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Myc protein: function, pathway and mutation

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Abstract

Myc protein belongs to Myc family of transcription factors, there are 3 types of Myc: c-Myc, N-Myc, and L-Myc expression by dendritic cells is required for optimal T cell priming. Myc family of transcription factors contain bHLH/LZ (basic Helix-Loop-Helix Leucine Zipper) domain. Through its bHLH domain can bind to DNA, while the leucine zipper domain allows the dimerization with its partner Max, another bHLH transcription factor. Myc protien is a transcription factor that activates expression of many genes through binding on consensus sequences (Enhancer Box sequences (E-boxes)) and recruiting histone acetyltransferase (HATs). The first to be discovered was its capability to drive cell proliferation (upregulates cyclins, downregulates p21), but it also plays a very important role in regulating cell growth (upregulates ribosomal RNA and proteins), apoptosis (downregulates Bcl-2), differentiation, and stem cell self-renewal. Myc is a very strong proto-oncogene and it is very often found to be upregulated in many types of cancers. Myc overexpression stimulates gene amplification, presumably through DNA over-replication. It can also act as a transcriptional repressor. By binding Miz-1 transcription factor and displacing the p300 co-activator, it inhibits expression of Miz-1 target genes. In addition, myc has a direct role in the control of DNA replication. Myc is activated upon various mitogenic signals such as Wnt, Shh and EGF (via the MAPK/ERK pathway). By modifying the expression of its target genes, Myc activation results in numerous biological effects. This paper will provide researchers with a critical appraisal of Myc in cell biology.

Key words: Myc protein, bHLH domain, Cancer, Histone, p300 co-activator

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Introduction

Myc protein belongs to Myc family of transcription factors, there are 3 types of Myc: c-Myc, N-Myc, and L-Myc expression by dendritic cells is required for optimal T cell priming. Myc family of transcription factors contain bHLH/LZ (basic Helix-Loop-Helix Leucine Zipper) domain. Through its bHLH domain can bind to DNA, while the leucine zipper domain allows the dimerization with its partner Max, another bHLH transcription factor [1].

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The original discoveries of several important cancer drivers, including *MYC* and *RAF*, are closely linked to animal retrovirology [2]. The first biochemical identification of a cancer gene [1] the v-*src* oncogene of Rous sarcoma virus – and the striking proof of its origin from a normal cellular gene [4] were landmark discoveries in molecular cancer research. They immediately stimulated the search for the transforming principles of other highly oncogenic retroviruses [4]. A specific class of retroviruses, the avian acute leukemia viruses, proved to be a rich source of novel cancer genes, including *myc*, *mil*(*raf*), *erb*B, *erb*A, *myb*, and *ets* (Bister and Jansen 1986). In 1977, specific protein products and nucleic acid sequences of the *myc* oncogene were discovered by biochemical analyses of acute leukemia virus MC29 [5].

The viral genome was shown to be defective in all replicative genes (*gag*, *pol*, and *env*), to contain a contiguous novel insert unrelated to *src* and later termed v-*myc* [6], and to encode a single protein product, a Gag-Myc hybrid protein [7]. Subsequently, v-*myc* alleles were identified in all other members of the MC29 subgroup of acute leukemia viruses, CMII, OK10, and MH2 [8]. Following the *src* paradigm, the cellular origin of the v-*myc* alleles was soon proven and the chicken c-*myc* gene identified [9]. The c-*myc* gene has been conserved throughout metazoan evolution and may even have pre-metazoan ancestors [10]. The discovery of chromosomal translocations of the human *MYC* gene in Burkitt lymphoma cells provided the first evidence for the involvement of the cellular homolog of the v-*myc* retroviral oncogene in human tumorigenesis [11]. Today, deregulated *MYC* expression is established as an important driving force in the majority of all human cancers [12].

