaka H, Fero ML, Eisenman RN. Gene regulation and epigenetic remodeling in murine embryonic stem cells by c-Myc. *PLoS One*. 2009; 4:e7839. [PubMed: 19915707]
84. Martinato F, Cesaroni M, Amati B, Guccione E. Analysis of Myc-induced histone modifications on target chromatin. *PLoS One*. 2008; 3:e3650. [PubMed: 18985155]
85. Havula E, Hietakangas V. Glucose sensing by ChREBP/MondoA-Mlx transcription factors. *Semin Cell Dev Biol*. 2012; 23:640–647. [PubMed: 22406740]
86. McFerrin LG, Atchley WR. A novel N-terminal domain may dictate the glucose response of Mondo proteins. *PLoS One*. 2012; 7:e34803. [PubMed: 22506051]
87. Kaadige MR, Looper RE, Kamalanaadhan S, Ayer DE. Glutamine-dependent anapleurosis dictates glucose uptake and cell growth by regulating MondoA transcriptional activity. *Proc Natl Acad Sci U S A*. 2009; 106:14878–14883. [PubMed: 19706488]
88. Chen JL, Merl D, Peterson CW, Wu J, Liu PY, Yin H, Muoio DM, Ayer DE, West M, Chi JT. Lactic acidosis triggers starvation response with paradoxical induction of TXNIP through MondoA. *PLoS Genet*. 2010; 6:e1001093. [PubMed: 20844768]
89. Stoltzman CA, Kaadige MR, Peterson CW, Ayer DE. MondoA senses non-glucose sugars: regulation of thioredoxin-interacting protein (TXNIP) and the hexose transport curb. *J Biol Chem*. 2011; 286:38027–38034. [PubMed: 21908621]
90. Han KS, Ayer DE. MondoA senses adenine nucleotides: transcriptional induction of thioredoxin-interacting protein. *Biochem J*. 2013; 453:209–218. [PubMed: 23631812]
91. Sans CL, Satterwhite DJ, Stoltzman CA, Breen KT, Ayer DE. MondoA-Mlx heterodimers are candidate sensors of cellular energy status: mitochondrial localization and direct regulation of glycolysis. *Mol Cell Biol*. 2006; 26:4863–4871. [PubMed: 16782875]
92. Cha-Molstad H, Saxena G, Chen J, Shalev A. Glucose-stimulated expression of Txnip is mediated by carbohydrate response element-binding protein, p300, and histone H4 acetylation in pancreatic beta cells. *J Biol Chem*. 2009; 284:16898–16905. [PubMed: 19411249]
93. Ogawa H, Ishiguro K, Gaubatz S, Livingston DM, Nakatani Y. A complex with chromatin modifiers that occupies E2F- and Myc-responsive genes in G0 cells. *Science*. 2002; 296:1132–1136. [PubMed: 12004135]
94. Yuhua S, Wei-Chia T, Xiang F, Rebecca B, Scott T D. Extra-embryonic signals under the control of Mga, Max and Smad4 are required for dorsoventral patterning. *Developmental Cells*. in press.
95. De Paoli L, Cerri M, Monti S, Rasi S, Spina V, Brusca A, Greco M, Ciardullo C, Fama R, Cresta S, Maffei R, Ladetto M, Martini M, Laurenti L, Forconi F, Marasca R, Larocca LM,

- Bertoni F, Gaidano G, Rossi D. MGA, a suppressor of MYC, is recurrently inactivated in high risk chronic lymphocytic leukemia. *Leuk Lymphoma*. 2013; 54:1087–1090. [PubMed: 23039309]
96. Romero OA, Torres-Diz M, Pros E, Savola S, Gomez A, Moran S, Saez C, Iwakawa R, Villanueva A, Montuenga LM, Kohno T, Yokota J, Sanchez-Cespedes M. MAX inactivation in small-cell lung cancer disrupts the MYC-SWI/SNF programs and is synthetic lethal with BRG1. *Cancer Discov*. 2013
 97. Dang CV. c-Myc target genes involved in cell growth, apoptosis, and metabolism. *Mol Cell Biol*. 1999; 19:1–11. [PubMed: 9858526]
 98. Meyer N, Kim SS, Penn LZ. The Oscar-worthy role of Myc in apoptosis. *Semin Cancer Biol*. 2006; 16:275–287. [PubMed: 16945552]
 99. Eilers M. Control of cell proliferation by Myc family genes. *Mol Cells*. 1999; 9:1–6. [PubMed: 10102563]
 100. Singh AM, Dalton S. The cell cycle and Myc intersect with mechanisms that regulate pluripotency and reprogramming. *Cell Stem Cell*. 2009; 5:141–149. [PubMed: 19664987]
 101. Amati B, Alevizopoulos K, Vlach J. Myc and the cell cycle. *Front Biosci*. 1998; 3:d250–d268. [PubMed: 9468463]
 102. Dang CV. Therapeutic targeting of Myc-reprogrammed cancer cell metabolism. *Cold Spring Harb Symp Quant Biol*. 2011; 76:369–374. [PubMed: 21960526]
 103. Eilers M, Eisenman RN. Myc's broad reach. *Genes Dev*. 2008; 22:2755–2766. [PubMed: 18923074]
 104. Dang CV. MYC, metabolism, cell growth, and tumorigenesis. *Cold Spring Harb Perspect Med*. 2013; 3
 105. Lahoz EG, Xu L, Schreiber-Agus N, DePinho RA. Suppression of Myc, but not E1a, transformation activity by Max-associated proteins, Mad and Mxi1. *Proc Natl Acad Sci U S A*. 1994; 91:5503–5507. [PubMed: 8202517]
 106. Vastrik I, Kaipainen A, Penttilä TL, Lymboussakis A, Alitalo R, Parvinen M, Alitalo K. Expression of the mad gene during cell differentiation in vivo and its inhibition of cell growth in vitro. *J Cell Biol*. 1995; 128:1197–1208. [PubMed: 7896882]
 107. Koskinen PJ, Ayer DE, Eisenman RN. Repression of Myc-Ras cotransformation by Mad is mediated by multiple protein-protein interactions. *Cell Growth Differ*. 1995; 6:623–629. [PubMed: 7669717]
 108. Hurlin PJ, Queva C, Eisenman RN. Mnt, a novel Max-interacting protein is coexpressed with Myc in proliferating cells and mediates repression at Myc binding sites. *Genes Dev*. 1997; 11:44–58. [PubMed: 9000049]
 109. Cerni C, Bousset K, Seelos C, Burkhardt H, Henriksson M, Luscher B. Differential effects by Mad and Max on transformation by cellular and viral oncoproteins. *Oncogene*. 1995; 11:587–596. [PubMed: 7630643]
 110. Cerni C, Skrzypek B, Popov N, Sasgary S, Schmidt G, Larsson LG, Luscher B, Henriksson M. Repression of in vivo growth of Myc/Ras transformed tumor cells by Mad1. *Oncogene*. 2002; 21:447–459. [PubMed: 11821957]
 111. Schreiber-Agus N, Chin L, Chen K, Torres R, Rao G, Guida P, Skoultchi AI, DePinho RA. An amino-terminal domain of Mxi1 mediates anti-Myc oncogenic activity and interacts with a homolog of the yeast transcriptional repressor SIN3. *Cell*. 1995; 80:777–786. [PubMed: 7889571]
 112. Chen J, Willingham T, Margraf LR, Schreiber-Agus N, DePinho RA, Nisen PD. Effects of the MYC oncogene antagonist, MAD, on proliferation, cell cycling and the malignant phenotype of human brain tumour cells. *Nat Med*. 1995; 1:638–643. [PubMed: 7585143]
 113. Gehring S, Rottmann S, Menkel AR, Mertsching J, Krippner-Heidenreich A, Luscher B. Inhibition of proliferation and apoptosis by the transcriptional repressor Mad1. Repression of Fas-induced caspase-8 activation. *J Biol Chem*. 2000; 275:10413–10420. [PubMed: 10744730]
 114. Roussel MF, Ashmun RA, Sherr CJ, Eisenman RN, Ayer DE. Inhibition of cell proliferation by the Mad1 transcriptional repressor. *Mol Cell Biol*. 1996; 16:2796–2801. [PubMed: 8649388]

