MYC and Transcription Elongation

Peter B. Rahl[1] and Richard A. Young[1,2]

[1]Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142

[2]Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02142

Correspondence: young@wi.mit.edu

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Most transcription factors specify the subset of genes that will be actively transcribed in the cell by stimulating transcription initiation at these genes, but MYC has a fundamentally different role. MYC binds E-box sites in the promoters of active genes and stimulates recruitment of the elongation factor P-TEFb and thus transcription elongation. Consequently, rather than specifying the set of genes that will be transcribed in any particular cell, MYC's predominant role is to increase the production of transcripts from active genes. This increase in the transcriptional output of the cell's existing gene expression program, called transcriptional amplification, has a profound effect on proliferation and other behaviors of a broad range of cells. Transcriptional amplification may reduce rate-limiting constraints for tumor cell proliferation and explain MYC's broad oncogenic activity among diverse tissues.

TRANSCRIPTIONAL REGULATION

Transcription factors bind specific DNA sequences and regulate the recruitment and activity of the transcription apparatus at genes (Ptashne and Gann 1997; Lee and Young 2013). The process of transcription consists of at least three steps: initiation, elongation, and termination (Fuda et al. 2009; Malik and Roeder 2010; Zhou et al. 2012). During initiation, the transcription apparatus, which consists of RNA polymerase II (Pol II) and various cofactors, is recruited to genes by transcription factors. A short transcript is produced by Pol II and pause factors typically induce pausing 20–50 bp downstream of the transcriptional start site. Elongation proceeds after the elongation factor P-TEFb, which consists of Cdk9 and cyclin T, is recruited, and phosphorylates the pause factors and Pol II. Transcription termination is stimulated by recognition of polyadenylation site sequences by factors associated with Pol II during elongation.

It has long been clear that specific transcription factors are responsible for recruiting Pol II to selected genes during transcription initiation, but evidence emerged in the last decade that argues for an additional level of control at the pause-release and/or elongation stage of transcription for a large number of genes (Fuda et al. 2009; Nechaev and Adelman 2011; Zhou et al. 2012; Conaway and Conaway 2013). For example, in various human cells, Pol II was found to occupy the promoters of the majority (~70%) of protein-coding genes, but full-length transcripts were detected at only a subset of these genes (Guenther et al. 2007). Similarly, a large fraction of *Drosophila* genes with roles in devel-

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opment were found to show evidence of transcription initiation, but not elongation (Muse et al. 2007; Zeitlinger et al. 2007). These results indicated that Pol II pausing occurs at many genes and suggested that pause control is an important step in global gene regulation.

Further investigation in mammalian cells revealed that Pol II initiates transcription bidirectionally and this divergent transcription produces short RNA species at active promoters, with full-length transcripts occurring predominantly across protein-coding genes following pause release (Core et al. 2008; Seila et al. 2008). Recent studies indicate that the RNAs produced by antisense transcription from promoters of protein-coding genes account for a large fraction of long noncoding RNA (lncRNA) species in mammalian cells (Sigova et al. 2013). Thus, Pol II molecules initiate divergent transcription at a large fraction of the genes in the genome, are subjected to pausing in both directions, and only a portion of the initiated Pol II molecules are released to produce the longer transcripts recognized as messenger RNAs (mRNAs) and lncRNAs. This further supports the idea that promoter-proximal pausing is a general feature of Pol II transcription and suggests that regulation of pause release influences both mRNA and lncRNA levels. Genome-wide studies show that the negative elongation factors NELF, DSIF, and Gdown1 co-occupy most promoters with paused Pol II, and that the positive elongation factors P-TEFb and TFIIS, generally, control pause release at actively transcribed genes (Chao and Price 2001; Core et al. 2008; Gilchrist et al. 2010; Nechaev et al. 2010; Rahl et al. 2010; Cheng et al. 2012; Jishage et al. 2012). Thus, the control of promoter-proximal pausing and transcription elongation by these and other factors is important to global gene regulation.