MYC Proteins

The domain structure of the human MYC protein with a total length of 439 amino acids. MYC belongs to a family of proteins with a characteristic hallmark, a dimerization and DNA binding domain (bHLH-LZ) encompassing a basic region (b) as DNA contact surface, a helix-loop-helix

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(HLH) and a leucine repeat (zipper) (LZ) region as protein-protein interaction (PPI) domains [13].

The preferred binding partner for MYC is another member of the bHLH-LZ protein family, MAX, and PPI between these proteins leads to formation of a stable MYC:MAX heterodimer. The discovery of MAX and the recognition of MYC:MAX as a sequence-specific DNA binding complex enabled crucial leaps forward in the understanding of MYC biochemistry [14].

In the absence of MAX and at physiological concentrations, MYC is monomeric in solution and displays properties of an intrinsically disordered protein (IDP) with isolated regions of dynamic secondary structure elements and helical fraying [15]. MAX forms homodimers, albeit with lower stability than that of the MYC:MAX heterodimer. X-ray structures of the bHLH-LZ domains in MAX homodimers [16] or in MYC:MAX heterodimers (Nair and Burley 2003) revealed the structural details of the dimer-specific selective PPIs between the parallel protein chains.

The α-helical basic regions of MYC:MAX heterodimers bind to specific DNA sequence elements (E-boxes) with the preferred structure 5'-CACGTG-3' by making specific base contacts in the major groove of DNA [17]. MYC:MAX complexes induce transcription by binding to E-boxes in promoter or enhancer regions of target genes and form PPIs with a variety of other factors.

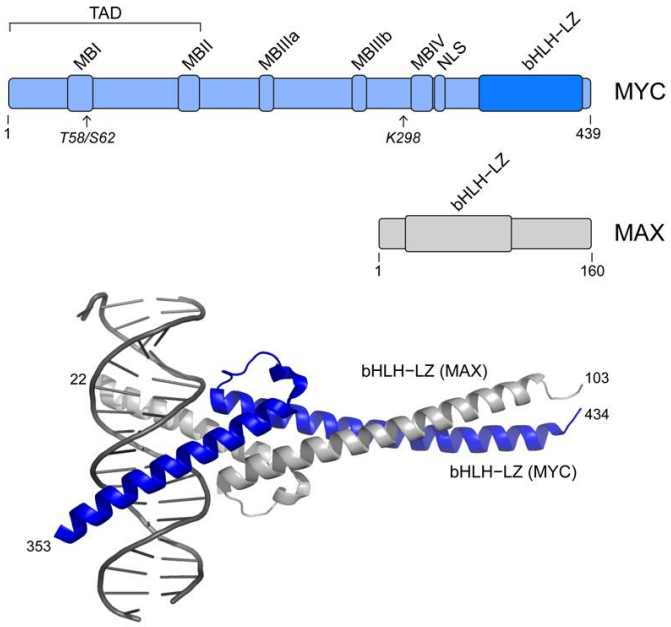

Figure 1.

Structure of the human MYC protein and its dimerization partner MAX. The dimerization and DNA binding domains (bHLH-LZ) are indicated. On the MYC protein, conserved MYC boxes (MBI-IV), the transactivation domain (TAD), the nuclear localization signal (NLS), critical phosphorylation sites (Thr58, Ser62), and a calpain cleavage site (Lys298) are depicted. The X-ray structure of a dimer of the MYC and MAX bHLH-LZ domains bound to DNA.

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Molecular functions of Myc

The MYC mRNA generates Myc polypeptides including one that initiates at a CUG upstream of the canonical AUG start codon, and another that starts at an internal AUG [18]. The Myc protein translated from the canonical AUG contains an N-terminal transcriptional regulatory domain followed by a nuclear localization signal and a C-terminal region with a basic DNA binding domain tethered to a helix-loop-helix-leucine zipper (HLH-Zip) dimerization motif.