115. Sommer A, Hilfenhaus S, Menkel A, Kremmer E, Seiser C, Loidl P, Luscher B. Cell growth inhibition by the Mad/Max complex through recruitment of histone deacetylase activity. *Curr Biol.* 1997; 7:357–365. [PubMed: 9197243]
116. Wechsler DS, Shelly CA, Petroff CA, Dang CV. MXI1, a putative tumor suppressor gene, suppresses growth of human glioblastoma cells. *Cancer Res.* 1997; 57:4905–4912. [PubMed: 9354456]
117. Bejarano MT, Albiñan A, Cornvik T, Brijker SO, Asker C, Osorio LM, Henriksson M. Inhibition of cell growth and apoptosis by inducible expression of the transcriptional repressor Mad1. *Exp Cell Res.* 2000; 260:61–72. [PubMed: 11010811]
118. Zou L, Zhang P, Luo C, Tu Z. Mad1 suppresses bladder cancer cell proliferation by inhibiting human telomerase reverse transcriptase transcription and telomerase activity. *Urology.* 2006; 67:1335–1340. [PubMed: 16765199]
119. Ohta Y, Hamada Y, Saitoh N, Katsuoka K. Effect of the transcriptional repressor Mad1 on proliferation of human melanoma cells. *Exp Dermatol.* 2002; 11:439–447. [PubMed: 12366697]
120. Queva C, McArthur GA, Ramos LS, Eisenman RN. Dwarfism and dysregulated proliferation in mice overexpressing the MYC antagonist MAD1. *Cell Growth Differ.* 1999; 10:785–796. [PubMed: 10616903]
121. Manni I, Tunici P, Cirenei N, Albarosa R, Colombo BM, Roz L, Sacchi A, Piaggio G, Finocchiaro G. Mxi1 inhibits the proliferation of U87 glioma cells through down-regulation of cyclin B1 gene expression. *Br J Cancer.* 2002; 86:477–484. [PubMed: 11875718]
122. Taj MM, Tawil RJ, Engstrom LD, Zeng Z, Hwang C, Sanda MG, Wechsler DS. Mxi1, a Myc antagonist, suppresses proliferation of DU145 human prostate cells. *Prostate.* 2001; 47:194–204. [PubMed: 11351349]
123. Ayer DE, Lawrence QA, Eisenman RN. Mad-Max transcriptional repression is mediated by ternary complex formation with mammalian homologs of yeast repressor Sin3. *Cell.* 1995; 80:767–776. [PubMed: 7889570]
124. Iritani BM, Delrow J, Grandori C, Gomez I, Klacking M, Carlos LS, Eisenman RN. Modulation of T-lymphocyte development, growth and cell size by the Myc antagonist and transcriptional repressor Mad1. *EMBO J.* 2002; 21:4820–4830. [PubMed: 12234922]
125. Rudolph B, Hueber AO, Evan GI. Expression of Mad1 in T cells leads to reduced thymic cellularity and impaired mitogen-induced proliferation. *Oncogene.* 2001; 20:1164–1175. [PubMed: 11313860]
126. Foley KP, McArthur GA, Queva C, Hurlin PJ, Soriano P, Eisenman RN. Targeted disruption of the MYC antagonist MAD1 inhibits cell cycle exit during granulocyte differentiation. *EMBO J.* 1998; 17:774–785. [PubMed: 9451002]
127. McArthur GA, Foley KP, Fero ML, Walkley CR, Deans AJ, Roberts JM, Eisenman RN. MAD1 and p27(KIP1) cooperate to promote terminal differentiation of granulocytes and to inhibit Myc expression and cyclin E-CDK2 activity. *Mol Cell Biol.* 2002; 22:3014–3023. [PubMed: 11940659]
128. Schreiber-Agus N, Meng Y, Hoang T, Hou H Jr. Chen K, Greenberg R, Cordon-Cardo C, Lee HW, DePinho RA. Role of Mxi1 in ageing organ systems and the regulation of normal and neoplastic growth. *Nature.* 1998; 393:483–487. [PubMed: 9624006]
129. Hurlin PJ, Zhou ZQ, Toyo-oka K, Ota S, Walker WL, Hirotsune S, Wynshaw-Boris A. Deletion of Mnt leads to disrupted cell cycle control and tumorigenesis. *EMBO J.* 2003; 22:4584–4596. [PubMed: 12970171]
130. Toyo-oka K, Hirotsune S, Gambello MJ, Zhou ZQ, Olson L, Rosenfeld MG, Eisenman R, Hurlin P, Wynshaw-Boris A. Loss of the Max-interacting protein Mnt in mice results in decreased viability, defective embryonic growth and craniofacial defects: relevance to Miller-Dieker syndrome. *Hum Mol Genet.* 2004; 13:1057–1067. [PubMed: 15028671]
131. Nilsson JA, Maclean KH, Keller UB, Pendeville H, Baudino TA, Cleveland JL. Mnt loss triggers Myc transcription targets, proliferation, apoptosis, and transformation. *Mol Cell Biol.* 2004; 24:1560–1569. [PubMed: 14749372]
132. Toyo-oka K, Bowen TJ, Hirotsune S, Li Z, Jain S, Ota S, Escoubet-Lozach L, Garcia-Bassets I, Lozach J, Rosenfeld MG, Glass CK, Eisenman R, Ren B, Hurlin P, Wynshaw-Boris A. Mnt-

deficient mammary glands exhibit impaired involution and tumors with characteristics of myc overexpression. *Cancer Res.* 2006; 66:5565–5573. [PubMed: 16740691]