MYC and MAX

MYC is a master regulator of cellular proliferation. Under normal physiologic conditions it connects growth-factor stimulation to cellular proliferation and cell-cycle progression. MYC coordinates these cellular events by forming a heterodimer with MAX and binding E-box sequences (Blackwood and Eisenman 1991). The MYC basic helix-loop-helix and leucine zipper (bHLH-LZ) domains, which are located at its carboxyl terminus, are responsible for dimerization with MAX and for DNA binding. MYC has multiple transcription activation domains (TADs) in its amino terminus that recruit transcription cofactors and chromatin regulators (McMahon et al. 1998, 2000; Park et al. 2001; Knoepfler et al. 2006). MAX also contains a bHLH-LZ domain, but lacks TADs. Similarly, other MAX dimerization partners such as Mnt and Mad contain bHLH-LZ domains to facilitate dimerization with MAX, but lack TADs (Ayer et al. 1993; Hurlin et al. 1997). MYC protein levels increase following growth-factor stimulation resulting in MYC binding to increasing amounts of the constitutively expressed MAX. MAX/MAX, Mad/MAX, and Mnt/MAX dimers can also bind E-box sequences, and because these proteins lack transcriptional activation domains, these are thought to have a different transcriptional impact than MYC/MAX heterodimers. MAX/MAX and Mad/MAX binding to these sites may maintain an open chromatin structure at MYC/MAX binding sites that would allow for rapid activation of MYC-regulated genes following MYC protein induction (Ayer and Eisenman 1993; Baudino and Cleveland 2001). Consistent with this idea, MYC appears to require active chromatin modifications to bind the genome (Guccione et al. 2006; Nie et al. 2012; Soufi et al. 2012). There is evidence that RNA Pol II and other components of the transcription machinery can be loaded at promoters before MYC binding (Guccione et al. 2006; Lin et al. 2012; Nie et al. 2012), suggesting that MYC is not required to recruit the transcription apparatus to these promoters (see Sabò and Amati 2013).

MYC Regulates Transcriptional Elongation

Eberhardy and Farnham first reported that MYC regulated transcription of the human *CAD* gene through a P-TEFb-dependent regulatory mechanism (Eberhardy and Farnham 2001; Eberhardy and Farnham 2002). RNA Pol

II was found to be constitutively bound to the *CAD* promoter, whereas full-length mRNA and RNA Pol II at the 3′ end of genes was detected only in S phase coincident with MYC occupancy. Furthermore, the E-box sites at the *CAD* promoter were dispensable for RNA Pol II recruitment. Thus, for the *CAD* gene, MYC binding was apparently required for transcription elongation, but not for RNA Pol II initiation.

MYC has been shown to interact with P-TEFb subunits CycT1 and CDK9 in vitro and in vivo via Myc's TAD (Eberhardy and Farnham 2002; Kanazawa et al. 2003; Gargano et al. 2007; Rahl et al. 2010). The MYC and CycT1 interaction requires MYC Box I and MYC Box II in the TAD—the ability of these MYC domains to activate expression of a Gal4 transactivation assay correlated with their CycT1 binding (Eberhardy and Farnham 2002). Cyclin T1 interacts with MYC through its cyclin boxes, which is similar to this cyclin's binding mode to the acidic activation domains of other transcription factors such as CIITA and RelA (Kanazawa et al. 2000; Barboric et al. 2001). The MYC, CycT1, and Cdk9 complex isolated by Peterlin and colleagues can phosphorylate the RNA Pol II carboxy-terminal domain in vitro (Kanazawa et al. 2003). Furthermore, direct recruitment of P-TEFb can substitute for Myc binding in *CAD* transcriptional activation (Eberhardy and Farnham 2002).

Studies of the control of the *CCND2* gene also suggested a role for MYC in transcriptional steps subsequent to initiation. Eilers and colleagues found that MYC and FoxO regulate distinct steps in the transcription cycle at the *CCND2* gene (Bouchard et al. 2004). Here the PI3K pathway, by regulating FoxO function, is responsible for preinitiation complex formation. Pol II and other components of the transcription initiation apparatus were found to be loaded at *CCND2* in the absence of MYC activity.

Rahl et al. (2010) described multiple lines of evidence that MYC's dominant transcriptional role at most genes in embryonic stem cells is to regulate transcriptional pause release. For example, they found that reducing the levels of MYC caused a reduction in the levels of elongating Pol II, but had little effect on the levels of promoter-proximal Pol II in genome-wide chromatin immunoprecipitation-sequencing (ChIP-seq) assays. This is in contrast to the effect of reducing the levels of the pluripotency transcription factor Oct4, which reduced the levels of both promoter-proximal Pol II and elongating Pol II at its target genes. As described below, further studies revealed the significance of this mode of transcriptional regulation in cancer cells, in which elevated levels of MYC cause transcriptional amplification by increasing transcriptional pause release (Lin et al. 2012).