Myc dimerizes with Max to bind DNA and mediates many of its functions [19]. A distinct function for the longer Myc polypeptide initiated at the upstream CUG is not known [20], but the shorter one initiated from an internal AUG appears to play a role in stress response and perhaps serves as a dominant negative Myc protein [3]. Myc biology is further complicated by the finding that a cytoplasmic cleavage product of Myc (Myc-nick), which lacks the nuclear localization signal and DNA binding domain, can promote alpha-tubulin acetylation by recruiting GCN5 and promote cell differentiation in a non-transcriptional manner [6].

Myc also appears to recruit DNA replication licensing factors to catalyze DNA replication, although whether its transcriptional function at replication origins is part-and-parcel of its DNA replication activity is not yet clear [8]. Myc also plays an important non-transcriptional role in stimulating cap-dependent translation [19].

Lastly, Myc appears to function even in the absence of functional Max protein as documented in PC12 cells and more recently in Drosophila [11]. Whether Myc could homo-oligomerize or hetero-oligomerize with other helix-loop-helix proteins to regulate transcription in the absence of Max in cells remains unknown [4].

The Myc protein contains an unstructured N-terminal transcriptional regulatory domain, which contains conserved Myc Boxes I and II, followed by Myc Box lll and IV and a nuclear targeting sequence [21]. The C-terminal domain comprises a basic HLH-Zip domain, which is largely unstructured until it dimerizes with Max [22]. The monomers assemble on DNA, and the heterodimer locks onto and bend DNA through binding motifs (5′-CACGTG-3′) termed E-boxes [12].

The N-terminal domain has been documented to form complexes with many factors including TRRAP, GCN5, and TBP, which are likely to induce more structured folding of the N-terminal Myc transcriptional regulatory domain [16]. Hence, it is envisioned that when bound to DNA, the Myc-Max heterodimer would recruit complexes that modify chromatin.

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Myc, checkpoints and neoplastic transformation

Early in vitro studies of MYC revealed its potential to transform normal embryonic fibroblasts in cooperation with other oncogenes [22]. These studies set the stage for transgenic mouse studies that provided the evidence that deregulated expression of MYC is sufficient to drive tumorigenesis in a number of transgenic mouse tissues [23]. Retroviral insertional mutagenesis further identified c-Myc as a major murine oncogene [11].

In each of these models, however, additional mutagenic events are necessary for tumor formation as evidenced by a predictable time delay before the onset of tumors [19]. Hence, MYC requires other genetic alterations in vivo to enable its tumorigenic potential. Mammary carcinoma triggered by transgenic Myc expression acquired K-ras mutations that rendered tumors aggressive [22]. Acute overexpression of MYC in normal cells triggers checkpoints including ARF or p53, such that many MYC-induced transgenic lymphomas lacks functional Arf or p53 [23]. The findings from transgenic mouse studies underscore a causal role for MYC in murine cancers and support its tumorigenic role in human cancers.

MYC is documented to play a role in tumor initiation; however, whether MYC participates in tumor maintenance was previously unclear. Knock-down of MYC in established cancer cell lines in vitro appears to uniformly reduce cell proliferation and in some instances induce apoptosis [20]. In transgenic mouse models with inducible MYC, established tumors regress upon withdrawal of MYC ectopic expression, indicating that MYC plays a role in tumor maintenance, and once established these tumors are addicted to MYC [11].

In fact, expression of a dominant negative inhibitor of Myc heterodimerization in vivo has resulted in tumor regression, suggesting that inhibiting Myc function could be a feasible therapeutic strategy [24].

C-MYC transcription factor, its binding partner max, and mad proteins

The c-*myc* gene, located on human chromosome 8, is comprised of three exons. Translation of the major 64-kDa polypeptide is initiated at the canonical AUG start codon (exon 2), and a longer polypeptide of 67 kDa results from translation initiated 15 codons upstream of the AUG at a CUG codon (exon 1). An internal translationally initiated c-Myc 45-kDa polypeptide was recently recognized [22].

