Control of Vertebrate Development by MYC

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Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

The study of MYC has led to pivotal discoveries in cancer biology, induced pluripotency, and transcriptional regulation. In this review, continuing advances in our understanding of the function of MYC as a transcription factor and how its transcriptional activity controls normal vertebrate development and contributes to developmental disorders is discussed.

During embryonic development, a great diversity of cell types are rapidly produced concomitant with their organization into the different functional tissues and organ structures needed to sustain life. A common theme underlying higher-order organ development is the initial establishment of stem cells or multipotent progenitor cells at distinct spatial locations. These stem and progenitor populations then respond to local environmental cues by implementing selective activation and/or silencing of specific transcription programs that drive the generation and ordered proliferative expansion of the different cell types and lineages responsible for organ-specific tissue formation. There is intense interest in how these transcriptional programs are established and maintained, both with respect to the signaling pathways and critical transcription factors involved, because their manipulation may permit the in vivo or ex vivo generation of diverse cell types for therapeutic purposes, and because their misregulation appears to be a root cause of diverse cancers and developmental disorders. Prominent among the transcription factors involved are members of the MYC family of proteins, particularly MYC and MYCN.

MYC and *MYCN*, together with the other *MYC* gene family member, *MYCL*, encode basic-helix-loop-helix-leucine zipper (BHLHZIP) proteins that function primarily as nuclear transcription factors. However, additional activities of MYC have been identified in the control of DNA replication (Dominguez-Sola et al. 2007), and in the cytoplasm where a cleaved form of MYC that lacks the BHLHZIP region can promote differentiation (Conacci-Sorrell et al. 2010). MYC proteins are best known for their frequent involvement in a great variety of cancers and the ability of ectopic MYC to contribute to pluripotency (Cartwright et al. 2005; Takahashi and Yamanaka 2006; Wernig et al. 2007). The role of MYC family proteins in cancer and induced pluripotency is thought to stem from the appropriation of MYC activities that generally, but not universally, tend to maintain cells in a proliferative state and prevent differentiation. In the context of cancer, the activities

Editors: Chi V. Dang and Robert N. Eisenmann
Additional Perspectives on *MYC* and the Pathway to Cancer available at www.perspectivesinmedicine.org

 doi: 10.1101/cshperspect.a014332
Cite this article as *Cold Spring Harb Perspect Med* 2013;3:a014332

of MYC are essentially constitutive due to mutations in signaling pathways that regulate MYC, as well as to MYC gene amplification and translocations that prevent the normal down-regulation of MYC expression. But during embryonic development, the activities of MYC are harnessed through Myc's tightly regulated temporal and spatial expression to perform critical functions in generating functional organ systems and tissues.

TRANSCRIPTIONAL CONTROL BY MYC AND MYC–MAX COMPLEXES

MYC does not bind DNA on its own, but relies on interaction with the protein MAX through shared BHLHZIP motifs to form complexes that bind E-box (CACGTG) and closely related sequences (Blackwood and Eisenman 1991). The original thinking was that MYC–MAX complexes functioned like traditional sequence-specific DNA-binding transcription factors to activate transcription of a restricted set of target genes encoding proteins that then directly promoted cell growth and proliferation (Blackwood et al. 1992; Torres et al. 1992). Although conceptually not far off the mark, the transcriptional activities of MYC have proven to be far more complicated and multifaceted. For example, MYC–MAX heterodimers can associate with the POZ domain/zinc finger transcription factor MIZ, and MYC–MIZ complexes can repress transcription of MIZ target genes (Herkert and Eilers 2010). Additionally, the central tenet that MAX is an obligate partner for MYC's ability to activate transcription and for its biological activities is under considerable revision. Evidence for MAX-independent functions for MYC in transcriptional regulation is found in *Drosophila* mutants that lack MAX (Steiger et al. 2008; Gallant and Steiger 2009) and in the rat pheochromocytoma cell line PC12 that lacks MAX (Ribon et al. 1994; Hopewell and Ziff 1995). In both of these settings, MYC can activate transcription, but it is not clear how widespread such MAX-independent transcription might be when MAX is present in cells.

The physiological significance of MAX loss in PC12 cells was reconsidered recently when it was shown that MAX is often deleted in human pheochromocytoma and potentially in other neuronal cancers (Comino-Méndez et al. 2011; Burnichon et al. 2012). These findings, together with previous studies showing that ectopic overexpression of MAX can repress MYC-dependent transcription and suppress oncogenic transformation by MYC (Grandori et al. 2000), are consistent with the idea that MAX has important functions beyond acting to mediate MYC DNA binding and oncogenic activity. Some of the MYC-independent functions of MAX include forming heterodimeric DNA-binding complexes with several other BHLHZIP proteins related to MYC and MAX that function as transcriptional repressors (Ayer et al. 1993; Zervos et al. 1993; Hurlin et al. 1997, 1999, 2003). Additional MAX-independent functions include the potential for direct and indirect association between MYC and transcriptional cofactors already associated with chromatin and between MYC and members of the basal transcriptional machinery and with RNA polymerases as described below.