133. Meroni G, Reymond A, Alcalay M, Borsani G, Tanigami A, Tonlorenzi R, Lo Nigro C, Messali S, Zollo M, Ledbetter DH, Brent R, Ballabio A, Carrozzo R. Rox, a novel bHLHZip protein expressed in quiescent cells that heterodimerizes with Max, binds a non-canonical E box and acts as a transcriptional repressor. *EMBO J.* 1997; 16:2892–2906. [PubMed: 9184233]
134. Popov N, Wahlstrom T, Hurlin PJ, Henriksson M. Mnt transcriptional repressor is functionally regulated during cell cycle progression. *Oncogene.* 2005; 24:8326–8337. [PubMed: 16103876]
135. Berns K, Hijmans EM, Bernards R. Repression of c-Myc responsive genes in cycling cells causes G1 arrest through reduction of cyclin E/CDK2 kinase activity. *Oncogene.* 1997; 15:1347–1356. [PubMed: 9315103]
136. Steiner P, Philipp A, Lukas J, Godden-Kent D, Pagano M, Mittnacht S, Bartek J, Eilers M. Identification of a Myc-dependent step during the formation of active G1 cyclin-cdk complexes. *EMBO J.* 1995; 14:4814–4826. [PubMed: 7588611]
137. Rudolph B, Saffrich R, Zwicker J, Henglein B, Muller R, Ansorge W, Eilers M. Activation of cyclin-dependent kinases by Myc mediates induction of cyclin A, but not apoptosis. *EMBO J.* 1996; 15:3065–3076. [PubMed: 8670807]
138. Walker W, Zhou ZQ, Ota S, Wynshaw-Boris A, Hurlin PJ. Mnt-Max to Myc-Max complex switching regulates cell cycle entry. *J Cell Biol.* 2005; 169:405–413. [PubMed: 15866886]
139. Pierce SB, Yost C, Anderson SA, Flynn EM, Delrow J, Eisenman RN. Drosophila growth and development in the absence of dMyc and dMnt. *Dev Biol.* 2008; 315:303–316. [PubMed: 18241851]
140. Pierce SB, Yost C, Britton JS, Loo LW, Flynn EM, Edgar BA, Eisenman RN. dMyc is required for larval growth and endoreplication in Drosophila. *Development.* 2004; 131:2317–2327. [PubMed: 15128666]
141. Dezfouli S, Bakke A, Huang J, Wynshaw-Boris A, Hurlin PJ. Inflammatory disease and lymphomagenesis caused by deletion of the Myc antagonist Mnt in T cells. *Mol Cell Biol.* 2006; 26:2080–2092. [PubMed: 16507988]
142. 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; 13:2658–2669. [PubMed: 10541552]
143. Zindy F, Eischen CM, Randle DH, Kamijo T, Cleveland JL, Sherr CJ, Roussel MF. Myc signaling via the ARF tumor suppressor regulates p53-dependent apoptosis and immortalization. *Genes Dev.* 1998; 12:2424–2433. [PubMed: 9694806]
144. Link JM, Ota S, Zhou ZQ, Daniel CJ, Sears RC, Hurlin PJ. A critical role for Mnt in Myc-driven T-cell proliferation and oncogenesis. *Proc Natl Acad Sci U S A.* 2012; 109:19685–19690. [PubMed: 23150551]
145. Sloan EJ, Ayer DE. Myc, mondo, and metabolism. *Genes Cancer.* 2010; 1:587–596. [PubMed: 21113411]
146. Kaadige MR, Elgort MG, Ayer DE. Coordination of glucose and glutamine utilization by an expanded Myc network. *Transcription.* 2010; 1:36–40. [PubMed: 21327161]
147. O'Shea JM, Ayer DE. Coordination of nutrient availability and utilization by MAX- and MLX-centered transcription networks. *Cold Spring Harb Perspect Med.* 2013; a014258; 3
148. Peterson CW, Ayer DE. An extended Myc network contributes to glucose homeostasis in cancer and diabetes. *Front Biosci (Landmark Ed).* 2011; 16:2206–2223. [PubMed: 21622171]
149. Metukuri MR, Zhang P, Basantani MK, Chin C, Stamateris RE, Alonso LC, Takane KK, Gramignoli R, Strom SC, O'Doherty RM, Stewart AF, Vasavada RC, Garcia-Ocana A, Scott DK. ChREBP mediates glucose-stimulated pancreatic beta-cell proliferation. *Diabetes.* 2012; 61:2004–2015. [PubMed: 22586588]
150. Tong X, Zhao F, Mancuso A, Gruber JJ, Thompson CB. The glucose-responsive transcription factor ChREBP contributes to glucose-dependent anabolic synthesis and cell proliferation. *Proc Natl Acad Sci U S A.* 2009; 106:21660–21665. [PubMed: 19995986]
151. Wernicke CM, Richter GH, Beinvogl BC, Plehm S, Schlitter AM, Bandapalli OR, Prazeres da Costa O, Hattenhorst UE, Volkmer I, Staeger MS, Esposito I, Burdach S, Grunewald TG.

- MondoA is highly overexpressed in acute lymphoblastic leukemia cells and modulates their metabolism, differentiation and survival. *Leuk Res.* 2012; 36:1185–1192. [PubMed: 22748921]
152. Rabbitts PH, Watson JV, Lamond A, Forster A, Stinson MA, Evan G, Fischer W, Atherton E, Sheppard R, Rabbitts TH. Metabolism of c-myc gene products: c-myc mRNA and protein expression in the cell cycle. *EMBO J.* 1985; 4:2009–2015. [PubMed: 4065102]
 153. Queva C, Hurlin PJ, Foley KP, Eisenman RN. Sequential expression of the MAD family of transcriptional repressors during differentiation and development. *Oncogene.* 1998; 16:967–977. [PubMed: 9519870]
 154. Queva C, McArthur GA, Iritani BM, Eisenman RN. Targeted deletion of the S-phase-specific Myc antagonist Mad3 sensitizes neuronal and lymphoid cells to radiation-induced apoptosis. *Mol Cell Biol.* 2001; 21:703–712. [PubMed: 11154258]
 155. Fox EJ, Wright SC. S-phase-specific expression of the Mad3 gene in proliferating and differentiating cells. *Biochem J.* 2001; 359:361–367. [PubMed: 11583582]
 156. Leon J, Ferrandiz N, Acosta JC, Delgado MD. Inhibition of cell differentiation: a critical mechanism for MYC-mediated carcinogenesis? *Cell Cycle.* 2009; 8:1148–1157. [PubMed: 19282668]
 157. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell.* 2006; 126:663–676. [PubMed: 16904174]
 158. Chappell J, Dalton S. Roles for MYC in the Establishment and Maintenance of Pluripotency. *Cold Spring Harb Perspect Med.* 2013; 3
 159. Ayer DE, Eisenman RN. A switch from Myc:Max to Mad:Max heterocomplexes accompanies monocyte/macrophage differentiation. *Genes Dev.* 1993; 7:2110–2119. [PubMed: 8224841]
 160. Hurlin PJ, Ayer DE, Grandori C, Eisenman RN. The Max transcription factor network: involvement of Mad in differentiation and an approach to identification of target genes. *Cold Spring Harb Symp Quant Biol.* 1994; 59:109–116. [PubMed: 7587059]
 161. Larsson LG, Pettersson M, Oberg F, Nilsson K, Luscher B. Expression of mad, mx1, max and c-myc during induced differentiation of hematopoietic cells: opposite regulation of mad and c-myc. *Oncogene.* 1994; 9:1247–1252. [PubMed: 8134128]
 162. Larsson LG, Bahram F, Burkhardt H, Luscher B. Analysis of the DNA-binding activities of Myc/Max/Mad network complexes during induced differentiation of U-937 monoblasts and F9 teratocarcinoma cells. *Oncogene.* 1997; 15:737–748. [PubMed: 9264414]
 163. Acosta JC, Ferrandiz N, Bretones G, Torrano V, Blanco R, Richard C, O'Connell B, Sedivy J, Delgado MD, Leon J. Myc inhibits p27-induced erythroid differentiation of leukemia cells by repressing erythroid master genes without reversing p27-mediated cell cycle arrest. *Mol Cell Biol.* 2008; 28:7286–7295. [PubMed: 18838534]
 164. Cultraro CM, Bino T, Segal S. Function of the c-Myc antagonist Mad1 during a molecular switch from proliferation to differentiation. *Mol Cell Biol.* 1997; 17:2353–2359. [PubMed: 9111304]
 165. Hopewell R, Ziff EB. The nerve growth factor-responsive PC12 cell line does not express the Myc dimerization partner Max. *Mol Cell Biol.* 1995; 15:3470–3478. [PubMed: 7791753]
 166. Greenberg ME, Greene LA, Ziff EB. Nerve growth factor and epidermal growth factor induce rapid transient changes in proto-oncogene transcription in PC12 cells. *J Biol Chem.* 1985; 260:14101–14110. [PubMed: 3877054]
 167. Noda M, Ko M, Ogura A, Liu DG, Amano T, Takano T, Ikawa Y. Sarcoma viruses carrying ras oncogenes induce differentiation-associated properties in a neuronal cell line. *Nature.* 1985; 318:73–75. [PubMed: 4058592]
 168. Bar-Sagi D, Feramisco JR. Microinjection of the ras oncogene protein into PC12 cells induces morphological differentiation. *Cell.* 1985; 42:841–848. [PubMed: 2996779]
 169. Hishida T, Nozaki Y, Nakachi Y, Mizuno Y, Okazaki Y, Ema M, Takahashi S, Nishimoto M, Okuda A. Indefinite self-renewal of ESCs through Myc/Max transcriptional complex-independent mechanisms. *Cell Stem Cell.* 2011; 9:37–49. [PubMed: 21726832]
 170. Gallant P, Steiger D. Myc's secret life without Max. *Cell Cycle.* 2009; 8:3848–3853. [PubMed: 19887915]
 171. Steiger D, Furrer M, Schwinkendorf D, Gallant P. Max-independent functions of Myc in *Drosophila melanogaster*. *Nat Genet.* 2008; 40:1084–1091. [PubMed: 19165923]