The primary sequence of the c-Myc protein suggests that it contains a potential transactivation domain within its N-terminal 140 amino acids and a dimerization interface consisting of a helix-loop-helix leucine zipper (HLH/LZ) domain at its C-terminal end. Evidence from fusion proteins consisting of GAL4 and c-Myc suggested that the c-Myc transactivation domain is localized to its first 143 amino acids. Immediately N terminal to the dimerization domain is a domain rich in basic amino acids which directly contacts specific DNA sequences within the DNA major groove [24].

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c-Myc DNA binding sites (both canonical [5′-CACGTG-3′] and noncanonical) have been identified by using a variety of in vitro protein-DNA binding assays [25]. The search for a Myc binding partner protein resulted in the breakthrough discovery of an HLH/LZ human Max protein [26]. Initial models proposed that Myc/Max heterodimers bind to target sites to transactivate genes via the Myc transactivation domain.

Max homodimers were thought to counter the function of the Myc/Max heterodimers through competitive binding to target DNA sites [23]; however, functional Max homodimers are not readily detectable in vivo [19].

Transcriptional properties of C-MYC

The c-Myc protein binds to and transactivates through consensus 5′-CACGTG-3′ sequences or E boxes in transient transfection experiments; however, the potency of transactivation by c-Myc pales when compared to those of other transcription factors, such as the HLH/LZ transcriptional factor USF, which also binds 5′-CACGTG-3′ [26].

The variability of c-Myc transactivation has been questioned, and a study has provided evidence that endogenous levels of c-Myc may affect the outcome of transient-transfection experiments. Others suggest that the transactivation properties of c-Myc depend on whether the 64- or 67-kDa form is produced. The ability of c-Myc to interact with the TATA binding protein (TBP) and the transcriptional machinery [27] may be modulated by its interaction with other factors, such as BIN1, MIZ1, PAM, p107, TFII-I, TRRAP, YY1 [28].

Understanding of how each of these proteins modulates the transcriptional activity of c-Myc requires further studies. Another as yet unresolved quagmire in the study of c-Myc is the inability to easily detect c-Myc gel shift activities in nuclear extracts of mammalian cells, although some progress has been achieved recently [29]. Notwithstanding these unresolved concerns, evidence accumulated to date supports the model in which c-Myc is able to bind E boxes and transactivate genes.

In addition to its ability to activate transcription, c-Myc is able to repress transcription in in vitro transcription and transient-transfection assays [30]. The in vitro data are compatible with the ability of c-Myc to inhibit transcription through the initiator or Inr element, which is a consensus transcriptional initiation motif found in certain gene promoters [5]. Likewise, transfection studies using model promoter reporter constructs suggest that c-Myc is able to repress Inr-mediated transcription [9].

c-Myc also represses genes that do not contain Inr sequences [6] and may modulate transcription through interactions with other transcription factors, such as C/EBP or AP-2 b [5]. Since many genes bearing Inr sequences are differentiation marker genes, it is surmised that in addition to its ability to activate growth related genes through E boxes, c-Myc is also able to repress differentiation-related genes. The transcriptional repression function of c-Myc and its transactivation ability are both required for its transforming activity.

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C-MYC target genes

The mechanisms by which c-Myc induces neoplastic transformation and apoptosis are beginning to emerge with the identification of authentic target genes, both direct and indirect (Figure 1). A direct target gene is one whose expression is altered by direct interaction of the c-Myc protein with the gene regulatory elements or with *trans*-acting factors that bind these *cis* elements.

The time course of induction of a direct target gene should closely follow the expression of Myc. The Myc-estrogen receptor (Myc-ER) fusion protein system has become a standard for establishing the direct regulation of a candidate target gene by c-Myc [9]. In this system, the Myc-ER fusion protein is retained in the cytoplasm via chaperone proteins. Upon exposure of cells expressing the Myc-ER protein to estrogenic ligands, the ligand-bound fusion protein is translocated into the nucleus. The Myc-ER protein then activates Myc target genes without requiring new intervening protein synthesis. Thus, exposure of cells simultaneously to estrogenic compounds and cycloheximide will result in the activation or repression of direct target genesbb[11].