The chromatin landscape of MYC- and MYC–MAX-binding sites suggests that MYC preferentially binds to active promoters or promoters associated with a pre-engaged basal transcription machinery (Fig. 1) (Eberhardy and Farnham 2001; Frank et al. 2001; Guccione et al. 2006; Kidder et al. 2008). MYC associates with a wide assortment of histone- and chromatin-modifying proteins and protein complexes (Adhikary and Eilers 2005; Cowling and Cole 2006; Eilers and Eisenman 2008; He et al. 2013), and once bound to its target promoters, MYC-associated activities generate additional chromatin modifications that create a chromatin environment favorable for DNA and chromatin binding by additional factors that cooperate in stimulating polymerase activity (Frank et al. 2001; Martinato et al. 2008; Lin et al. 2009, 2012; Nie et al. 2012; Soufi et al. 2012). A major effect of MYC binding is attenuation of RNA polymerase II pausing through its recruitment of P-TEFb complexes and P-TEPb-dependent phosphorylation of serine 2 of the RNA polymerase II carboxy-terminal domain (CTD) (Fig. 1) (Eberhardy and Farnham 2001; Rahl

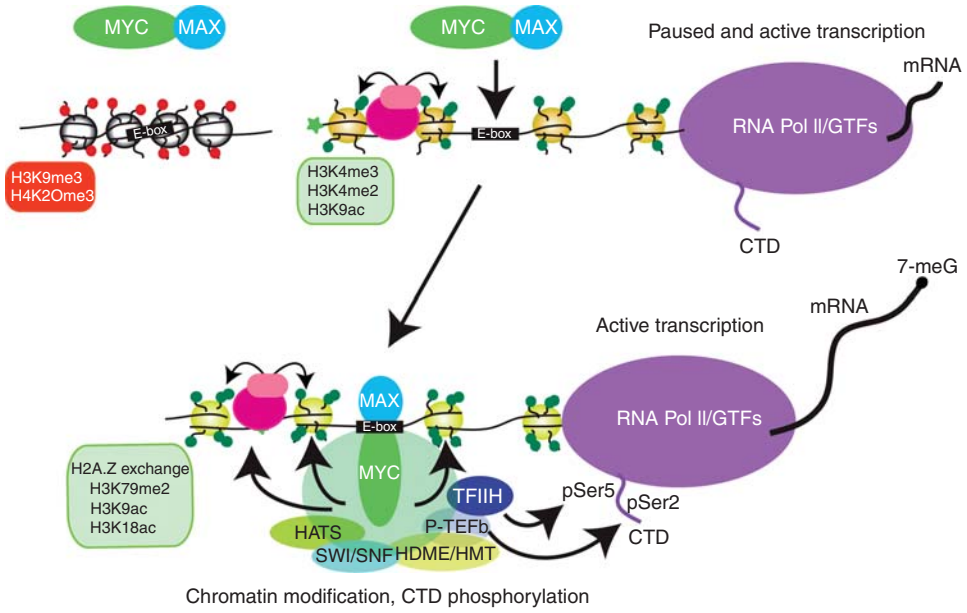

Figure 1. Regulation of RNA polymerase II by MYC. MYC associates with MAX to form DNA-binding complexes that preferentially bind E-box sites that are associated with euchromatic islands marked by specific histone modifications and bound RNA polymerase II. Such euchromatic islands may be formed through the activities of other transcription factors (pink), including "pioneer" transcription factors that can actively modify and open up the local chromatin environment. Chromatin marked by repressive histone modifications (red) and not poised for transcription is generally not accessible to MYC. MYC binding mediates further chromatin modifications that favor active transcription, including representative ones shown, through the recruitment and actions of histone-modifying proteins. These include histone acetyltransferases (HATs), histone demethylases and methyltransferases (HDME/HMT), and SWI/SNF chromatin-remodeling proteins. Although MYC binding amplifies transcription of actively transcribed genes, MYC also attenuates transcriptional pausing by interacting with P-TEFb-promoting pTEFb-dependent phosphorylation at serine 2 of the carboxy-terminal domain of RNA polymerase II. Finally, MYC interacts with the general transcription factor TFIIH to promote CTD phosphorylation at serine 5 and RNA methyltransferase-dependent 5′ methyl capping of nascent transcripts, a function that supports ribosome loading and translation.

et al. 2010; Lin et al. 2012; Nie et al. 2012). MYC also interacts with TFIIH and stimulates TFIIH-dependent phosphorylation of serine 5 of the CTD (Fig. 1) (Cowling and Cole 2007). Serine 5 phosphorylation facilitates translation of mRNAs by functioning as a signal for the recruitment of RNA methyltransferase, the enzyme responsible for 7-methyl guanine capping of nascent mRNA (Cowling and Cole 2010).

In addition to RNA polymerase II, MYC directly or indirectly interacts with and stimulates the activities of RNA polymerases I and III (Gomez-Roman et al. 2003; Arabi et al. 2005; Grandori et al. 2005; Grewal et al. 2005). Finally, the affects of MYC on transcription can be compounded through the induction of various chromatin-modifying and -binding proteins (Knoepfler et al. 2006; Lin et al. 2009). In aggregate, the various proximal promoter-specific functions of MYC that have been defined describe a unique transcription factor, capable of broadly impacting transcription, translation, and the epigenetic state of cells.