172. Burnichon N, Cascon A, Schiavi F, Morales NP, Comino-Mendez I, Abermil N, Inglada-Perez L, de Cubas AA, Amar L, Barontini M, de Quiros SB, Bertherat J, Bignon YJ, Blok MJ, Bobisse S, Borrego S, Castellano M, Chanson P, Chiara MD, Corssmit EP, Giacche M, de Krijger RR, Ercolino T, Girerd X, Gomez-Garcia EB, Gomez-Grana A, Guilhem I, Hes FJ, Honrado E, Korpershoek E, Lenders JW, Leton R, Mensenkamp AR, Merlo A, Mori L, Murat A, Pierre P, Plouin PF, Prodanov T, Quesada-Charneco M, Qin N, Rapizzi E, Raymond V, Reisch N, Roncador G, Ruiz-Ferrer M, Schillo F, Stegmann AP, Suarez C, Taschin E, Timmers HJ, Tops CM, Urioste M, Beuschlein F, Pacak K, Mannelli M, Dahia PL, Opocher G, Eisenhofer G, Gimenez-Roqueplo AP, Robledo M. MAX mutations cause hereditary and sporadic pheochromocytoma and paraganglioma. *Clin Cancer Res.* 2012; 18:2828–2837. [PubMed: 22452945]
173. Comino-Mendez I, Gracia-Aznarez FJ, Schiavi F, Landa I, Leandro-Garcia LJ, Leton R, Honrado E, Ramos-Medina R, Caronia D, Pita G, Gomez-Grana A, de Cubas AA, Inglada-Perez L, Maliszewska A, Taschin E, Bobisse S, Pica G, Loli P, Hernandez-Lavado R, Diaz JA, Gomez-Morales M, Gonzalez-Neira A, Roncador G, Rodriguez-Antona C, Benitez J, Mannelli M, Opocher G, Robledo M, Cascon A. Exome sequencing identifies MAX mutations as a cause of hereditary pheochromocytoma. *Nat Genet.* 2011; 43:663–667. [PubMed: 21685915]
174. Vaque JP, Fernandez-Garcia B, Garcia-Sanz P, Ferrandiz N, Bretones G, Calvo F, Crespo P, Marin MC, Leon J. c-Myc inhibits Ras-mediated differentiation of pheochromocytoma cells by blocking c-Jun up-regulation. *Mol Cancer Res.* 2008; 6:325–339. [PubMed: 18314492]
175. Iizuka K, Bruick RK, Liang G, Horton JD, Uyeda K. Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis. *Proc Natl Acad Sci U S A.* 2004; 101:7281–7286. [PubMed: 15118080]
176. Prendergast GC. Mechanisms of apoptosis by c-Myc. *Oncogene.* 1999; 18:2967–2987. [PubMed: 10378693]
177. Pelengaris S, Rudolph B, Littlewood T. Action of Myc in vivo - proliferation and apoptosis. *Curr Opin Genet Dev.* 2000; 10:100–105. [PubMed: 10679391]
178. Nieminen AI, Partanen JI, Klefstrom J. c-Myc blazing a trail of death: coupling of the mitochondrial and death receptor apoptosis pathways by c-Myc. *Cell Cycle.* 2007; 6:2464–2472. [PubMed: 17914284]
179. Nilsson JA, Cleveland JL. Myc pathways provoking cell suicide and cancer. *Oncogene.* 2003; 22:9007–9021. [PubMed: 14663479]
180. Eischen CM, Roussel MF, Korsmeyer SJ, Cleveland JL. Bax loss impairs Myc-induced apoptosis and circumvents the selection of p53 mutations during Myc-mediated lymphomagenesis. *Mol Cell Biol.* 2001; 21:7653–7662. [PubMed: 11604501]
181. Wagner AJ, Small MB, Hay N. Myc-mediated apoptosis is blocked by ectopic expression of Bcl-2. *Mol Cell Biol.* 1993; 13:2432–2440. [PubMed: 8455620]
182. Waikel RL, Wang XJ, Roop DR. Targeted expression of c-Myc in the epidermis alters normal proliferation, differentiation and UV-B induced apoptosis. *Oncogene.* 1999; 18:4870–4878. [PubMed: 10490820]
183. Chen CH, Zhang J, Ling CC. Transfected c-myc and c-Ha-ras modulate radiation-induced apoptosis in rat embryo cells. *Radiat Res.* 1994; 139:307–315. [PubMed: 8073113]
184. Rupnow BA, Alarcon RM, Giaccia AJ, Knox SJ. p53 mediates apoptosis induced by c-Myc activation in hypoxic or gamma irradiated fibroblasts. *Cell Death Differ.* 1998; 5:141–147. [PubMed: 10200458]
185. Shim H, Chun YS, Lewis BC, Dang CV. A unique glucose-dependent apoptotic pathway induced by c-Myc. *Proc Natl Acad Sci U S A.* 1998; 95:1511–1516. [PubMed: 9465046]
186. 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; 178:93–105. [PubMed: 17606868]
187. Nieminen AI, Partanen JI, Hau A, Klefstrom J. c-Myc primed mitochondria determine cellular sensitivity to TRAIL-induced apoptosis. *EMBO J.* 2007; 26:1055–1067. [PubMed: 17268552]