The control of transcriptional pause release by MYC plays a key role in control of the pluripotent ground state in murine embryonic stem cells (mESCs) (Marks et al. 2012). mESCs can be grown in two different conditions, referred to here as 2i and serum conditions, which produce two distinguishable cell states. When grown under 2i conditions, mESCs express low levels of c-Myc and show relatively low RNA Pol II pause release across the genome (a high ratio of initiating vs. elongating RNA Pol II). When grown in serum conditions, mESCs express relatively high levels of c-Myc and show higher levels of pause release across the genome. Thus, MYC's role in regulating transcriptional pause release appears to be key to the control of embryonic stem cell pluripotency.

GENERAL TRANSCRIPTION ELONGATION CONTROL FACTORS

The promoters of many genes in mammalian cells can be found occupied by Pol II together with the negative elongation factors DSIF, NELF, and Gdown1 at positions located approximately 30–50 bp downstream of the transcription start site (Fig. 1), also known as promoter-proximal pause sites (Fuda et al. 2009; Nechaev and Adelman 2011; Zhou et al. 2012). DSIF, which consists of Spt4 and Spt5 subunits, was isolated as a factor that is essential for 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole-induced transcriptional inhibition (Wada et al. 1998). NELF is a multisubunit complex that functions with DSIF to repress transcriptional elongation through binding Pol II in the promoter-proxi-

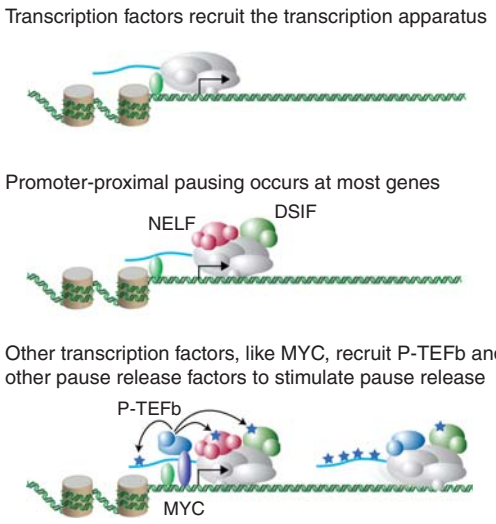

Figure 1. Key regulatory steps leading to transcriptional pause release. (*Top*) Transcription factors bind to specific DNA elements and recruit the transcription apparatus and Pol II to the transcription start site. (*Middle*) DSIF, NELF, and other pausing factors co-occupy regions near transcription start sites with Pol II. Pol II begins transcription from the initiation site, but pausing factors cause it to stall approximately 50 bp downstream from the start site. (*Bottom*) Transcription factors, including MYC, and cofactors recruit pause-release factors such as P-TEFb, which phosphorylates the pausing factors, DSIF and NELF, and Pol II, leading to elongation. Additional pause-release factors, including TFIIS and FACT, facilitate pause release and elongation. Adapted from Rahl et al. (2010) with permission from the author.

mal region (Yamaguchi et al. 1999). Gdown1 adds an additional layer of promoter-proximal negative regulation that plays an important role in Mediator-dependent gene activation (Hu et al. 2006; Cheng et al. 2012; Jishage et al. 2012). Thus, Pol II occupies pause sites at a large population of genes together with this set of negative elongation factors, and the functions of these negative factors must be overcome to allow full-length transcript production.

Pause release and processive transcript elongation requires the recruitment of pause-release factors, which include P-TEFb, TFIIS, and FACT (Fuda et al. 2009; Nechaev and Adelman 2011; Zhou et al. 2012). P-TEFb phosphorylates multiple key substrates important for stimulating pause release including DSIF, NELF, and the carboxy-terminal heptapeptide repeat domain of Pol II. TFIIS activity counteracts backtracking and arrests (Adelman et al. 2005). The FACT complex aids transcript elongation by remodeling nucleosomes to allow for Pol II transit through the gene (Orphanides et al. 1998; Winkler and Luger 2011). It is the recruitment of these factors by various transcriptional regulators that therefore plays a key role in effecting the gene expression program of cells.