THE EVOLVING DEFINITION OF A MYC TARGET GENE

During the last decade, the notion that MYC and MYC–MAX complexes regulate a restricted and standard set of target genes that account for

its various activities in cell growth, proliferation, apoptosis, and oncogenesis has been largely dispelled. Instead, genome-wide DNA-binding studies reveal that MYC and MAX are highly enriched at thousands of overlapping genomic sites (Fernandez et al. 2003; Li et al. 2003a,b; Orian et al. 2003; Cawley et al. 2004; Lin et al. 2009; Ji et al. 2011). MYC–MAX-binding sites are typically enriched for E-box sequences and most frequently located in the proximal promoters of genes (Fernandez et al. 2003; Zeller et al. 2006; Lin et al. 2012; Nie et al. 2012). Given these findings and the ability of MYC to engage and stimulate RNA polymerases I, II, and III, it is not surprising that MYC induces broad changes in gene expression in the wide variety of cellular settings that have been investigated (Patel et al. 2004; Eilers and Eisenman 2008). However, it remains unresolved whether MYC has a dedicated set of target genes that do not vary from cell type to cell type, or whether it functions as a general transcription factor with no universal targets.

A recent study by Ji et al. (2011) identified a set of approximately 50 MYC target genes that were common to several cancer cell lines and mouse and human embryonic stem cells. Expression of these genes was found to correlate with MYC levels in more than 300 other cell and tissue types (Ji et al. 2011). This core set of target genes highlights a role for MYC in RNA processing, ribosome biogenesis, and macromolecular synthesis (Ji et al. 2011), processes previously identified as controlled by MYC (Eilers and Eisenman 2008; Dang 2012). However, other attempts to identify a core signature of MYC target genes in different cell types have failed (Chandriani et al. 2009; Lee et al. 2012). Indeed, the study by Lee et al. (2012) found genomic MYC binding was highly cell-type specific.

A potential explanation for the difficulty in identifying a core MYC signature comes from two recent studies examining genome-wide binding by MYC and MAX and its relationship to global gene transcription (Lin et al. 2012; Nie et al. 2012). These studies found that MYC, in a largely uniform and dose-dependent manner, increased or "amplified" transcription of genes already undergoing active transcription (Lin et al. 2012; Nie et al. 2012). Moreover, they found that MYC's amplifier effect was independent of cell type—that is, MYC did not alter the unique diversity of gene transcription found in different, albeit very limited, cell types tested (Lin et al. 2012; Nie et al. 2012). The transcriptional amplification phenomenon was closely associated with MYC-enhanced P-TEFb-dependent CTD serine 2 phosphorylation and pause release (Fig. 1) (Lin et al. 2012; Nie et al. 2012). These findings are in general agreement with the finding that MYC broadly targets both active and transcriptionally poised chromatin (Guccione et al. 2006), and that MYC and RNA polymerase II are often found at the same promoters (Martinato et al. 2008; Lee et al. 2012). Perhaps most importantly, these findings suggest that many, if not all, of the "MYC target genes" identified over the years, including various protein coding genes and nonprotein coding genes (Eilers and Eisenman 2008; Dang 2012), may be largely conditional targets dependent on cellular context and their related epigenetic status.

Thus, although there are a number of issues to reconcile with the amplification model, including how MYC-dependent repression and differential gene activation of MYC targets fits in (Nature Medicine Commentaries 2013), and the seemingly general ability of MYC to promote growth and proliferation (Littlewood et al. 2012), it helps explain the longstanding conundrum of how MYC can elicit such a wide variety of cellular behaviors depending on extracellular inputs and cell types. As discussed further below, this model may also provide considerable insight into how MYC controls embryonic development and disease.

DOSE-DEPENDENT CONTROL OF EMBRYONIC DEVELOPMENT BY MYCN AND MYC

Homozygous null MYCN mouse embryos die at approximately E11.5, with different organ systems showing severe hypoplasia, including the heart, lungs, kidneys, gut, skeleton, central nervous system, and genitourinary system (Charron et al. 1992; Stanton et al. 1992; Sawai et al. 1993). Studies of MYCN heterozygotes

and mice engineered to express hypomorphic MYCN alleles (Moens et al. 1992, 1993) show clear dose-dependent effects of MYCN on the development of different organs. For example, mice homozygous for a MYCN hypomorphic allele expressing ~20%–25% MYCN survived to birth, but showed pronounced lung hypoplasia and associated defects in branching morphogenesis that led to early postnatal lethality (Moens et al. 1992; Nagy et al. 1998). And mice engineered to express ~15% MYCN showed severe cardiac muscle hypoplasia and lethality between E12.5 and E14.5 (Moens et al. 1993). The essential roles of MYCN in lung and heart development (Okubo et al. 2005; Harmelink and Jiao 2010; Harmelink et al. 2013), as well as in the development of the central nervous system (CNS) (Knoepfler et al. 2002), limbs (Ota et al. 2007), kidneys (Nakhai et al. 2008), and inner ear (Domínguez-Frutos et al. 2011) were confirmed and/or further defined by conditional MYCN deletion at these sites (see below for further discussion).