An indirect target gene of c-Myc is one whose expression is altered as a consequence of expression of the direct Myc target genes and whose expression is connected to c-Myc-dependent phenotypes such as cellular proliferation, transformation, or apoptosis. The search for target genes usually implies identification of the direct targets; however, it stands to reason that indirect targets may provide the missing links between deregulated c-Myc expression and neoplastic transformation or apoptosis.

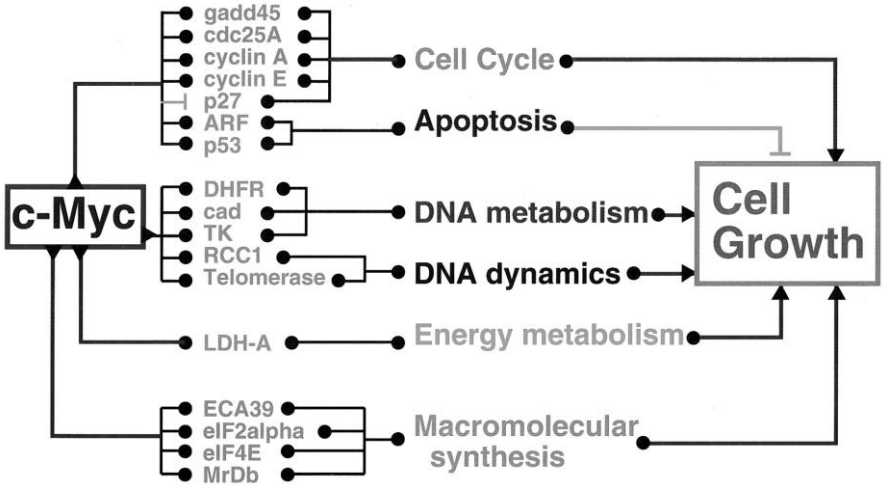

Figure 2.

Links between c-Myc, selected putative target genes, cellular functions, and cell growth. This diagram illustrates the complexity of the connections between c-Myc and its putative target genes, which are shown clustered according to their functions. The various cellular functions cooperate to promote cell growth. It should be noted that this diagram does not reflect the controversies over the authentication of the various target genes.

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RAF Proteins

The domain structure of the human RAF1 protein (648 amino acids) is depicted in Figure 2. The X-ray structure of the RAS binding domain (RBD) of RAF1 in complex with the G domain of HRAS, a member of the RAS protein family of small GTPases [31].

RAF proteins are cytoplasmic serine/threonine-specific protein kinases that share three conserved regions (CR). CR1 is located in the N-terminal auto-inhibitory domain of RAF and comprises the RBD and the cysteine-rich domain (CRD), which are both involved in a GTP-dependent interaction with RAS. The central serine/threonine-rich CR2 is essential for phosphorylation-dependent regulation of RAF, and the C-terminal CR3 encompasses the catalytic kinase domain [22]. Notably, the transduced v-*mil* and v-*raf* alleles in MH2 und 3611-MSV contain only the 3' segment of the coding domains of c-*mil* and c-*raf*, respectively, and hence the Gag-Mil and Gag-Raf hybrid proteins lack the auto-inhibitory N-terminal domain.

RAF proteins are essential effectors within the mitogen activated protein kinase (MAPK) pathway and phosphorylate MEK [12] which then activates ERK signaling (Lavoie and Therrien 2015; Desideri et al. 2015). They are activated and relieved from intramolecular auto-inhibition by (i) interaction with GTP-loaded RAS proteins [19], (ii) recruitment to the plasma membrane, and (iii) formation of allosterically regulated homo- and heterodimers [28].

These activating interactions are regulated by several phosphorylation and dephosphorylation events at crucial residues. Phosphorylation of Ser259 in CR2 by PKA generates a binding site for 14-3-3 scaffold proteins and this interferes with RAS binding and membrane recruitment. For RAF activation, Ser259 has to be dephosphorylated by protein phosphatase 2A [30]. In contrast, several other phosphorylation sites have positive effects on RAF activation. Phosphorylation of the C terminal 14-3-3 binding site Ser621 facilitates RAF dimerization [24]. Other positive phosphorylation events involve Ser338 and Tyr341 in the N-region (*Negative* charge required for RAF activation) adjacent to CR3, and Thr491 and Ser494 within the activation segment of the kinase domain [22].