188. Cho K, Shin HW, Kim YI, Cho CH, Chun YS, Kim TY, Park JW. Mad1 mediates hypoxia-induced doxorubicin resistance in colon cancer cells by inhibiting mitochondrial function. *Free Radic Biol Med*. 2013; 60:201–210. [PubMed: 23459071]
189. Rottmann S, Speckgens S, Luscher-Firzlaff J, Luscher B. Inhibition of apoptosis by MAD1 is mediated by repression of the PTEN tumor suppressor gene. *FASEB J*. 2008; 22:1124–1134. [PubMed: 17998413]
190. James L, Eisenman RN. Myc and Mad bHLHZ domains possess identical DNA-binding specificities but only partially overlapping functions in vivo. *Proc Natl Acad Sci U S A*. 2002; 99:10429–10434. [PubMed: 12149476]
191. Staller P, Peukert K, Kiermaier A, Seoane J, Lukas J, Karsunky H, Moroy T, Bartek J, Massague J, Hanel F, Eilers M. Repression of p15INK4b expression by Myc through association with Miz-1. *Nat Cell Biol*. 2001; 3:392–399. [PubMed: 11283613]
192. Seoane J, Pouppnot C, Staller P, Schader M, Eilers M, Massague J. TGFbeta influences Myc, Miz-1 and Smad to control the CDK inhibitor p15INK4b. *Nat Cell Biol*. 2001; 3:400–408. [PubMed: 11283614]
193. Seoane J, Le HV, Massague J. Myc suppression of the p21(Cip1) Cdk inhibitor influences the outcome of the p53 response to DNA damage. *Nature*. 2002; 419:729–734. [PubMed: 12384701]
194. Patel JH, McMahon SB. Targeting of Miz-1 is essential for Myc-mediated apoptosis. *J Biol Chem*. 2006; 281:3283–3289. [PubMed: 16352593]
195. Vasilevsky NA, Ruby CE, Hurlin PJ, Weinberg AD. OX40 engagement stabilizes Mxd4 and Mnt protein levels in antigen-stimulated T cells leading to an increase in cell survival. *Eur J Immunol*. 2011; 41:1024–1034. [PubMed: 21400495]
196. Corn PG, Ricci MS, Scata KA, Arsham AM, Simon MC, Dicker DT, El-Deiry WS. Mxi1 is induced by hypoxia in a HIF-1-dependent manner and protects cells from c-Myc-induced apoptosis. *Cancer Biol Ther*. 2005; 4:1285–1294. [PubMed: 16319523]
197. Kaelin WG Jr. The von Hippel-Lindau tumour suppressor protein: O2 sensing and cancer. *Nat Rev Cancer*. 2008; 8:865–873. [PubMed: 18923434]
198. Tsao CC, Teh BT, Jonasch E, Shreiber-Agus N, Efstathiou E, Hoang A, Czerniak B, Logothetis C, Corn PG. Inhibition of Mxi1 suppresses HIF-2alpha-dependent renal cancer tumorigenesis. *Cancer Biol Ther*. 2008; 7:1619–1627. [PubMed: 19018165]
199. Pongvarin N, Lee JK, Yechoor VK, Li MV, Assavapokee T, Suksaranjit P, Thepsongwajja JJ, Saha PK, Oka K, Chan L. Carbohydrate response element-binding protein (ChREBP) plays a pivotal role in beta cell glucotoxicity. *Diabetologia*. 2012; 55:1783–1796. [PubMed: 22382520]
200. Bello-Fernandez C, Cleveland JL. c-myc transactivates the ornithine decarboxylase gene. *Curr Top Microbiol Immunol*. 1992; 182:445–452. [PubMed: 1490383]
201. Miltenberger RJ, Sukow KA, Farnham PJ. An E-box-mediated increase in cad transcription at the G1/S-phase boundary is suppressed by inhibitory c-Myc mutants. *Mol Cell Biol*. 1995; 15:2527–2535. [PubMed: 7739536]
202. Mai S. Overexpression of c-myc precedes amplification of the gene encoding dihydrofolate reductase. *Gene*. 1994; 148:253–260. [PubMed: 7958952]
203. Pusch O, Soucek T, Hengstschlager-Ottnd E, Bernaschek G, Hengstschlager M. Cellular targets for activation by c-Myc include the DNA metabolism enzyme thymidine kinase. *DNA Cell Biol*. 1997; 16:737–747. [PubMed: 9212167]
204. Shim H, Dolde C, Lewis BC, Wu CS, Dang G, Jungmann RA, Dalla-Favera R, Dang CV. c-Myc transactivation of LDH-A: implications for tumor metabolism and growth. *Proc Natl Acad Sci U S A*. 1997; 94:6658–6663. [PubMed: 9192621]
205. Dang CV, Le A, Gao P. MYC-induced cancer cell energy metabolism and therapeutic opportunities. *Clin Cancer Res*. 2009; 15:6479–6483. [PubMed: 19861459]
206. Gomez-Roman N, Grandori C, Eisenman RN, White RJ. Direct activation of RNA polymerase III transcription by c-Myc. *Nature*. 2003; 421:290–294. [PubMed: 12529648]
207. Grandori C, Gomez-Roman N, Felton-Edkins ZA, Ngouenet C, Galloway DA, Eisenman RN, White RJ. c-Myc binds to human ribosomal DNA and stimulates transcription of rRNA genes by RNA polymerase I. *Nat Cell Biol*. 2005; 7:311–318. [PubMed: 15723054]