These findings from mouse studies have significance to human disease as MYCN haploinsufficiency causes Feingold syndrome (van Bokhoven et al. 2005; Cognet et al. 2011). The constellation of organs and tissues affected in Feingold syndrome (Celli et al. 2003) is largely recapitulated by MYCN deficiency in the mouse (Charron et al. 1992; Stanton et al. 1992; Sawai et al. 1993). Defects in intellectual development, deafness, and esophageal and gastrointestinal atresia are additional phenotypes associated with Feingold syndrome (Celli et al. 2003) that were not described in the mouse studies. The atresia malformations can lead to death without surgical intervention (de Jong et al. 2010). In addition to MYCN haploinsufficiency, haploinsufficiency of the microRNA miR-17 ~ 92 polycistronic cluster causes a human disorder related to Feingold syndrome (de Pontual et al. 2011). Although MYCN can up-regulate miR-17 ~ 92 transcription and miR-17 ~ 19 deficiency phenocopies skeletal phenotypes in Feingold syndrome (de Pontual et al. 2011), it remains to be determined whether miR-17 ~ 92 is broadly controlled by MYCN during development.

Studies of heterozygous and homozygous null MYC embryos together with embryos containing hypomorphic MYC alleles show dose-dependent MYC activities in controlling proliferation, growth, and cellularity of multiple tissues and organs (Davis et al. 1993; Trumpp et al. 2001). MYC null embryos die at approximately E10.5, with lethality associated with a number of abnormalities including defects in heart development and neural tube closure (Davis et al. 1993; Trumpp et al. 2001). However, studies in which MYC was deleted either in the epiblast, such that the extraembryonic placental tissues were spared, or conditionally in all hematopoietic lineages, showed that MYC was dispensable for the development of most organs and tissues up to E11.5 and that lethality at this time was due to a failure in definitive hematopoiesis taking place in the extraembryonic tissues (Dubois et al. 2008; He et al. 2008; reviewed in Laurenti et al. 2009; Delgado and León 2010). The importance of MYC in extraembryonic definitive hematopoiesis corresponds to its strong expression in extraembryonic tissues (Downs et al. 1989).

In contrast to the essential activities of MYC and MYCN, MYCL-deficient mice lacked a discernible phenotype (Hatton et al. 1996). This is most likely explained by redundant expression between family members and evidence that MYC protein functions are largely although not completely redundant (Nesbit et al. 1999; Malynn et al. 2000). The development of conditional MYCL knockout mice (Kopecky et al. 2012; R Eisenman, pers. comm.) will allow the redundancy model to be rigorously examined.

Homozygous deletion of MAX causes lethality early after postimplantation (Shen-Li et al. 2000). The deterioration of MAX-deficient embryos appears to coincide with the exhaustion of maternally supplied stores of MAX (Shen-Li et al. 2000). Mice heterozygous for MAX had no apparent phenotype, raising the possibility that MAX is not limiting for the embryonic functions of MYC and MYCN. Although early embryonic lethality caused by loss of MAX supports the concept that MYC functions are dependent on MAX, given the emerg-

ing evidence of separate and independent functions of MYC and MAX as discussed above, it will be important to further evaluate MAX function using conditional, site-, and temporal-specific deletion systems.

COORDINATED ACTIONS OF MYCN AND MYC IN LINEAGE DEVELOPMENT AND PROLIFERATIVE EXPANSION

Epiblast deletion of MYC clearly shows that it is not required for proliferation and development of most tissues in the early embryo (up until E11.5) (Dubois et al. 2008). However, this might be expected because MYC RNA appears to be expressed at very low levels in tissues of early mouse, chick, xenopus, and human embryos during the time between exhaustion of maternal supplies of MYC RNA and commencement of organogenesis (Pfeifer-Ohlsson et al. 1985; King et al. 1986; Downs et al. 1989; Jaffredo et al. 1989; Stanton et al. 1992). In contrast to MYC, MYCN is not expressed or present only at low levels in extraembryonic tissues and not required for hematopoiesis (Laurenti et al. 2008), but is widely expressed in the embryo proper at developmental stages immediately before organogenesis (late gastrulation) and during early organogenesis (Mugrauer et al. 1988; Downs et al. 1989; Schmid et al. 1989; Hirvonen et al. 1990; Kato et al. 1991; Stanton et al. 1992; Hurlin et al. 1997). After organogenesis commences, MYCN is down-regulated in most if not all tissues, whereas MYC typically increases (Slamon and Cline 1984; Zimmerman et al. 1986; Mugrauer et al. 1988; Kato et al. 1991; Stanton et al. 1992). These data, together with the widespread failure in organogenesis that occurs in the absence of MYCN (Charron et al. 1992; Stanton et al. 1992; Sawai et al. 1993), are consistent with the notion that MYCN, perhaps with contributions from MYCL in some settings, is instrumental in the initial establishment and expansion of stem and progenitor populations at various strategic positions within developing organ systems (Fig. 2A).