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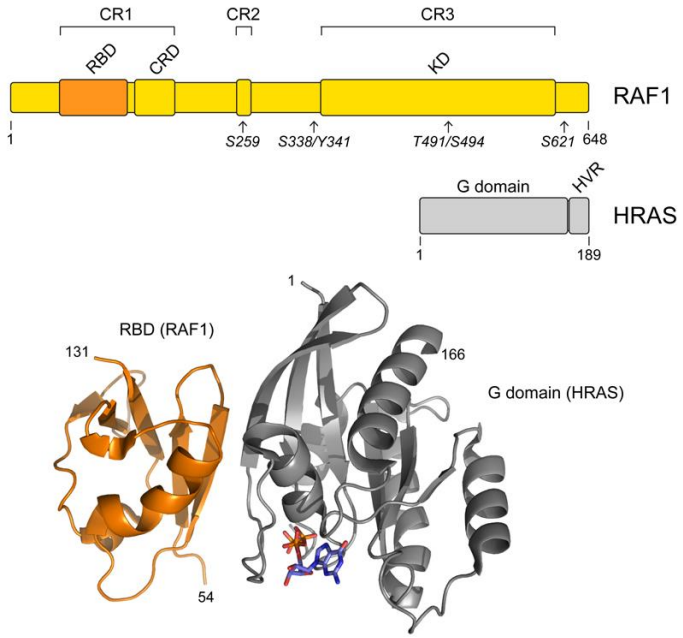

Figure 3.

Structure of the human RAF1 and HRAS proteins. The conserved regions (CR1-3), the RAS binding domain (RBD), the cysteine-rich domain (CRD), and the kinase domain (KD) of RAF1 are indicated. Critical phosphorylation sites in CR2 (Ser259), in the C-terminal region (Ser621), in the N-region upstream of CR3 (Ser338, Tyr341), and in the activation segment (Thr491, Ser494) are marked. On the HRAS protein, the G domain and the hypervariable region (HVR) are depicted.

Transcription: upstream and downstream of Myc

The MYC proto-oncogene itself is under tight transcriptional control as are the mRNA and Myc protein. In fact, MYC is not only regulated by a whole host of transcription factors, such as CNBP, FBP, and TCF that is downstream of the Wnt pathway, but it is also regulated by non-B DNA structures including single-stranded bubbles, G-quadruplexes and Z-DNA [30]. The FUSE (Far UpStream Element), melts when bound by FBP (FUSE binding protein), which relieves torsional stresses on DNA from ongoing transcription of MYC [22]. TCF is a transcription factor that plays a role in deregulated MYC expression downstream of the WNT pathway, such as with the loss of the tumor suppressor APC that results in constitutive nuclear localization of the TCF co-factor β-catenin.

Genome wide association studies further identified common polymorphisms nearby MYC that are associated with multiple cancers [10]. Such SNPs lie in enhancers that involve TCF binding and DNA looping, which connects the enhancer to the MYC proximal promoter [32]. Recently, the BET domain containing transcriptional regulator, BRD4, was shown to bind to the MYC promoter region and play a critical role in MYC expression in human cancer cells such that a drug-like BET domain chemical inhibitor could inhibit in vivo tumorigenesis [30].

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The MYC mRNA, which is short-lived, is affected by microRNAs (let-7, miR-34, and miR-145) resulting in translational modulation [33].

The Myc protein itself is post-translationally modified, ubiquitinated and degraded, with a half-life in the order of 15–20 minutes [34]. Myc transcriptional activity is regulated by phosphorylation at Ser-62 followed by Thr-58, and subsequent proteasomal degradation after performing its function [33]. Mutations of Myc residues Thr-58 and Ser-62, prevalently found in Burkitt lymphoma, are associated with stabilized mutant proteins that could perturbed transgenic mammary tumorigenesis [21].