208. Gomez-Roman N, Felton-Edkins ZA, Kenneth NS, Goodfellow SJ, Athineos D, Zhang J, Ramsbottom BA, Innes F, Kantidakis T, Kerr ER, Brodie J, Grandori C, White RJ. Activation by c-Myc of transcription by RNA polymerases I, II and III. *Biochem Soc Symp.* 2006;141–154. [PubMed: 16626295]
209. Yeung SJ, Pan J, Lee MH. Roles of p53, MYC and HIF-1 in regulating glycolysis - the seventh hallmark of cancer. *Cell Mol Life Sci.* 2008; 65:3981–3999. [PubMed: 18766298]
210. Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK, Nissim I, Daikhin E, Yudkoff M, McMahon SB, Thompson CB. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. *Proc Natl Acad Sci U S A.* 2008; 105:18782–18787. [PubMed: 19033189]
211. Dang CV. Rethinking the Warburg effect with Myc micromanaging glutamine metabolism. *Cancer Res.* 2010; 70:859–862. [PubMed: 20086171]
212. Wang R, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, McCormick LL, Fitzgerald P, Chi H, Munger J, Green DR. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity.* 2011; 35:871–882. [PubMed: 22195744]
213. Kim JW, Zeller KI, Wang Y, Jegga AG, Aronow BJ, O'Donnell KA, Dang CV. Evaluation of myc E-box phylogenetic footprints in glycolytic genes by chromatin immunoprecipitation assays. *Mol Cell Biol.* 2004; 24:5923–5936. [PubMed: 15199147]
214. Osthus RC, Shim H, Kim S, Li Q, Reddy R, Mukherjee M, Xu Y, Wonsey D, Lee LA, Dang CV. Deregulation of glucose transporter 1 and glycolytic gene expression by c-Myc. *J Biol Chem.* 2000; 275:21797–21800. [PubMed: 10823814]
215. Hu S, Balakrishnan A, Bok RA, Anderton B, Larson PE, Nelson SJ, Kurhanewicz J, Vigneron DB, Goga A. 13C-pyruvate imaging reveals alterations in glycolysis that precede c-Myc-induced tumor formation and regression. *Cell Metab.* 2011; 14:131–142. [PubMed: 21723511]
216. Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T, Zeller KI, De Marzo AM, Van Eyk JE, Mendell JT, Dang CV. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. *Nature.* 2009; 458:762–765. [PubMed: 19219026]
217. Morrish F, Giedt C, Hockenbery D. c-MYC apoptotic function is mediated by NRF-1 target genes. *Genes Dev.* 2003; 17:240–255. [PubMed: 12533512]
218. Morrish F, Noonan J, Perez-Olsen C, Gafken PR, Fitzgibbon M, Kelleher J, VanGilst M, Hockenbery D. Myc-dependent mitochondrial generation of acetyl-CoA contributes to fatty acid biosynthesis and histone acetylation during cell cycle entry. *J Biol Chem.* 2010; 285:36267–36274. [PubMed: 20813845]
219. Li F, Wang Y, Zeller KI, Potter JJ, Wonsey DR, O'Donnell KA, Kim JW, Yustein JT, Lee LA, Dang CV. Myc stimulates nuclearly encoded mitochondrial genes and mitochondrial biogenesis. *Mol Cell Biol.* 2005; 25:6225–6234. [PubMed: 15988031]
220. van Riggelen J, Yetil A, Felsher DW. MYC as a regulator of ribosome biogenesis and protein synthesis. *Nat Rev Cancer.* 2010; 10:301–309. [PubMed: 20332779]
221. Zhang Z, Sun H, Dai H, Walsh RM, Imakura M, Schelter J, Burchard J, Dai X, Chang AN, Diaz RL, Marszalek JR, Bartz SR, Carleton M, Cleary MA, Linsley PS, Grandori C. MicroRNA miR-210 modulates cellular response to hypoxia through the MYC antagonist MNT. *Cell Cycle.* 2009; 8:2756–2768. [PubMed: 19652553]
222. Iizuka K, Horikawa Y. ChREBP: a glucose-activated transcription factor involved in the development of metabolic syndrome. *Endocr J.* 2008; 55:617–624. [PubMed: 18490833]
223. Davies MN, O'Callaghan BL, Towle HC. Glucose activates ChREBP by increasing its rate of nuclear entry and relieving repression of its transcriptional activity. *J Biol Chem.* 2008; 283:24029–24038. [PubMed: 18591247]
224. Davies MN, O'Callaghan BL, Towle HC. Activation and repression of glucose-stimulated ChREBP requires the concerted action of multiple domains within the MondoA conserved region. *Am J Physiol Endocrinol Metab.* 2010; 299:E665–E674. [PubMed: 20682844]
225. Peterson CW, Stoltzman CA, Sighinolfi MP, Han KS, Ayer DE. Glucose controls nuclear accumulation, promoter binding, and transcriptional activity of the MondoA-Mlx heterodimer. *Mol Cell Biol.* 2010; 30:2887–2895. [PubMed: 20385767]

226. Ma L, Robinson LN, Towle HC. ChREBP**Mlx* is the principal mediator of glucose-induced gene expression in the liver. *J Biol Chem*. 2006; 281:28721–28730. [PubMed: 16885160]
227. Ishii S, Iizuka K, Miller BC, Uyeda K. Carbohydrate response element binding protein directly promotes lipogenic enzyme gene transcription. *Proc Natl Acad Sci U S A*. 2004; 101:15597–15602. [PubMed: 15496471]
228. Wang Y, Botolin D, Xu J, Christian B, Mitchell E, Jayaprakasam B, Nair MG, Peters JM, Busik JV, Olson LK, Jump DB. Regulation of hepatic fatty acid elongase and desaturase expression in diabetes and obesity. *J Lipid Res*. 2006; 47:2028–2041. [PubMed: 16790840]
229. Jeong YS, Kim D, Lee YS, Kim HJ, Han JY, Im SS, Chong HK, Kwon JK, Cho YH, Kim WK, Osborne TF, Horton JD, Jun HS, Ahn YH, Ahn SM, Cha JY. Integrated expression profiling and genome-wide analysis of ChREBP targets reveals the dual role for ChREBP in glucose-regulated gene expression. *PLoS One*. 2011; 6:e22544. [PubMed: 21811631]
230. Ma L, Tsatsos NG, Towle HC. Direct role of ChREBP. *Mlx* in regulating hepatic glucose-responsive genes. *J Biol Chem*. 2005; 280:12019–12027. [PubMed: 15664996]
231. Collier JJ, Doan TT, Daniels MC, Schurr JR, Kolls JK, Scott DK. c-Myc is required for the glucose-mediated induction of metabolic enzyme genes. *J Biol Chem*. 2003; 278:6588–6595. [PubMed: 12480946]
232. Collier JJ, Zhang P, Pedersen KB, Burke SJ, Haycock JW, Scott DK. c-Myc and ChREBP regulate glucose-mediated expression of the L-type pyruvate kinase gene in INS-1-derived 832/13 cells. *Am J Physiol Endocrinol Metab*. 2007; 293:E48–56. [PubMed: 17341548]
233. Zhang P, Metukuri MR, Bindom SM, Prochownik EV, O'Doherty RM, Scott DK. c-Myc is required for the CHREBP-dependent activation of glucose-responsive genes. *Mol Endocrinol*. 2010; 24:1274–1286. [PubMed: 20382893]
234. Qing G, Li B, Vu A, Skuli N, Walton ZE, Liu X, Mayes PA, Wise DR, Thompson CB, Maris JM, Hogarty MD, Simon MC. ATF4 regulates MYC-mediated neuroblastoma cell death upon glutamine deprivation. *Cancer Cell*. 2012; 22:631–644. [PubMed: 23153536]
235. Le A, Cooper CR, Gouw AM, Dinavahi R, Maitra A, Deck LM, Royer RE, Vander Jagt DL, Semenza GL, Dang CV. Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. *Proc Natl Acad Sci U S A*. 2010; 107:2037–2042. [PubMed: 20133848]
236. Greenberg RA, O'Hagan RC, Deng H, Xiao Q, Hann SR, Adams RR, Lichtsteiner S, Chin L, Morin GB, DePinho RA. Telomerase reverse transcriptase gene is a direct target of c-Myc but is not functionally equivalent in cellular transformation. *Oncogene*. 1999; 18:1219–1226. [PubMed: 10022128]
237. Gunes C, Lichtsteiner S, Vasserot AP, Englert C. Expression of the hTERT gene is regulated at the level of transcriptional initiation and repressed by Mad1. *Cancer Res*. 2000; 60:2116–2121. [PubMed: 10786671]
238. Oh S, Song YH, Yim J, Kim TK. Identification of Mad as a repressor of the human telomerase (hTERT) gene. *Oncogene*. 2000; 19:1485–1490. [PubMed: 10723141]
239. Xu D, Popov N, Hou M, Wang Q, Bjorkholm M, Gruber A, Menkel AR, Henriksson M. Switch from Myc/Max to Mad1/Max binding and decrease in histone acetylation at the telomerase reverse transcriptase promoter during differentiation of HL60 cells. *Proc Natl Acad Sci U S A*. 2001; 98:3826–3831. [PubMed: 11274400]
240. Lutz W, Leon J, Eilers M. Contributions of Myc to tumorigenesis. *Biochim Biophys Acta*. 2002; 1602:61–71. [PubMed: 11960695]
241. Bouchard C, Thieke K, Maier A, Saffrich R, Hanley-Hyde J, Ansorge W, Reed S, Sicinski P, Bartek J, Eilers M. Direct induction of cyclin D2 by Myc contributes to cell cycle progression and sequestration of p27. *EMBO J*. 1999; 18:5321–5333. [PubMed: 10508165]
242. Bouchard C, Dittrich O, Kiermaier A, Dohmann K, Menkel A, Eilers M, Luscher B. Regulation of cyclin D2 gene expression by the Myc/Max/Mad network: Myc-dependent TRRAP recruitment and histone acetylation at the cyclin D2 promoter. *Genes Dev*. 2001; 15:2042–2047. [PubMed: 11511535]
243. Rottmann S, Menkel AR, Bouchard C, Mertsching J, Loidl P, Kremmer E, Eilers M, Luscher-Firzlaff J, Lilischkis R, Luscher B. Mad1 function in cell proliferation and transcriptional