As organogenesis proceeds, MYCN levels subside and low levels of MYC appear to support stem and progenitor cell maintenance in a number of different settings, including the skin epidermis (Gandarillas and Watt 1997; Waikel et al. 2001), the intestinal epithelium (Bettess et al. 2005; Muncan et al. 2006), kidney (Couillard and Trudel 2009), pancreas (Nakhai et al. 2008; Bonal et al. 2009), lung (Okubo et al. 2005; Dong et al. 2011), and mammary gland (Stoelzle et al. 2009; Moumen et al. 2012). In these organ systems, MYC induction, which is often controlled by WNT/β-catenin signaling (Waikel et al. 2001; Hu and Rosenblum 2005; Shu et al. 2005; Nakhai et al. 2008), is then associated with the mobilization of stem cells and the proliferative expansion of specific lineages or differentiated cell types, including keratinocytes committed to terminal differentiation (Fig. 2A) (Gandarillas and Watt 1997; Gandarillas et al. 2000; Waikel et al. 2001; Zanet et al. 2005). Additionally, although MYCN does not play an essential role in establishing the hematopoietic system, it is expressed in hematopoietic stem cells and cooperates with MYC in hematopoietic stem cell (HSC) proliferation and self-renewal (Laurenti et al. 2008). Similar to its effects in skin keratinocytes, ectopic MYC in HSCs stimulates their exit from the stem cell niche and promotes the proliferative expansion of differentiated cell types at the expense of self-renewal (Wilson et al. 2004). In contrast to skin epithelial stem cells, the absence of MYC in HSCs leads to their accumulation at the expense of differentiation (Wilson et al. 2004). The latter phenomenon is attributed to defects in maintenance of the HSC niche (Wilson et al. 2004; Laurenti et al. 2008).

In the developing intestine at mid-gestation, MYCN is required for formation of the initial epithelial component of the intestinal tract (Stanton et al. 1992). Subsequent MYCN expression and activities in stem cells of the crypts of *Lieberkühn* contribute to homeostasis of the organ (Bettess et al. 2005; Muncan et al. 2006). Here, it is interesting to note that MYCN expression is retained in a single cell at the base of the intestinal crypt that lacks MYC and may be the intestinal stem cell (Bettess et al. 2005). Additionally, MYCN protein was found to be abundant in postmitotic villi (Bettess et al. 2005), suggesting potential proliferation-in-