The resulting sustained levels of Myc contributes to tumorigenesis, which in some instances may not require total elevated average levels of Myc but rather depend on deregulated expression of Myc throughout the cell cycle. How then does Myc transcriptional activity contribute to tumorigenesis?

The canonical Myc E-box 5′-CACGTG-3′ is among the most frequently occurring DNA binding motifs in the human genome [35]. This motif, however, could be bound by different transcription factors such as ChREBP, SREBP, HIF-1, NRF1, USF, TFE3, Clock, and Bmal (Figure 4). It stands to reason that in non-proliferating cells, non-Myc E-box transcription factors regulate basal metabolism to maintain cellular structural and functional integrity. When cells are stimulated to proliferate, Myc levels rise, permitting it to occupy E-box driven genes normally bound by other transcription factors and activate a program of biomass accumulation and enhanced cellular bioenergetics. As such, which of the many E-boxes are occupied by Myc in proliferating cells and does occupancy trigger changes in transcription and mRNA levels of the target genes?

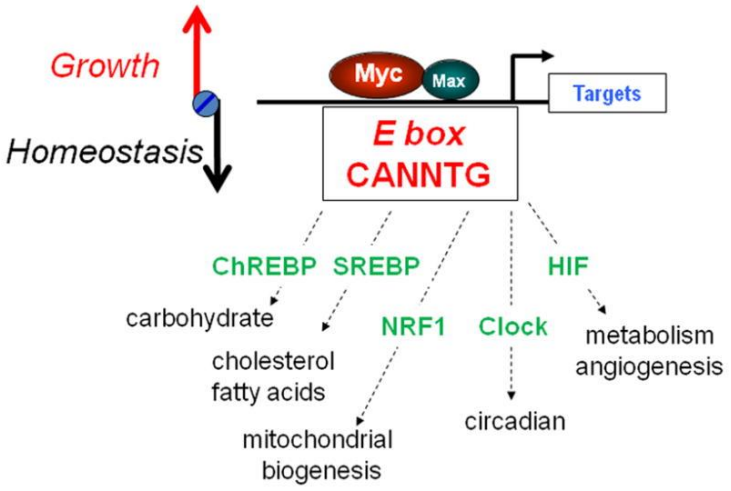

Figure 4

Myc-Max is shown bound to E-box driven genes, which could also be regulated by other E-box transcription factors, such as the carbohydrate response element binding protein (ChREBP), sterol response element binding protein (SREBP), nuclear respiratory factor 1 (NRF1), circadian transcription factor Clock (and Bmal), and hypoxia inducible factor (HIF).

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Conclusion

The c-Myc molecule has continued to emerge as a centerpiece and key to the many secrets of cancer biology. Recent studies suggest that c-Myc is able to activate the cell cycle machinery and its safeguards. Intriguingly, its ability to activate glycolysis suggests that in addition to triggering the cell cycle, c-Myc also sustains the fuel necessary to run the cell cycle machinery. Indeed, its ability to enhance the activities of specific enzymes involved in DNA metabolism and other metabolic pathways further suggests that it is a key molecular integrator of cell cycle machinery and cellular metabolism.

The future of the study of c-Myc target genes lies in the use of arrayed gene expression analysis to determine the common and divergent patterns of c-Myc target gene expression in a variety of physiological and neoplastic conditions. The benefits from such advances in technology, however, will require the expertise of biologists who are able to tease out the roles of the target genes in producing the multitude of c-Myc-mediated phenotypes.

The greatest challenge, however, is the development of a discipline that is capable of dynamically and comprehensively linking transcription factor activities to their target genes and, in turn, to cellular phenotypes.

Competing interests

The authors declare that they have no competing interests.

Ethics Statement

The study was approved by the ethics committees at the Department of Cell Biology and Biochemistry, Tech University Health Sciences Center, Texas, USA

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