RECRUITMENT OF TRANSCRIPTION ELONGATION CONTROL FACTORS

Transcription elongation is regulated by at least three types of protein complexes: the Mediator complex, a protein associated with both Mediator and acetylated nucleosomes called BRD4, and a variety of DNA-binding transcription factors.

The Mediator coactivator complex functions as a molecular switch capable of regulating both the initiation and elongation stages of transcription. DNA-binding transcription factors bind directly to the Mediator complex, which, in turn, binds to the transcriptional machinery (Malik and Roeder 2010; Meyer et al. 2010; Knuesel and Taatjes 2011). The human Mediator complex is approximately 1.2 mDa and consists of about 30 subunits. Mediator coordinates transcription and higher-ordered chromatin structure through interactions with numerous transcription factors, cofactors, and Pol II. A domain in the MED26 subunit has been identified that can interact with either the general initiation factor TFIID or P-TEFb, and can contribute alternately to initiation and pause release (Takahashi et al. 2011). The MED23 subunit can also contribute to P-TEFb recruitment and pause release (Wang et al. 2013). This is consistent with evidence that Mediator is required for activator-dependent stimulation of RNA Pol II transcription when RNA Pol II is associated with the negative elongation factor Gdown1 in in vitro transcription assays (Hu et al. 2006). Furthermore, Mediator can be co-purified with BRD4 and co-occupies promoters genome-wide with BRD4 (Jiang et al. 1998;

Loven et al. 2013; Whyte et al. 2013), and BRD4 is involved in P-TEFb recruitment, as described in more detail below. These studies argue that Mediator plays an important role in coordinating initiation and elongation.

The BET bromodomain protein BRD4, which contains two bromodomains that interact with acetylated lysines in the nucleosomal histones of active promoter regions, binds the active form of P-TEFb and thereby stimulates pause release (Jang et al. 2005; Yang et al. 2005; Krueger et al. 2010). BRD4 has been shown to interact with the transcription factors MYC/MAX, c-Jun, AP2, YY1, p53, C/EBPα, and C/EBPβ, suggesting that all these factors may influence elongation (Wu et al. 2013). Interestingly, Brd4 interacts with MYC/MAX heterodimers, but not MAX or MXD/MAX complexes, suggesting that a structural feature present on MYC but not on the structurally similar MAX or Mad accounts for the interaction with BRD4 (Wu et al. 2013). BRD4 thus serves as an adaptor protein to link active P-TEFb complex to transcriptional activators and chromatin to coordinate pause release.

Several families of sequence-specific transcription factors can recruit pause-release factors and may function through postinitiation mechanisms. Basic helix-loop-helix transcription factors including MYC, nuclear hormone receptors such as ERα, and cytokine-responsive factors including NFκB and CIITA have all been shown to recruit P-TEFb to control postinitiation regulation at regulated genes (Peterlin and Price 2006). Aire induces expression of peripheral tissue antigens in thymic epithelial cells via pause release in which *Aire* deficiency has been shown to have little effect on initiation, but results in a block in elongation (Oven et al. 2007; Giraud et al. 2012). The transcription factor p53 can regulate transcription through postinitiation mechanisms by modulating Mediator structure and function (Donner et al. 2010).

MYC Oncogenic Activity Alters Cellular Gene Expression Programs

MYC is one of the most potent oncogenes and possesses broad oncogenic activity in a wide range of human cancers. MYC's primary mode of deregulation in cancer is through altered levels of MYC protein, resulting in deregulated MYC activity. A broad spectrum of cellular roles has been attributed to MYC in cancer, including regulation of cell cycle, cell proliferation, response to growth factors, ribosome biogenesis, protein synthesis, cell adhesion and cytoskeleton, angiogenesis, metabolic pathways, apoptosis, DNA replication, mRNA capping, and chromatin structure (Amati et al. 1998, 2001; Facchini and Penn 1998; Nilsson and Cleveland 2003; Hurlin and Dezfouli 2004; Secombe et al. 2004; Gallant 2005; Bernard and Eilers 2006; Dang et al. 2006, 2009; Kuttler and Mai 2006; Meyer et al. 2006; Cowling and Cole 2007, 2010; Lebofsky and Walter 2007; Nieminen et al. 2007; Shchors and Evan 2007; Sutphin et al. 2007; Cole and Cowling 2008; Dai and Lu 2008; Eilers and Eisenman 2008; Hoffman and Liebermann 2008; Meyer and Penn 2008; Prochownik 2008; Herold et al. 2009; Lin et al. 2009; Ruggero 2009; Singh and Dalton 2009; Dang 2010; van Riggelen et al. 2010; Hanahan and Weinberg 2011; Peterson and Ayer 2012; Conacci-Sorrell et al. 2013). How does oncogenic MYC activity produce these broad effects?