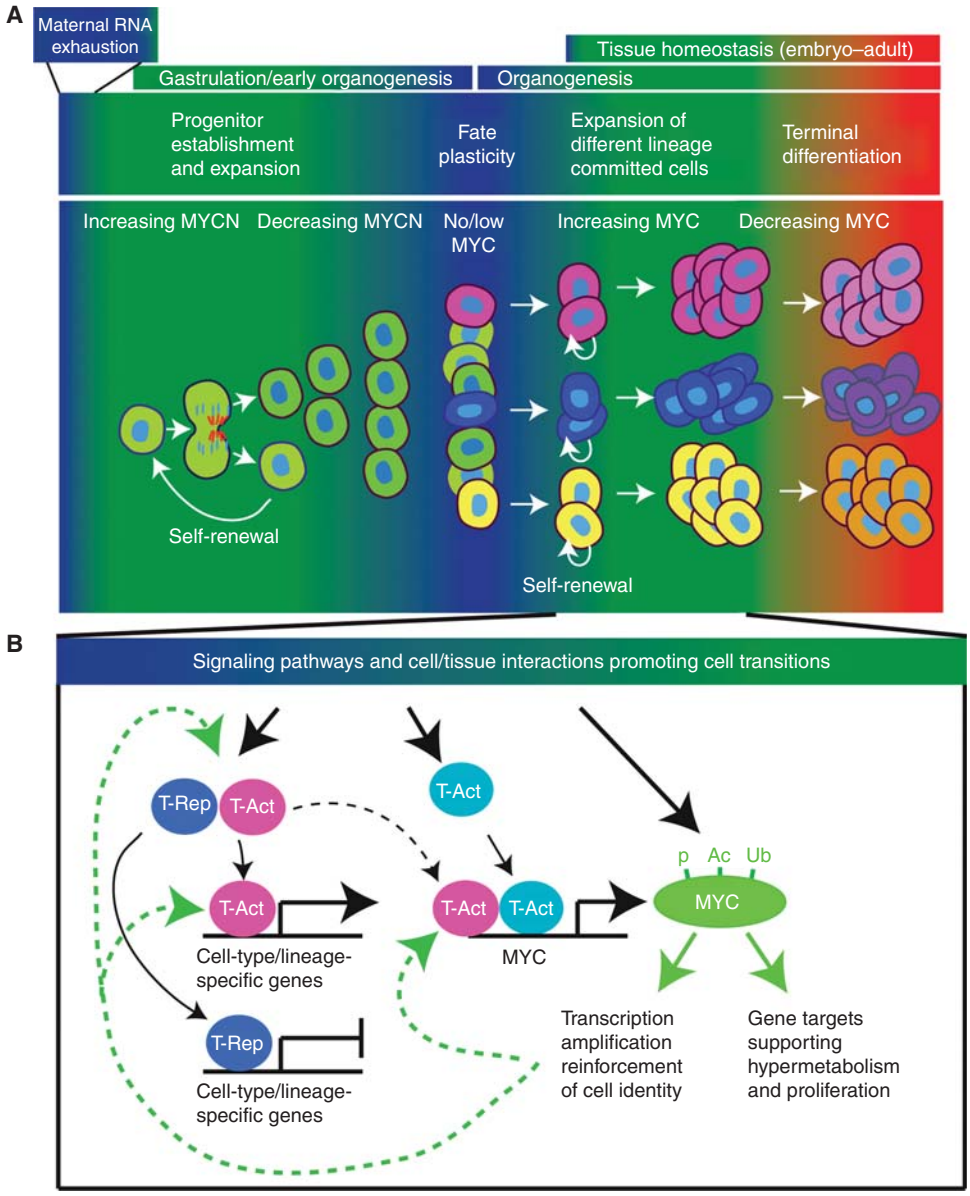

Figure 2. Speculative model for MYC-dependent regulation of cell plasticity and cell fate transitions during embryonic development. (*A*) Low levels of MYC expression and activity are proposed to contribute to increased cell plasticity in the early gastrulating embryo and again during early organogenesis. Increasing MYCN during gastrulation and early organogenesis contributes to the establishment and expansion of stem and progenitor populations at discreet locations within emerging organ systems. Following proliferative expansion, declining MYCN levels contribute to increased plasticity of these progenitor populations. Signaling that promotes the formation of new fate-restricted progenitors and stem cells also induces low levels of MYC, which functions to maintain their identity and self-renewal. Strong induction of MYC during commitment to specific lineages or to differentiation states within a given lineage couples reinforcement of cell identity with robust proliferative expansion. Subsequent MYC down-regulation then contributes to terminal differentiation. (*B*) Signaling that drives the development of cell identity and lineage transitions couples induction of specific transcription activators (T-Act) and repressors (T-Rep) with induction of MYC. The cell- or lineage-specific factors drive transcriptional and epigenetic reprogramming events that confer a unique cell identity (i.e., mesenchymal progenitor) or committed lineage (i.e., B cell). Coordinated MYC induction would act to stimulate and amplify transcription at poised and actively transcripted genes. MYC targets in this context would include the pioneer factors responsible for initiating fate transitions, as well as their transcriptional targets that ultimately define the functional identity of a cell or committed lineage. MYC induction may also couple fate transitions with proliferation by targeting a core set of genes involved in macromolecule biosynthesis and metabolism either undergoing active transcription or that exist in a transcriptionally poised state and therefore subject to regulation by MYC.

dependent activities. Based on these findings, conditional MYCN deletion at these specific locations should be informative in defining how MYCN and MYC coordinate the development and maintain homeostasis of the intestine.

The coordinated actions of MYCN and MYC are particularly apparent in the development of the skeleton and connective tissues of the developing limb. There, undifferentiated mesenchymal progenitors of the emergent limb bud are dependent on MYCN for their proliferative expansion (Sawai et al. 1993; Ota et al. 2007). As the undifferentiated mesenchyme expands, the most centrally located cells, ones furthest from proproliferative WNT and FGF signaling emanating from the surface ectoderm, down-regulate MYCN, condense, and exit the cell cycle (Ten Berge et al. 2008). Chondrogenic progenitors that express little or no MYC and show minimal proliferation emerge from the condensing mesenchyme (Ota et al. 2007; Zhou et al. 2011). Subsequent induction of MYC, which may also be under the control of WNT/β-catenin signaling (Shung et al. 2012), contributes to the proliferative expansion of chondrocytes within the emerging cartilaginous growth plate of the long bones that drives bone growth (Zhou et al. 2011). Osteoblasts and other connective tissue lineages are also produced from the condensing mesenchyme or immediately adjacent cells in the central limb bud, and may also be dependent on the sequential actions of MYCN and MYC (Ota et al. 2007; Zhou et al. 2011). These findings are consistent with the idea that the severe down-regulation or absence of MYC expression and associated cell-cycle exit, and declining RNA polymerase II activity in the prechondrogenic condensation may contribute to or be necessary for epigenetic reprogramming to the chondrogenic lineage and other lineages (Fig. 2) (Zhou et al. 2011).