- repression is antagonized by cyclin E/CDK2. *J Biol Chem.* 2005; 280:15489–15492. [PubMed: 15722557]
244. Galaktionov K, Chen X, Beach D. Cdc25 cell-cycle phosphatase as a target of c-myc. *Nature.* 1996; 382:511–517. [PubMed: 8700224]
 245. Zhao H, Xu YH. Mad-overexpression down regulates the malignant growth and p53 mediated apoptosis in human hepatocellular carcinoma BEL-7404 cells. *Cell Res.* 1999; 9:51–59. [PubMed: 10321688]
 246. Lin CJ, Cencic R, Mills JR, Robert F, Pelletier J. c-Myc and eIF4F are components of a feedforward loop that links transcription and translation. *Cancer Res.* 2008; 68:5326–5334. [PubMed: 18593934]
 247. Jones RM, Branda J, Johnston KA, Polymenis M, Gadd M, Rustgi A, Callanan L, Schmidt EV. 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; 16:4754–4764. [PubMed: 8756633]
 248. Postic C, Dentin R, Denechaud PD, Girard J. ChREBP, a transcriptional regulator of glucose and lipid metabolism. *Annu Rev Nutr.* 2007; 27:179–192. [PubMed: 17428181]
 249. Uyeda K, Repa JJ. Carbohydrate response element binding protein, ChREBP, a transcription factor coupling hepatic glucose utilization and lipid synthesis. *Cell Metab.* 2006; 4:107–110. [PubMed: 16890538]
 250. Shih HM, Liu Z, Towle HC. Two CACGTG motifs with proper spacing dictate the carbohydrate regulation of hepatic gene transcription. *J Biol Chem.* 1995; 270:21991–21997. [PubMed: 7665621]
 251. Ma L, Sham YY, Walters KJ, Towle HC. A critical role for the loop region of the basic helix-loop-helix/leucine zipper protein Mlx in DNA binding and glucose-regulated transcription. *Nucleic Acids Res.* 2007; 35:35–44. [PubMed: 17148476]
 252. Stoeckman AK, Ma L, Towle HC. Mlx is the functional heteromeric partner of the carbohydrate response element-binding protein in glucose regulation of lipogenic enzyme genes. *J Biol Chem.* 2004; 279:15662–15669. [PubMed: 14742444]
 253. Schuhmacher M, Kohlhuber F, Holzel M, Kaiser C, Burtscher H, Jarsch M, Bornkamm GW, Laux G, Polack A, Weidle UH, Eick D. The transcriptional program of a human B cell line in response to Myc. *Nucleic Acids Res.* 2001; 29:397–406. [PubMed: 11139609]
 254. Littlewood TD, Kreuzaler P, Evan GI. All things to all people. *Cell.* 2012; 151:11–13. [PubMed: 23021211]
 255. Walz S, Eilers M, Eisenman R, Dang CV. Unlocking the mysterious mechanisms of Myc. *Nat Med.* 2013; 19:26–27. [PubMed: 23296005]
 256. McCarthy N. Tumorigenesis: Megaphone MYC. *Nat Rev Cancer.* 2012; 12:733. [PubMed: 23037452]
 257. Sabo' A, Amati B. Genome Recognition by Myc. *Cold Spring Harb Perspect Med.* 2013 in press.
 258. Grignani F, Lombardi L, Inghirami G, Sternas L, Cechova K, Dalla-Favera R. Negative autoregulation of c-myc gene expression is inactivated in transformed cells. *EMBO J.* 1990; 9:3913–3922. [PubMed: 2249657]
 259. Breit S, Schwab M. Suppression of MYC by high expression of NMYC in human neuroblastoma cells. *J Neurosci Res.* 1989; 24:21–28. [PubMed: 2810395]
 260. Rosenbaum H, Webb E, Adams JM, Cory S, Harris AW. N-myc transgene promotes B lymphoid proliferation, elicits lymphomas and reveals cross-regulation with c-myc. *EMBO J.* 1989; 8:749–755. [PubMed: 2785918]
 261. Kime L, Wright SC. Mad4 is regulated by a transcriptional repressor complex that contains Miz-1 and c-Myc. *Biochem J.* 2003; 370:291–298. [PubMed: 12418961]
 262. Marcotte R, Chen JM, Huard S, Wang E. c-Myc creates an activation loop by transcriptionally repressing its own functional inhibitor, hMad4, in young fibroblasts, a loop lost in replicatively senescent fibroblasts. *J Cell Biochem.* 2005; 96:1071–1085. [PubMed: 16167342]
 263. Poloni A, Serrani F, Berardinelli E, Maurizi G, Mariani M, Costantini B, Trappolini S, Mancini S, Olivieri A, Leoni P. Telomere length, c-myc and mad-1 expression could represent prognosis markers of myelodysplastic syndrome. *Leuk Res.* 2013; 37:1538–1544. [PubMed: 24095110]