Two models have been proposed to explain the impact of oncogenic MYC activity on the cellular gene expression program. Distinct thresholds of MYC expression are required for increasing proliferation and apoptosis in vivo (Murphy et al. 2008). In their preview of this study, Freie and Eisenman (2008) proposed two models to explain how increased MYC levels can account for these cellular effects. In the first model, MYC binds and activates a new set of genes when expressed at increased levels. In the second model, MYC binds more of the genes it occupies when expressed at lower levels, whereby increased binding results in increased expression of the same set of genes.

It has been widely assumed that MYC, when expressed at high levels, binds and activates a new set of genes. Numerous gene expression studies have identified specific sets of genes whose expression levels are altered by changes in MYC levels; these so-called MYC targets might thus explain MYC's role in cancer (Schuh-

macher et al. 2001; Schlosser et al. 2005; Dang et al. 2006; Kim et al. 2006; Ji et al. 2011). However, it is evident that these "MYC signatures" tend to vary greatly across cell types (Chandriani et al. 2009), which has made it difficult to ascribe MYC's oncogenic properties to a specific set of target genes.

Transcriptional Amplification

MYC's dominant transcriptional role in embryonic stem cells is to regulate transcriptional pause release genome-wide (Rahl et al., 2010), but it was not clear from this study how exceptionally high levels (oncogenic levels) of MYC might impact tumor cells. The MYC-inducible P493-6 B cell lymphoma cell line model and various other MYC-dependent human cancer cell lines were recently used to study this issue (Lin et al. 2012). The effect of elevated levels of MYC on its occupancy was analyzed using ChIP-seq analysis. In general, MYC occupied the core promoter of active genes together with RNA Pol II. Increasing MYC protein levels 28-fold in P493-6 cells had little effect on the total number of genes bound by MYC or the number of genes that were actively transcribed. Rather, the predominant effect on MYC occupancy was increased levels of MYC binding at the promoters of the same set of active genes. Increased levels of MYC also caused it to occupy the enhancers of actively transcribed genes. MYC occupied lower affinity E-box sequences at core promoters and enhancers when expressed at high levels (Fig. 2). Similar results were obtained with human cancer cell lines overexpressing MYC. The predominant effect of increased MYC occupancy at genes was increased transcriptional pause release. Increased MYC occupancy led to increased P-TEFb occupancy, increased levels of RNAPII Serine 2 (a modification associated with elongation), increased levels of elongating RNAPII, and increased levels of mRNA for the active gene expression program. Thus, the primary effect of elevated levels of MYC is transcriptional amplification: the production of increased levels of transcripts within the cell's gene expression program (Fig. 3).

MYC acts as a transcriptional amplifier in nonpathological settings as well. By studying MYC activity in murine lymphocyte activation and embryonic stem cells, Levens and colleagues found that MYC does not specifically activate or repress genes, rather it is a nonlinear amplifier of most actively transcribed genes (Nie et al. 2012). For example, RNA Pol II loading at promoters of resting and activated B cells is highly similar. MYC expression in activated B cells simply elevates the expression level of genes already expressed. Therefore, MYC is an amplifier under normal physiologic conditions and cancer exploits this function through deregulating its activity (see Levens 2013).

MYC overexpression consistently results in global transcriptional amplification with widespread increases in transcripts per cell following increases in MYC levels (Loven et al. 2012). Importantly, however, a prolonged increase of MYC activity can lead to repression of certain genes as secondary effects begin to occur. For example, increased expression of repressors, including miRNAs and Polycomb proteins, can lead to repression of some genes (Neri et al. 2012). Thus, the net effect of global transcriptional amplification can ultimately cause repression of certain genes.

Transcriptional amplification of the cell's gene expression program can account for Myc's diverse roles in cancer and explain why MYC plays a critical role in tumorigenesis in a wide variety of human tissues. MYC signatures vary greatly across multiple cell types (Chandriani et al. 2009). The transcriptional amplification model provides an explanation for this variation. The set of genes whose expression is altered by MYC should, in fact, be different in different cell types, as oncogenic MYC will amplify each cell's inherent gene expression program and not an MYC-specific program.

MYC's broad oncogenic activity suggests that it can reduce different rate-limiting constraints for cellular proliferation in different cells (Vita and Henriksson 2006). In this model of transcription amplification, genes rate limiting for growth should be amplified provided they are transcriptionally active before MYC elevation. For example, MYC-mediated tran-

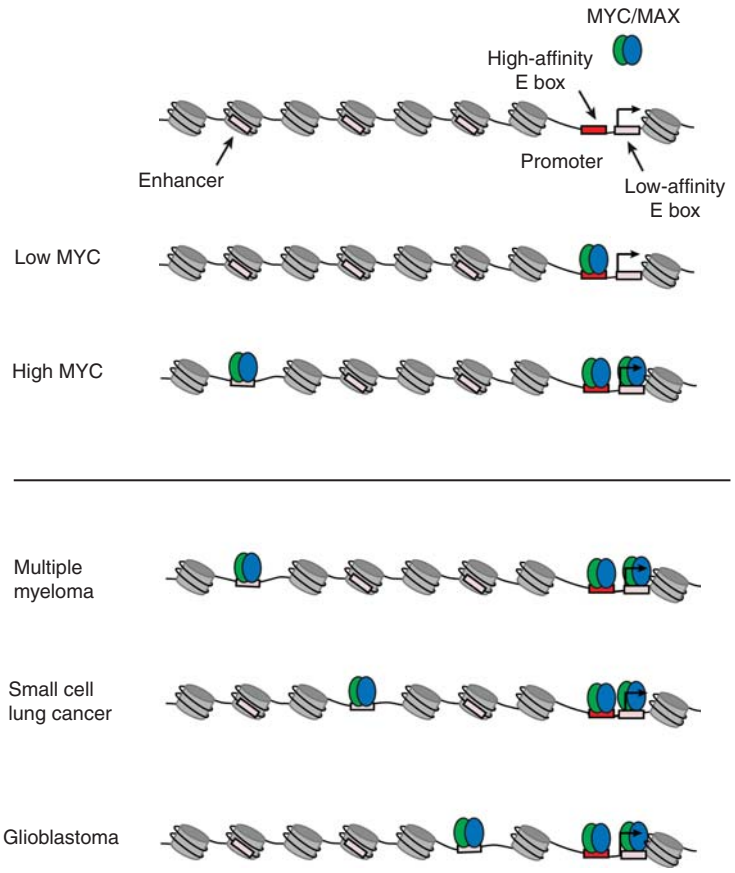

Figure 2. Elevated levels of MYC leads to altered genome-wide occupancy. (*Top*) When expressed at low levels, MYC/MAX dimers occupy high-affinity E-box sites in the genome, which are generally located near transcriptional start sites. When expressed at elevated levels, MYC/MAX dimers saturate high-affinity E-box sites and occupy lower affinity binding sites near transcription start sites and at enhancer regions. (*Bottom*) When overexpressed in different cancer types, MYC/MAX dimers bind low-affinity sites at enhancers. Because many enhancers are used in a tissue-specific manner, open chromatin regions with low-affinity binding sites can vary between cancer types, thus leading to different MYC/MAX binding profiles at enhancers in different cancer types.

scriptional amplification of ribosomal subunits could increase translational capacity (Arabi et al. 2005; Grandori et al. 2005; Grewal et al. 2005; Dai and Lu 2008). For cellular functions that are limiting for the growth of tumorigenic cells such as translational capacity and aerobic energy metabolism, an increase in this machinery would provide a mechanism to explain how elevated MYC levels contribute to tumorigenesis (Ruggero et al. 2004; Dang et al. 2009; Feng and Levine 2010; Vander Heiden et al. 2010; Hanahan and Weinberg 2011; Bayley and Devilee 2012). It is also possible that the increase in essentially all components of the gene expression program provides cells with an advantage when adapting to the multiple mutated pathways that characterize most tumor cells.

There has been substantial progress in our understanding of MYC-dependent transcriptional control across the genome and how it influences cell state. N-MYC and L-MYC are also powerful oncogenes that function as transcription factors, although less is known about their transcriptional regulatory circuitry. Future studies of N-MYC and L-MYC function should

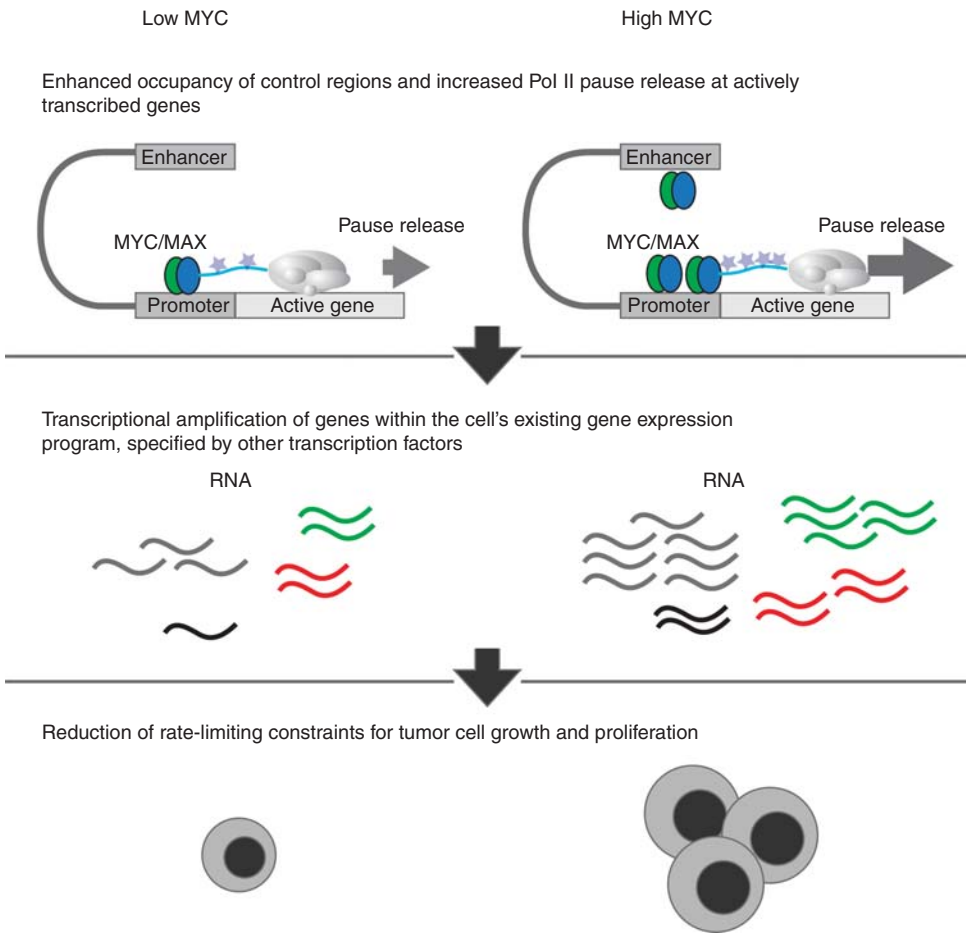

Figure 3. Elevated levels of MYC leads to transcriptional amplification. (*Top*) High levels of MYC lead to enhanced occupancy at transcriptional start sites and enhancer elements and increased Pol II pause release. (*Middle*) High levels of MYC lead to transcriptional amplification of the actively transcribed genes in the cell. The cell's existing gene expression program is specified by other transcription factors. (*Bottom*) The amplification of the gene expression program can reduce rate-limiting constraints for cell growth and proliferation. (From Lin et al. 2012; adapted, with permission, from the author.)

provide important insight into the similarities or differences among the MYC family regulatory circuitry. MYC appears to be a broader-acting oncogene with tumorigenic activity in diverse tissues, whereas N-MYC and L-MYC are often more restricted in the tissues they transform. Despite this difference, which may be largely due to tissue-specific expression, there are likely to be many mechanistic similarities among the MYC family as they are all powerful oncogenes. Such similarity is suggested by different subclasses of medulloblastoma that appear to activate MYC family transcription factors through any means necessary: MYC amplification, MYCN amplification, MYCL1 amplification, or deregulated upstream signaling pathways including Wnt (Northcott et al. 2012; Roussel and Robinson 2013).

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