AMPLIFYING CELL FATE DURING DEVELOPMENT

As illustrated in the above examples, once stem and progenitor cell compartments are established in organ systems and their homeostatic tissue compartments, the basal level of MYC or MYCN and the timing of MYC induction is essential for maintaining the proper balance between stem cell renewal and the production and proliferative expansion of differentiated cell types (Fig. 2). In several developmental settings in which MYCN, MYC, or both MYCN and MYC are deleted, both reduced proliferation and the emergence of differentiated cell types is observed (Knoepfler et al. 2002; Okubo et al. 2005; Nakhai et al. 2008; Couillard and Trudel 2009; Harmelink and Jiao 2010; Kuwahara et al. 2010). Moreover, forced MYC expression has been shown to both promote proliferation and prevent differentiation in a variety of cell types (Eilers and Eisenman 2008). These findings generally support the idea that MYC may function during development to reinforce transcriptional programs that confer functional cell or lineage identities, including stem and progenitor identities. However, it is notable that forced MYC expression is compatible with differentiation within different lineages, including the B-cell (Habib et al. 2007) and T-cell lineages (Link et al. 2012), epidermal keratinocytes (Gandarillas and Watt 1997; Waikel et al. 2001), and more generally in the hematopoietic system (Wilson et al. 2004). Thus, forced MYC expression, or normal regulated induction of endogenous MYC within a specific lineage, does not inherently impede the activity of factors that drive differentiation events, and may even stimulate latent, active, or newly directed differentiation programs.

The finding that MYC has the general effect of amplifying active and stimulating poised transcription in cells (Lin et al. 2012; Nie et al. 2012) provides a new paradigm for viewing how MYC activity might influence cellular plasticity and fate determination. According to the amplification model, the absence of MYC, or MYC below some threshold level, is predicted to increase cell plasticity by restraining or preventing the transcription of factors responsible for installing specific identity or fates (Fig. 2). For the same reason, cells with low MYC might be more responsive to epigenetic reprogramming by factors that drive transitions in cell identi-

ty and/or lineage. Many signaling pathways known to strongly impact cell identity and fate during embryonic development, such as the WNT, RAS/MAPK, NOTCH, HEDGEHOG, and NF-κB, include dedicated mechanisms for transcriptional and posttranscriptional MYC induction (Sears 2004; Knoepfler and Kenney 2006; Sharma et al. 2007; Gerondakis and Siebenlist 2010; Clevers and Nusse 2012). The induction of MYC by such pathways may serve to drive or reinforce changes in cell identity by amplifying transcription of both the pioneer factors that initiate fate change and their transcriptional targets (Fig. 2B). In the setting of induced pluripotency, MYC transcription would act, at least in part, by reinforcing the expression and transcriptional targets of pluripotency factors such as Nanog, Sox2, and Oct4 (Lin et al. 2012; Nie et al. 2012; Soufi et al. 2012).

Although largely speculative, support for such a model is suggested by studies examining the relationship between MYC and global gene expression in different cell types where MYC was found to stimulate or maintain cell-type-specific transcriptional programs rather than installing a unique, MYC-specific transcriptional program (Lawlor et al. 2006; Lin et al. 2009, 2012; Ji et al. 2011; Lee et al. 2012; Nie et al. 2012; Pello et al. 2012). Although a number of studies suggest that MYC preferentially stimulates expression of genes involved in ribosome biogenesis, protein synthesis, and metabolism (Patel et al. 2004; Eilers and Eisenman 2008), this may simply reflect classes of genes that typically exist in a transcriptionally poised or active state in most cell types, and are therefore more generally subject to MYC-dependent attenuation of pausing and transcriptional amplification (Fig. 2). The existence of such a class of genes involved in promoting metabolism and macromolecule synthesis might also help explain the competitive advantage that MYC expression confers to cells (de la Cova et al. 2004; Moreno and Basler 2004) and what seems to be a near universal ability of MYC to promote proliferation (Littlewood et al. 2012). However, it is important to note that this model does not yet integrate MYC-dependent repression as a mechanism that contributes to cell identity and lineage development during development (Adhikary et al. 2003; Varlakhanova et al. 2011).

In the great majority of studies in which cells have been subjected to forced MYC expression, the target cells, whether in culture or in vivo, are already in a proliferative state and MYC has the general effect of supporting proliferation and preventing cell-cycle exit (Grandori et al. 2000). But even in postmitotic cells, MYC can induce cell-cycle entry and proliferation. For example, in vivo forced expression of MYC in postmitotic skin keratinocytes, forebrain neurons, adult myocytes, and support cells of adult utricles, led to their reentry into the cell cycle (Pelengaris et al. 1999; Xiao et al. 2001; Lee et al. 2009; Burns et al. 2012). However, while adenoviral-mediated expression of MYCN in cultured postmitotic sympathetic neurons also supported cell-cycle reentry, its expression in postmitotic cortical neurons failed to induce S-phase entry or progression (Wartiovaara et al. 2002). Additionally, forced expression of MYC caused cell-cycle reentry in quiescent epithelial cells of structurally unorganized mammary acini in organotypic culture, but its expression in quiescent cells within mature acini failed to induce cell-cycle reentry (Partanen et al. 2007).

Together with the finding that MYC and MYCN are found in postmitotic cells in vivo (Grady et al. 1987; Mugrauer et al. 1988; Hirvonen et al. 1990; Wakamatsu et al. 1993), these results are consistent with the presence of different classes of postmitotic cells, with some cells more poised to respond to MYC and reenter the cell cycle than others. Moreover, there is evidence of diversity in the epigenetic state of chromatin in postmitotic cells (Srivastava et al. 2010). Superimposing the transcriptional amplification model on these findings leads to the prediction that only postmitotic cells retaining proliferation-associated genes in a transcriptionally poised or low-level active state (but below a threshold needed to support proliferation) and therefore subject to induction by MYC would be capable of reentering the cell cycle in response to MYC. For postmitotic cells that cannot enter the cell cycle in response to forced

MYC, their epigenetically repressed state may render them impervious to the actions of MYC (Fig. 1). For the latter cells, the actions of robust pioneer transcription factors capable of initiating epigenetic changes that increase accessibility to DNA binding by MYC (Fig. 1) would be required to make them responsive to the actions of MYC.

MYC IN DEVELOPMENTAL DISORDERS

As discussed above, MYCN haploinsufficiency causes the pleomorphic developmental disorder Feingold syndrome. There exist a number of developmental disorders related to Feingold syndrome referred to as VACTERL association, comprising vertebral defects, anal atresia, cardiac defects, tracheoesophageal fistula, renal malformations, and limb defects (Shaw-Smith 2006). Although MYCN mutations or deletions have not been identified in the wider collection of VACTERL association syndromes, the various causative genes in these syndromes (Shaw-Smith 2006) offer promising leads for upstream regulators of MYCN and perhaps for downstream targets and/or transcriptional programs that MYCN regulates.

There are no developmental disorders currently known that are directly caused by MYC mutations or deletion. However, MYC, as well as MYCN, is regulated by a variety of signaling pathways that not only influence cell identity and fate, but also cause developmental disorders. Thus, as in cancer, where MYC deregulation caused by mutations in pathways such as the RTK/RAS/MAPK and WNT/β-catenin is either proven or implicated to be critical for oncogenesis (Oskarsson et al. 2006; Sansom et al. 2007), it seems highly likely that misregulation of MYC plays an equally important underlying role in developmental disorders caused by defects in these and other signaling systems. For example, cardiac defects associated with the collection of developmental disorders known as rasopathies caused by mutations in the RAS-pathway (Sala et al. 2012) have features that often closely overlap with cardiac defects caused by ectopic MYC expression (Jackson et al. 1990). Additionally, in the developing skeleton, mutations in FGFR3 that cause achondroplasia lead to MYC down-regulation and impaired proliferation through a mechanism involving enhanced β-catenin degradation (Shung et al. 2012). MYC down-regulation appears to be a key mechanism in this disorder because conditional deletion of MYC in chondrocytes caused a similar achondroplasia-like phenotype (Zhou et al. 2011).

Finally, the collection of related developmental disorders known as cohesinopathies that include Cornelia de Lange syndrome and Roberts syndrome are linked to misregulation of MYC (Horsfield et al. 2012). Cohesinopathies are caused by mutations in components of the cohesin complex, including the cohesin subunits NIPPED-B, SMC1 and SMC3, RAD21, and the cohesin acetyltransferase ESCO2 (Horsfield et al. 2012). Cohesinopathies show considerable overlapping pathology with VACTERAL association and Feingold syndromes, suggesting potential involvement of MYCN. For example, these disorders typically feature short stature, dysmorphic facial features including micrognathia, limb defects, hearing loss, gastrointestinal defects including atresia, cardiac defects, genitourinary defects, and mental retardation (van Bokhoven et al. 2005; Shaw-Smith 2006; Horsfield et al. 2012). Interestingly, the cohesin complex, which is best known for its functions in sister chromatid exchange (Horsfield et al. 2012), shares with MYC the property of preferentially binding to genes being actively transcribed (Misulovin et al. 2008). Furthermore, cohesin has been linked to stimulation of transcription elongation through release of paused RNA polymerase II complexes (Fay et al. 2011). These results raise the intriguing possibility that the cohesin complex is mechanistically linked with MYC binding to transcriptionally paused and active genes (Guccione et al. 2006) and to transcription amplification (Lin et al. 2012; Nie et al. 2012).

CONCLUDING REMARKS

Although much is known about the activities of MYC and MYCN during embryonic develop-

ment, much less is known about how these activities are coordinated by developmental cues. This is complicated by the great variety of signaling pathways that are implicated in controlling MYC abundance in cells and the multitude of ways that MYC activities can be regulated. In addition to regulation at the transcriptional, translational, and posttranslational levels, MYC activity has the potential to be significantly impacted by members of the extended network of MAX and MAX-like factor (MLX)-interacting transcription factors (Hurlin and Huang 2006; Dang 2012). At one level, establishing the temporal relationship between MYC induction or suppression in different developmental settings with emergent epigenetic signatures and gene regulation may help clarify how MYC impacts cell fate. However, a major challenge going forward is to integrate these relationships with a more complete understanding of how MYC expression and activity is determined. The promise of such work is the recognition of effective strategies for the therapeutic manipulation of MYC and its key activities in diverse disease and injury settings.

ACKNOWLEDGMENTS

P.J.H. is supported by grants from Shriners Hospitals for Children.

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