264. Boulton JK, Tanieri P, Hallissey MT, Campbell MJ, Tselepis C. Oesophageal adenocarcinoma is associated with a deregulation in the MYC/MAX/MAD network. *Br J Cancer*. 2008; 98:1985–1992. [PubMed: 18493233]
265. Crona J, Maharjan R, Delgado Verdugo A, Stalberg P, Granberg D, Hellman P, Bjorklund P. MAX mutations status in Swedish patients with pheochromocytoma and paraganglioma tumours. *Fam Cancer*. 2013
266. Guo XL, Pan L, Zhang XJ, Suo XH, Niu ZY, Zhang JY, Wang F, Dong ZR, Da W, Ohno R. Expression and mutation analysis of genes that encode the Myc antagonists Mad1, Mxi1 and Rox in acute leukaemia. *Leuk Lymphoma*. 2007; 48:1200–1207. [PubMed: 17577784]
267. Li XJ, Wang DY, Zhu Y, Guo RJ, Wang XD, Lubomir K, Mukai K, Sasaki H, Yoshida H, Oka T, Machinami R, Shinmura K, Tanaka M, Sugimura H. Mxi1 mutations in human neurofibrosarcomas. *Jpn J Cancer Res*. 1999; 90:740–746. [PubMed: 10470286]
268. Prochownik EV, Eagle Grove L, Deubler D, Zhu XL, Stephenson RA, Rohr LR, Yin X, Brothman AR. Commonly occurring loss and mutation of the MXI1 gene in prostate cancer. *Genes Chromosomes Cancer*. 1998; 22:295–304. [PubMed: 9669667]
269. Kuczyk MA, Serth J, Bokemeyer C, Schwede J, Herrmann R, Machten S, Grunewald V, Hofner K, Jonas U. The MXI1 tumor suppressor gene is not mutated in primary prostate cancer. *Oncol Rep*. 1998; 5:213–216. [PubMed: 9458379]
270. Bartsch D, Peiffer SL, Kaleem Z, Wells SA Jr. Goodfellow PJ. Mxi1 tumor suppressor gene is not mutated in primary pancreatic adenocarcinoma. *Cancer Lett*. 1996; 102:73–76. [PubMed: 8603382]
271. Eagle LR, Yin X, Brothman AR, Williams BJ, Atkin NB, Prochownik EV. Mutation of the MXI1 gene in prostate cancer. *Nat Genet*. 1995; 9:249–255. [PubMed: 7773287]
272. Sommer A, Waha A, Tonn J, Sorensen N, Hurlin PJ, Eisenman RN, Luscher B, Pietsch T. Analysis of the Max-binding protein MNT in human medulloblastomas. *Int J Cancer*. 1999; 82:810–816. [PubMed: 10446446]
273. Takahashi T, Konishi H, Kozaki K, Osada H, Saji S, Takahashi T, Takahashi T. Molecular analysis of a Myc antagonist, ROX/Mnt, at 17p13.3 in human lung cancers. *Jpn J Cancer Res*. 1998; 89:347–351. [PubMed: 9617337]
274. Cvekl A Jr, Zavadi J, Birshtein BK, Grotzer MA, Cvekl A. Analysis of transcripts from 17p13.3 in medulloblastoma suggests ROX/MNT as a potential tumour suppressor gene. *Eur J Cancer*. 2004; 40:2525–2532. [PubMed: 15519529]
275. Vermeer MH, van Doorn R, Dijkman R, Mao X, Whittaker S, van Voorst Vader PC, Gerritsen MJ, Geerts ML, Gellrich S, Soderberg O, Leuchowius KJ, Landegren U, Out-Luiting JJ, Knijnenburg J, Ijszenga M, Szuhai K, Willemze R, Tensen CP. Novel and highly recurrent chromosomal alterations in Sezary syndrome. *Cancer Res*. 2008; 68:2689–2698. [PubMed: 18413736]
276. Link JM, Hurlin PJ. MYC needs MNT. *Cell Cycle*. 2013; 12:385–386. [PubMed: 23324353]
277. Kawamata N, Sugimoto KJ, Sakajiri S, Oshimi K, Koeffler HP. Mxi1 isoforms are expressed in hematological cell lines and normal bone marrow. *Int J Oncol*. 2005; 26:1369–1375. [PubMed: 15809730]
278. Edelmann J, Holzmann K, Miller F, Winkler D, Buhler A, Zenz T, Bullinger L, Kuhn MW, Gerhardinger A, Bloehdorn J, Radtke I, Su X, Ma J, Pounds S, Hallek M, Lichter P, Korbel J, Busch R, Mertens D, Downing JR, Stilgenbauer S, Dohner H. High-resolution genomic profiling of chronic lymphocytic leukemia reveals new recurrent genomic alterations. *Blood*. 2012; 120:4783–4794. [PubMed: 23047824]
279. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci U S A*. 1998; 95:14863–14868. [PubMed: 9843981]
280. Yuneva MO, Fan TW, Allen TD, Higashi RM, Ferraris DV, Tsukamoto T, Mates JM, Alonso FJ, Wang C, Seo Y, Chen X, Bishop JM. The metabolic profile of tumors depends on both the responsible genetic lesion and tissue type. *Cell Metab*. 2012; 15:157–170. [PubMed: 22326218]

Highlights

- A comprehensive model of the MAX/MLX intra-network interaction is proposed
- MAX/MLX network members are widely expressed in both normal and malignant tissues
- The MAX and MLX modules coordinate proliferation and metabolism
- Balanced expression among network members contributes to cell fate determination
- Different human cancers possess a distinct MAX/MLX network organization

Figure 1. Diagram of the MAX/MLX network

All members of the MAX/MLX network and their reciprocal heterodimerization partners (indicated by the two-headed arrows) are represented. Green arrows and red lines indicate transcriptional activation and repression respectively. E-Box, Enhancer-box; ChoRE, Carbohydrate response element.

Figure 2. The MAX/MLX network in the animal kingdom

A) Species specific distribution of MAX/MLX network members. “X”, the bHLHZ was found within a protein or expressed sequence tag; “*”, part or all of the sequence was found within a genetic region; “0”, the protein is known to be absent. B) Schematic representation of the four main network topologies. Solid arrows indicate experimentally verified interactions, whereas dotted arrows indicate debated or unknown interactions. Figure created from data in McFerrin and Atchley (c) 2011, with permission from the authors.

Figure 3. Domain organization of the MAX/MLX network members

A representative member of each subfamily is included. Colored box indicates functional domains. MBI-IV, MYC box domains; NLS, nuclear localization signal; BHLH, Basic helix loop helix; LZ, leucine zipper; SID, SID3-interacting domain; DCD, dimerization and cytoplasmic localization domain; TAD, transcriptional activation domain; TRD, transcriptional repression domain. The Glucose sensing domain contains six conserved regions named MONDO Conserved Region (MCR). Proteins size is not represented to scale.

Figure 4. Schematic representation of the biological activities regulated by the MAX/MLX network members

Green arrows and red lines indicate positive and negative regulation respectively. Dotted red lines indicate a functional connection that has not been directly investigated or for which only a limited amount of data is available. A) MYC centered view. B) MAX/MLX network centered view.

Figure 5. Comprehensive model of the MAX/MLX network and its role in determining cellular fates

External signals (top) determine the expression levels of the network members, which in turn promote alternative cellular state (bottom). High levels of MYCc sensitize cells to pro-apoptotic stimuli. Loss of function of MXD1, MNT or MONDOA further promotes apoptosis in MYC driven tumor models. MGA has been reported recurrently lost in leukemia and lung cancers. Because MAX and MLX are transcriptionally inert and have multiple heterodimerization partners, they have been excluded from the representation for simplicity.

Figure 6. Analysis of the MAX/MLX network in human cancer

A) Relative distribution of the network members across TCGA tissue types, each represented by a different color (“tumor type column”). “Sample” column: Pink normal tissue; Blue, Primary Tumor; Red, Acute Myeloid Leukemia; Grey, Metastatic tumor; Green Recurrent tumor. Due to the heterogeneity in expression, breast and lung samples have been excluded from this representation to more readily identify the distinct expression patterns of the other tumors. B) Pearson correlation of network member expression levels among all samples (including lung and breast). Orange: positive correlation, blue: negative correlation.

C) Median of expression values within each tumor type (colored coded squares) are shown for each network member. Black dashed line, median expression of all genes across tumor types; Grey dashed lines, top and bottom 25% of genes.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript