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MYC AND MNT IN TRANSCRIPTIONAL REGULATION AND CHROMATIN DYNAMICS

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TO MY FAMILY

ABSTRACT

The *MYC* proto-oncogene regulates several cellular processes including cell cycle progression, proliferation, apoptosis and differentiation. In normal cells, *MYC* expression is induced upon cell cycle entry and is thereafter expressed at low levels during proliferation. In contrast, *MYC* is de-regulated in the majority of human tumors and contributes to uncontrolled cell proliferation and immortalization. Both MYC and its antagonist MNT are transcription factors that bind to the same E-box sequences in target promoters as heterodimers with the MAX protein. Whereas MYC/MAX activates transcription by recruitment of co-factors containing histone acetyltransferase (HAT) activity, MNT/MAX interacts with the adaptor-protein SIN3 and recruits histone deacetyltransferases (HDAC) in order to repress transcription.

In this thesis I have studied MYC and MNT in transcriptional regulation and chromatin dynamics. More specifically, we demonstrated MNT to be a transcriptional repressor that is functionally regulated by mitogen-activated protein kinase (MAPK) induced phosphorylation at cell cycle entry. In turn, we showed that this phosphorylation inhibited MNT-mSIN3 interaction and recruitment of HDAC activity. In addition, relief of MNT-mediated transcriptional repression allowed activation of MYC target genes (Paper I). Phosphorylation at MNT S70 was shown to generate the 74 kDa form of the MNT protein, which was induced upon serum stimulation of quiescent cells (Paper I and II). However, the S70 phosphorylation did not inhibit MNT as a transcriptional repressor but was instead shown to regulate MNT protein stability. Interestingly, MYC/RAS induced cellular transformation was increased in cells expressing a mutant mimicking constitutive phosphorylation of MNT S70. Higher levels of MNT in these cells were suggested to antagonize both pro-proliferative and pro-apoptotic activities mediated by MYC (Paper II). Importantly, we have also found that MNT represses transcription by de-acetylation of histone tails and complete chromatin condensation. In contrast, we confirmed binding of MYC to active chromatin and its involvement in fine-tuning of gene expression. Our data show that MYC once bound to the promoter induces local hyper-acetylation and increased DNA accessibility, which allows transcriptional activation (Paper III).

Finally, we demonstrated transcriptional upregulation of the miR-17-92 cluster in neuroblastoma cells overexpressing the MYC family member MYCN. Two members of this cluster, miR-18a and miR-19a, was shown to interfere with the expression of the transcription factor estrogen receptor-α (ESR1). Based on our data we suggest that MYCN promotes tumorigenesis and development of neuroblastoma by preventing normal neuroblast differentiation through indirect regulation of ESR1 expression (Paper IV).

Taken together, the interplay between MYC and MNT regulate gene expression important for cellular activities, which will determine the biological outcome.

LIST OF PUBLICATIONS

This thesis is based on the following original papers, which are referred to in the text by their roman numerals:

Original publications

I. Nikita Popov*, **Therese Wahlström***, Peter J. Hurlin, and Marie Henriksson.
Mnt transcriptional repressor is functionally regulated during cell cycle progression.
Oncogene, 2005, 24(56): 8326-8337.
* Equal contribution

II. **Therese Wahlström***, Craig William Hooker*, Katannya Kapeli, Sara Ota, Roel van Eijk, Marie Arsenian Henriksson, and Peter J. Hurlin.
Phosphorylation at serine 70 controls MNT accumulation and MYC-dependent cell transformation.
Manuscript, 2014.
* Equal contribution

III. **Therese Wahlström**, Sergey Belikov, and Marie Arsenian Henriksson.
Chromatin dynamics at the *hTERT* promoter during transcriptional activation and repression by c-Myc and Mnt in *Xenopus leavis* oocytes.
Experimental Cell Research, 2013, 319(20): 3160-3169.

IV. Jakob Lovén, Nikolay Zinin, **Therese Wahlström**, Inga Müller, Petter Brodin, Erik Fredlund, Ulf Ribacke, Andor Pivarcsi, Sven Påhlman, and Marie Henriksson.
MYCN-regulated microRNAs repress estrogen receptor-α (ESR1) expression and neuronal differentiation in human neuroblastoma.
Proceeding of the National Academy of Science (PNAS), 2010, 107(4): 1553-1558.

Related publication

Therese Wahlström and Marie Henriksson.
Mnt takes control as key regulator of the myc/max/mxd network.
Advances in Cancer Research, 2007, 97:61-80. Review

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

3’ UTR	3’ untranslated region
APL	Acute promyelocytic leukemia
ATP	Adenosine triphosphate
BCL-2	B-cell lymphoma 2
bHLHZip	Basic helix-loop-helix leucine zipper
bp	Base pair
BRG1	Brahma related gene
BRM	Brahma
ChIP	Chromatin Immunoprecipitation
CAD	Cabamoylphosphate dihydroorotase
CBP	CREB binding protein
CDK	Cyclin dependent kinase
CDKI	Cyclin dependent kinase inhibitor
CLL	Chronic lymphocytic leukemia
CTD	C-terminal domain
DMS	Dimethyl sulfate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
DNMT	DNA methyl transferase
E-box	Enhancer box
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EMSA	Electrophoretic mobility shift assay
ERK	Extracellular signal regulated kinase
ESR1	Estrogen receptor-α
FBW7	F-box WD-40 domain protein 7
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
GDP	Guanosine diphosphate
GRB2	Growth factor receptor bound protein 2
GTP	Guanosine triphosphate
GSK3	Glycogen synthase kinase 3
HAT	Histone acetyl transferase
HIC1	Hypermethylated in cancer
HDAC	Histone deacetylase
hTERT	human telomerase reverse transcriptase
INK4A	Inhibitor of Cdk4
INO80	Inositol requiring 80
INR	Initiator
ISWI	Imitation SWI

KO	Knockout
LNA	Locked nucleic acid
LOH	Loss of heterozygosity
LUC	Luciferase
MAPK	Mitogen-activated protein kinase
MAR	Matrix attachment region
MAX	MYC associated protein X
MINE	MYC insulator element
MB	MYC box
MEF	Mouse embryo fibroblast
MGA	MAX gene associated
miRNA	microRNA
MIZ-1	MYC interacting zinc finger protein-1
MNT	MAX's next tango
MXD	MAX dimerization protein
MYC	Myelocytomatosis
MYCN	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived
NLS	Nuclear Localization Signal
NuRD	Nucleosome remodeling and deacetylation
ODC	Ornithine decarboxylase
PI3K	Phosphatidylinositol-3 kinase
pRB	Retinoblastoma protein
pTEFb	Positive transcription elongation factor b
PTEN	Phosphatase and tensin homolog
REF	Rat embryo fibroblast
RNA	Ribonucleic acid
RNAi	RNA interference
RNA Pol	RNA polymerase
ROS	Reactive oxygen species
rRNA	Ribosomal RNA
SAGE	Serial analysis of gene expression
SID	Sin3 interaction domain
SNP	Single nucleotide polymorphism
SP1	Specificity protein-1
SWI/SNF	Switching defective/sucrose non-fermenting
TAD	Transcriptional activation domain
TBP	TATA binding protein
TK	Thymidine kinase
tRNA	Transfer RNA
TRRAP	Transformation/Transcription domain-Associated Protein
wt	Wild type

1 INTRODUCTION

In this thesis I have studied the interplay between one of the most frequently deregulated oncogenes in human cancer, *MYC*, and its modulator and antagonist *MNT*. Both of these are transcription factors and exert their functions through regulation of gene expression. Various aspects about the MYC/MAX/MXD/MNT network as well as gene expression including chromatin structure/DNA accessibility, transcription, protein synthesis and regulation and the role of oncogenes and tumor suppressor genes in cancer will be discussed.

CANCER

Cancer is a group of diseases where cells divide and grow uncontrollably, form malignant tumors, which can invade nearby locations or spread through the lymphatic or blood system to more distant parts of the body. The causes of cancer are genetic (chromosomal translocations, mutations, gene amplifications, deletions, and insertions) and epigenetic alterations (modifications to the genome which do not change the nucleotide sequence i.e. DNA methylation, histone modifications and changes in the chromosomal architecture). These changes can be inherited or mediated by environmental factors such as exposure to tobacco smoke, ultraviolet radiation, certain infections, dietary factors, chemicals and pollutants causing genetic damage that may result in cancerous somatic mutations. A tumor can also be non-cancerous or benign which corresponds to a lump of dividing cells that do not metastasize. These masses of cells can however cause local damage on the tissue where it grows or become malignant through tumor progression.

Most if not all tumor cells obtain the same functional capabilities in order to develop into malignant cancers. These include: (1) sustained proliferative signaling, (2) evading growth suppressors, (3) resisting cell death, (4) enabling replicative immortality, (5) inducing angiogenesis, (6) activating invasion and metastasis, (7) evading immune destruction and (8) reprogramming of energy metabolism (Hanahan and Weinberg, 2000; Hanahan and Weinberg, 2011). Underlying these hallmarks are two enabling characteristics namely: genome instability and tumor-promoting inflammation. In addition, tumors contain recruited normal cells creating the tumor microenvironment which exhibit yet another dimension of complexity (Hanahan and Weinberg, 2011). These cellular mechanisms are aberrantly regulated due to activation of oncogenes and inactivation of tumor suppressor genes in cancer cells (Sherr, 2004; Todd and Wong, 1999).

The model for the multistep process of tumorigenesis suggests that accumulation of several mutations are needed in order to transform normal cells into tumor cells (Fearon and Vogelstein, 1990; Hanahan and Weinberg, 2000; Hanahan and Weinberg, 2011; Vogelstein and Kinzler, 1993) (Figure 1). The multi-hit hypothesis of carcinogenesis explains why most cancers occur in older people in which accumulation of mutations necessary to cause malignant cells have had time to occur (Vogelstein and Kinzler, 1993).

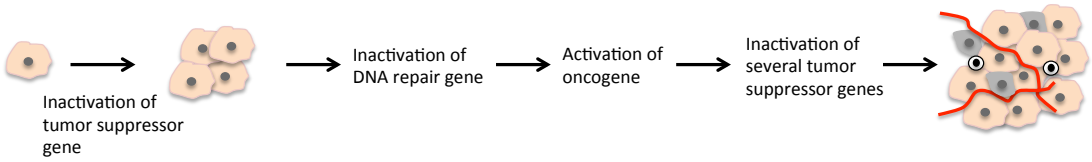

Figure 1. Suggested model for multistep process of tumorigenesis. Inactivation of a tumor suppressor gene by mutation leads to increased cell proliferation. Additional mutations causing inactivation of DNA repair gene, activation of oncogene and inactivation of more tumor suppressor genes drives tumor formation.

In contrast, childhood cancer cells often have fewer genetic defects and develop within a shorter period of time compared to adult tumors. Cancer in children arise from a progenitor cell, which is part of developmental programs. During prenatal and postnatal development cellular processes including cell division and apoptosis regulate tissue growth and differentiation. Defects in these processes promote transformation. In addition, developing tissues are highly proliferative, which allows more errors in DNA replication to occur. Childhood tumors include for example leukemia, Wilm's tumor, retinoblastomas, lymphomas, central nervous system (CNS) malignancies and neuroblastoma (Scotting et al., 2005).

Proto-oncogenes and oncogenes

Proto-oncogenes are necessary for normal physiological processes such as growth, proliferation, differentiation and survival. During malignant transformation proto-oncogenes are activated by mutations, increased expression or chromosomal translocations (Friedrich et al., 1976; Stehelin et al., 1976; Todd and Wong, 1999) to become oncogenes that give rise to cells with growth advantage compared to the normal counterpart (Hanahan and Weinberg, 2011). Tumor cells take advantage of the pro-proliferative properties of an oncogene resulting in enhanced growth without the effects of fail-safe mechanisms (Lowe et al., 2004). The first oncogene, *v-Src*, was described in 1970 (Martin, 1970) as an viral oncogene found in a chicken Rous sarcoma virus. Thereafter, cellular counterparts have been identified and include transcription factors, growth factors, receptor tyrosine kinases, signal transducers, and regulators of cell death. For example MYC, which will be discussed in more detail later, exert its function as a transcription factor and is de-regulated in the majority of human cancers (reviewed in (Vita and Henriksson, 2006)). In order to transform rodent primary cells it is sufficient to activate *MYC* together with *RAS* (Land et al., 1983). *RAS* encodes an oncogenic membrane-bound GTPase that signals from growth factors via

signaling pathways like the mitogen-activated protein kinases (MAPK) to regulate various transcription factors (Figure 4). Amplifications or point mutations in *RAS* are often found in human tumors (Karnoub and Weinberg, 2008). *BCR-ABL* is another example of an oncogene, which is created by fusions of the *BCR* and *ABL* genes located at the Philadelphia chromosome. This chimeric oncoprotein, which is often found in chronic myelogenous leukemia (CML), is created by translocations of chromosome 9 and 22 (Nowell and Hungerford, 1960). BCR-ABL has high protein tyrosine kinase activity that activates other proteins involved in the cell cycle and cell division.

Tumor suppressor genes

Tumor suppressor genes protect cells from immortalization by encoding for proteins involved in cellular activities including cell cycle checkpoint, detection and repair of DNA damage, protein ubiquitination and degradation, mitogenic signaling, induction of apoptosis (Sherr, 2004). These genes are inactivated by mutations, deletions or epigenetically to cause loss of function. In combination with other genetic alterations tumorigenesis can progress. Distinct from oncogenes in which single allele mutations are sufficient for gain-of-function, tumor suppressors usually follows the two-hit hypothesis meaning that both alleles has to be affected in order for the protein to loose its function (Knudson, 1971; Sherr, 2004). The two hit hypothesis was first shown for retinoblastoma (Knudson, 1971). The Retinoblastoma protein (pRB) is an important cell cycle regulator that controls the G1/S transition (McLaughlin et al., 2003) and has been shown to be altered in many human cancers (Weinberg, 1995). However, there are exceptions from this hypothesis where functional loss of only one allele in for example the cell-cycle inhibitor $p27^{Kip1}$ is enough promote tumor growth (Fero et al., 1998). Furthermore, the tumor suppressor *p53*, also called the "guardian of the genome" is mutated or deleted in at least 50 % of all human tumors (Lane, 1992). p53 induces p21-mediated cell cycle arrest or promotes apoptosis by transcriptionally repressing anti-apoptotic and activating pro-apoptotic proteins (Sherr, 2004). The tumor suppressor p19ARF (p14ARF in humans) binds and sequesters MDM2 to the nucleolus and protects thereby p53 from MDM2 mediated degradation (Weber et al., 1999). The function of inactivated p53 was re-activated using small molecules (RITA and Nutlin) which inhibit the interaction between p53 and human MDM-2 (HDM-2) (Issaeva et al., 2004; Vassilev et al., 2004). By activation of pro-apoptotic targets and inhibition of pro-proliferative oncogenes such as *MYC* re-activation of p53 induced robust apoptosis and has thus been proposed as a potential strategy for anticancer therapy (Grinkevich et al., 2009). Another tumor suppressor gene *PTEN* encodes for Phosphatase and tensin homolog (PTEN) and is involved in regulation of the cell cycle, preventing cells from growing and dividing too fast (Chu and Tarnawski, 2004). PTEN negatively regulates the PI3K and thereby the anti-apoptotic and pro-tumorigenic Protein kinase B (PKB)/Akt (Vivanco and Sawyers, 2002).

microRNAs in cancer

MicroRNAs (miRNAs) are small, approximately 23 nucleotides, non-coding regulatory RNAs, which control gene expression by binding to the 3´untranslated region (UTR) of mRNA resulting in mRNA degradation or inhibition of protein synthesis (Bartel, 2009; Flynt and Lai, 2008). miRNAs are involved in several processes including proliferation, differentiation and cell death (Flynt and Lai, 2008). The fact that every miRNA can target several different mRNAs and a single mRNA can be targeted by multiple miRNAs as well as the enormous number of miRNAs identified creates a complex network of pathways. The expression of miRNAs is often altered in cancer, where both oncogenic and tumor suppressive miRNAs have been described. For example, the miRNA let-7 that targets *K-RAS* mRNA (Johnson et al., 2005; Kumar et al., 2008), was shown to be down regulated in lung cancer resulting in increased level of K-RAS (Kumar et al., 2008). In addition, the polycistronic miRNA cluster, miR-17-92, encoding miR-17, miR-18a, miR-19a, miR-19b, miR-20a and miR-92a, was shown to possess oncogenic activity (He et al., 2005) and has been shown to be overexpressed in several tumor types (Volinia et al., 2006).

GENE EXPRESSION AND REGULATION

The genetic information stored in DNA is transcribed and translated into proteins. Gene expression is regulated at several levels including accessibility to DNA, regulation of transcription and post-transcriptional events. The level of protein is also dependent on translational and post-translational regulation including modifications involved in protein stability and degradation.

Chromatin structure and DNA accessibility

The DNA in every human cell is nearly 2 meter long and in order to find room for it in the cell nucleus it needs to be packed into chromatin, which is a complex structure of DNA and proteins (Kornberg, 1974). 146 bp of DNA is wrapped around the protein octamer, which consists of histone H3, H4, H2A and H2B forming the nucleosome structure (Luger et al., 1997). Nucleosomes are arranged into “bead-on-a-string”. This level of chromatin structure represents euchromatin with actively transcribed genes. Chromatin also contains linker histone H1 that binds in between nucleosomes and interacts with core histones to turn nucleosomal arrays into the 30 nm fiber, representing condensed heterochromatin. The chromatin can be further compacted to eventually form the chromosome structure (Figure 2).

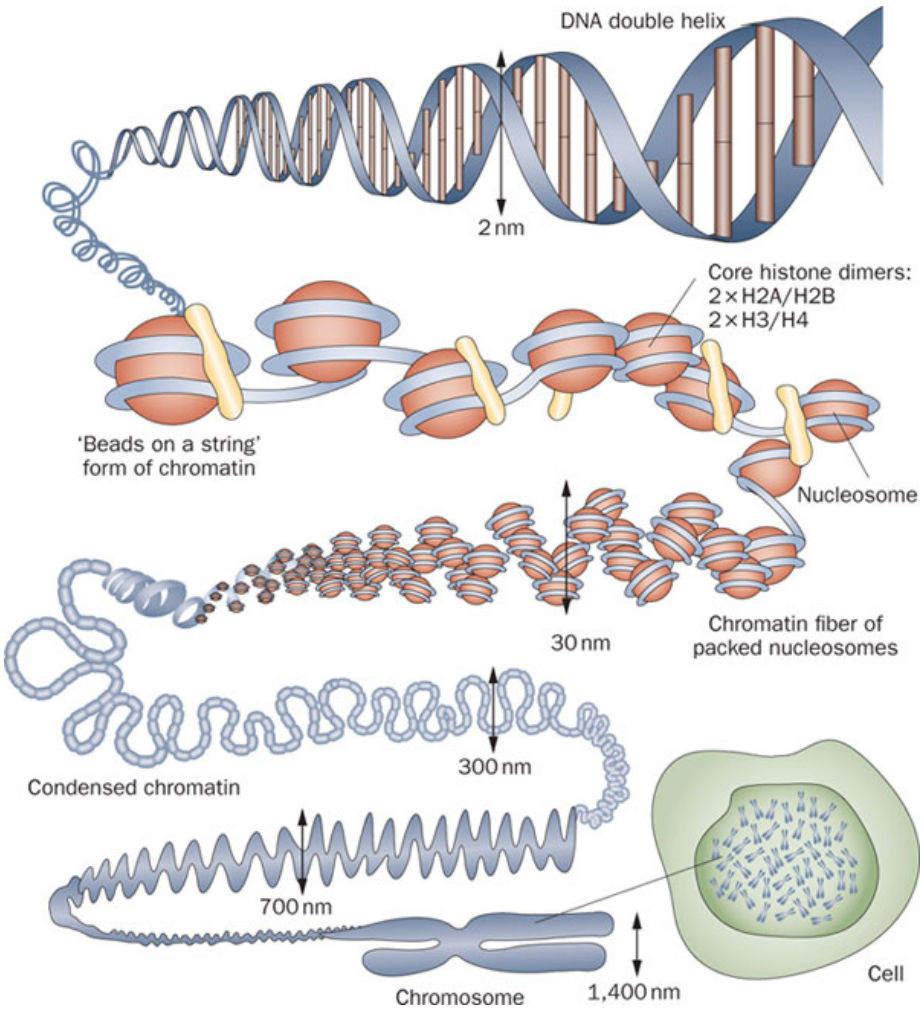

Figure 2. Organization of DNA and histones into nucleosomes, beads-on-a-string, 30 nm fiber and higher order structures packages the DNA into chromosomes in the cell nucleus. Re-printed from (Tonna et al., 2010) with permission from the publisher.

The chromatin structure is highly dynamic and can be locally modulated in order to allow DNA accessibility for processes like transcription, replication and repair to occur. Changes at every level of the chromatin structure are possible (Bell et al., 2011). Gene expression can be regulated at the level of nucleosomes where covalent histone modification and chromatin-remodeling complexes alter the histone-DNA contact in certain regions (reviewed in (Peterson and Laniel, 2004; Wang et al., 2007a; Wang et al., 2007b)).

Histone modification

Histone modifying complexes contain enzymes that covalently add or remove acetylation (ac) and methylation (me) of lysine (K) and arginine (R), phosphorylation (P) of serine (S) and threonine (T), ubiquitination, sumoylation, and ribosylation groups at histone tails. Transcriptional regulators recruit histone modifying enzymes to chromatin and these enzymes has different specificity for individual histone tails. This results in distinct combinations of histone marks. Different histone modifications and combinations of marks are coupled to different biological outcomes. Below are some examples of histone modifications involved in active or repressed chromatin/transcription (reviewed in (Peterson and Laniel, 2004)):

Active chromatin/transcription:

- H4K8ac, H3K14ac and H3S10P
- H3K9ac
- H3K4me3
- H3K79me2

Repressed chromatin/transcription:

- H3K9me3 and de-acetylation of H3 and H4
- H3K9me2/3
- H3K27me2/3
- H4K20me3

Histone acetylation/deacetylation is mediated by histone acetyl transferases (HATs) and histone de-acetyl transferases (HDACs) where HATs add and HDACs remove acetyl groups at histone tails (Allfrey et al., 1964). The first nuclear HAT, a *Tetrahymena* homolog of yeast GCN5 was identified in 1996 (Brownell et al., 1996). Studies in yeast had already shown GCN5 to be a transcriptional co-activator and the discovery of GCN5 as a HAT clarified that histone modifications directly regulate transcription. Subsequently, additional transcriptional co-activators including CBP/p300 was shown to have HAT activity. In contrast, co-repressors like SIN3-RPD3 were shown to have HDAC activity (reviewed in (Peterson and Laniel, 2004)).

In addition, histone methyltransferases and histone demethylases contribute to histone modifications by addition or removal of methyl groups on histone tails. For example, histone lysine N-methyltransferase (EZH2), which is a member of the polycomb-group methylate H3K27 and mediates transcriptional silencing (Peterson and Laniel, 2004).

Histone modifications affecting the affinity between DNA and histones was suggested to alter the chromatin structure (Wolffe and Hayes, 1999). However, the large number of different histone modifications representing the "histone code" has been shown to affect chromatin structure indirectly by controlling binding of non-histone proteins, which in turn alter the chromatin architecture (reviewed in (Peterson and Laniel, 2004; Strahl and Allis, 2000)). Many of these proteins bind specifically to certain histone

modifications and are part of chromatin remodeling complexes, which will be discussed below.

Histone modifications as well as miRNA mediated regulation of mRNA stability and methylation of cytosine residues in CpG sites of DNA all mediate differences in gene expression that occur without changes in the DNA sequence. These so called epigenetic changes are in many cases highly dynamic and reversible (reviewed in (Jakopovic et al., 2013)). Hypermethylation of promoter DNA are linked to transcriptional repression whereas hypomethylation leads to transcriptional activation (Zwart et al., 2001). DNA methylation is crucial for normal development and is associated with processes such as genomic imprinting, X-chromosome inactivation and tumorigenesis. Almost all types of cancer have alteration in DNA methylation status (reviewed in (Jaenisch and Bird, 2003)). For example, tumor suppressor genes are often inactivated by DNA hypermethylation. Transcription is affected by DNA methylation by interference with the binding of transcriptional proteins to the promoter (Choy et al., 2010) and by methyl-CpG-binding proteins that bind to the methylated DNA. These recruit additional proteins such as HDACs and chromatin remodeling factors, which result in repressed inactive chromatin (reviewed in (Ballestar and Wolffe, 2001)).

ATP-dependent chromatin remodeling

ATP-dependent chromatin remodeling enzymes are multi-subunit complexes. To regulate gene expression, ATP-dependent chromatin remodeling complexes uses energy from ATP hydrolysis to disrupt DNA-histone interactions, which regulates nucleosome mobility and DNA accessibility. SWI/SNF (Switching defective/sucrose non-fermenting), ISWI (Imitation SWI), NuRD (Nucleosome remodeling and deacetylation)/mi-2/CHD (Chromodomain, helicase, DNA binding) and INO80 (Inositol requiring 80) are different families of ATP-dependent chromatin remodeling factors. Each of these remodeling complexes have different functions due to their unique protein domain in the catalytic region that targets these factors selectively to different chromatin regions. For example, members of the SWI/SNF (BRM and BRG1) contain a bromodomain, which binds acetylated histone tails such as H4K8ac whereas ISWI family members (SNF2H and SNF2L) have a SANT and SLIDE domain that interacts with unmodified histone tails and linker DNA. Furthermore, Polycomb proteins bind to histone H3K27me2 via the chromodomain. NuRD/Mi-2/CHD has tandem chromodomains that recognized methylated histone tails and INO80 family members are characterized by split ATPase domain (reviewed in (Peterson and Laniel, 2004; Wang et al., 2007b)).

ATP-dependent chromatin remodeling complexes has an important role in regulating chromatin dynamics, which will allow the transcription machinery to bind to the otherwise well packed genome. SWI/SNF remodeling factors disorganize and reorganize nucleosome positioning to promote transcription factors to bind and activate or repress transcription depending on the conditions (reviewed in (Wang et al., 2007b)). In addition, the SWI/SNF subunit BRM was shown to bind nascent pre-mRNPs suggesting the role of SWI/SNF in regulation of pre-mRNA processing (Tyagi et al.,

2009). ISWI organize and orders the position of nucleosome to repress, activate or elongate transcription. NuRD/mi-2/CHD primarily mediate transcriptional repression in the cell nucleus whereas it is involved in transcriptional activation of rRNA in the nucleolus. INO80 is also involved in both activation and repression of transcription (reviewed in (Wang et al., 2007b)).

Apart from transcriptional regulation, ATP-dependent chromatin remodeling factors are involved in several important biological processes such as DNA replication and repair, cell cycle progression, chromosome assembly and embryonic development. Deregulation of chromatin remodeling factors result in a variety of diseases including cancer. For example, the SWI/SNF subunit SNF5, BRG1 and BRM have been suggested as tumor suppressors since biallelic loss or mutations has been found in human tumors (reviewed in (Wang et al., 2007b)).

Transcription

RNA polymerases transcribe the genetic information stored in DNA. There are three different RNA polymerases (Pol I, II, and III) that synthesize rRNA, mRNA and tRNA, respectively. Only RNA Pol II transcribes genes that encodes for proteins. RNA Pol II also transcribes non-coding RNAs such as miRNAs. Transcriptional regulation is mostly controlled at the level of transcriptional initiation. Control regions at promoters of protein encoding genes are the core promoter, the proximal and distal promoter, enhancers and silencers. Figure 3A shows a schematic picture of the promoter region of a representative RNA Pol II gene (Figure 3A). However, variability in gene structure occurs.

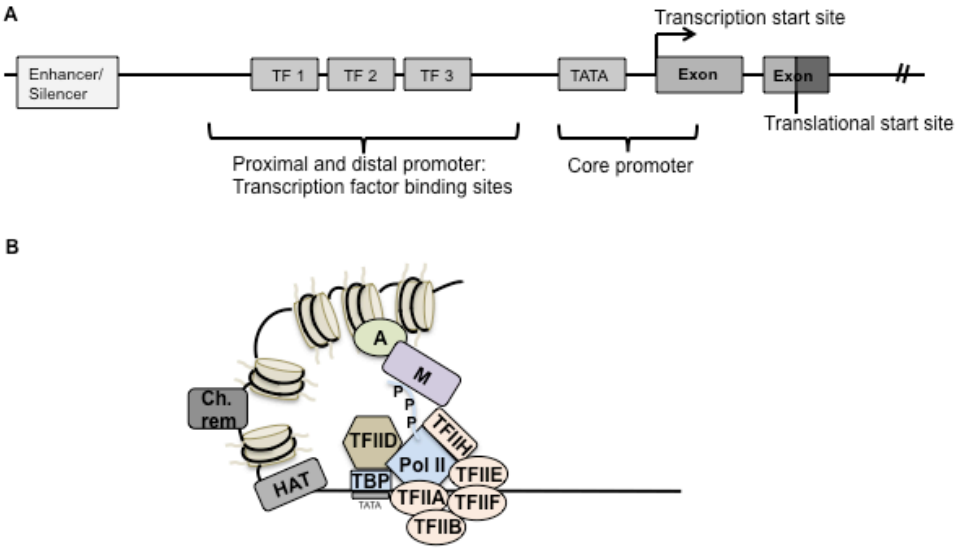

Figure 3. (A) Schematic picture of the promoter region of a representative RNA Pol II transcribed gene. Outlined in the picture are sites for Enhancer/Silencer, Transcription factor binding sites (TF 1, 2, 3), binding site for TATA binding protein (TATA), Transcription start site, coding (Exon) and non-coding regions and translational start site. (B) Transcriptional initiation is mediated by a variety of factors, which are described in more detail in the text. Activator (A), Chromatin remodeling factors (Ch. Rem), Mediator (M), Histone acetyl transferase (HAT), TBP (TATA binding protein), binding site for TBP (TATA-box), transcription factors (TFIID, TFIIA, TFIIB, TFIIE, TFIIF, THIIH), RNA Pol II (Pol II) and phosphorylation (P).

Transcription is initiated by binding of the activator(s) to its regulatory sequence, which is located upstream of the transcription start site (Figure 3B). Activators are transcription factors that bind to DNA both organized in nucleosomes and in nucleosome-free regions. By binding to enhancer elements, activators loop the DNA in order to bring a specific promoter to the transcription initiation complex. Enhancers are critical for tissue and developmental-stage specific expression of genes. In contrast, silencers are regions of DNA that are bound by transcription factors, which repress gene expression. Upon binding of the activator, chromatin remodeling complexes and HATs are recruited (Figure 3B). These mediate a chromatin structure with more accessible DNA for binding of transcription factors such as the TFIID complex, which contains TATA-binding protein (TBP) and TBP-associated factors (TAFs) (Figure 3B). The TATA-box is located in the core promoter, 25 – 30 bp upstream of the transcription start site (Figure 3A). Next the mediator complex, which is also a subunit of the RNA Pol II, is recruited. In addition, five more transcription factors (TFIIA, TFIIB, TFIIE, TFIIF and TFIIH) associates at the promoter (Figure 3B). One of the transcription factors TFIIH has helicase activity and is involved in separation of the double-stranded DNA to form the initial transcription bubble. The activator and the RNA Pol II are connected and the pre-initiation complex is formed. Upon assembly of this complex the C-terminal domain (CTD) of RNA Pol II is phosphorylated at Ser-5 by TFIIH and RNA Pol II leaves the pre-initiation complex and start synthesizing RNA (Figure 3B) (reviewed in (Delgado and Leon, 2006)).

Transcriptional elongation is thereafter mediated by positive transcription elongation factor (P-TEFb), which is recruited through chromatin remodeling factors or transcription factors. Once the mRNA is capped in the 5’ end, P-TEFb phosphorylates the CTD repeats of bound RNA Pol II at Ser-2 and recruits RNA processing and termination/polyadenylation factors needed for transcriptional elongation (reviewed in (Peterlin and Price, 2006)).

The traditional model of transcription describes how RNA polymerase moves along DNA to synthesize mRNA. An alternative model suggests that active enzymes are concentrated to distinct sites called transcription factories. These contain two or more RNA polymerases including associated machineries that are active on at least two different templates. The chromatin will then form loops around the transcription factories with heterochromatin regions apart from the factories and promoter and enhancer regions with active chromatin close in proximity (reviewed in (Papantonis and Cook, 2013)).

Post-transcriptional mechanisms involving 5’ capping and poly-adenylation of mRNA regulates the stability and protects mRNA from degradation until protein synthesis occur.

Protein translation and regulation

Protein synthesis occurs in ribosomes, where mRNA is translated to a specific protein. The ribosome consists of rRNA and proteins creating a multi-subunit structure. During translational initiation the small subunit of the ribosome binds to the 5' end of mRNA with help of initiation factors. tRNAs carries a specific amino acid and bind with the complimentary anticodon sequences to mRNA. By linking specific amino acids a polypeptide is created. Protein translation in the ribosomes is terminated once the stop codon (UAA, UAG, UGA) in the mRNA is reached since no tRNAs are able to bind this codon. Instead release factors bind and the ribosome/mRNA complex is disassembled. The polypeptide will thereafter fold into protein.

Proteins, including transcription factors, are often regulated by post-translational modifications i.e. addition of functional groups such as acetate, phosphate, lipids and carbohydrates. Phosphorylation is mediated by intrinsic signaling pathways, which are induced by extracellular activation of cell surface receptors such as Receptor tyrosine kinases. For example, extracellular mitogens such as Epidermal growth factor (EGF) or Fibroblast growth factor (FGF) bind to their corresponding receptor (EGFR or FGFR), which is located in the cell membrane. Tyrosine residues on the receptor are phosphorylated due to tyrosine kinase activity in the cytoplasmic domain of the receptor. Growth factor receptor bound protein 2 (GRB2) binds via the SH2 domain to phosphotyrosine residues on the activated receptor. GRB2 then binds and activates, via its SH3 domain, guanine nucleotide exchange factor (SOS) that remove GDP from RAS. RAS binds GTP and gets activated and a kinase cascade is started (Figure 4) (reviewed in (Katz et al., 2007)). Deregulation of *RAS*, which is a central player in MAPK signaling pathways, is often found in human tumors (reviewed in (Bos, 1989)). Activating mutations in *RAS* keeps it in its active GTP-bound state resulting in constant mitogenic signaling. RAS further activates the kinase activity of RAF, which phosphorylates MEK1/2 at serine/threonine residues that in turn phosphorylates and activates MAPK at Serine/Threonine sites (Figure 4). MAPK was originally called extracellular signal-regulated kinase (ERK). ERK translocate to the nucleus where it phosphorylates several transcription factors including c-FOS, c-JUN (reviewed in (Katz et al., 2007)) and MYC (reviewed in (Adhikary and Eilers, 2005)) (Figure 4). In addition, we showed that stability and degradation of MYC and its antagonist MNT to be co-regulated by MAPK/ERK signaling induced phosphorylation (paper II). Degradation of the MYC antagonist MXD1 is also regulated through the MAPK pathway (Zhu et al., 2008).

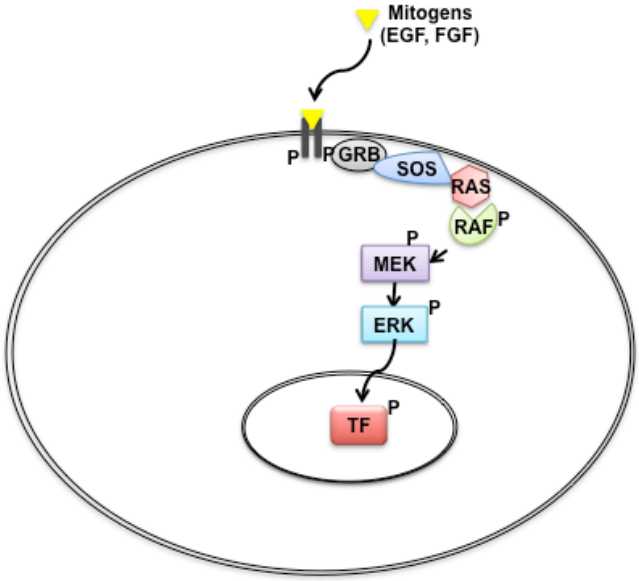

Figure 4. Growth factors such as Epidermal growth factor (EGF) or Fibroblast growth factor (FGF) bind to their corresponding tyrosine kinase receptor, which gets phosphorylated due to tyrosine kinase activity in the cytoplasmic domain of the receptor. Growth factor receptor-bound protein 2 (GRB2) binds to phosphotyrosine residues on the activated receptor followed by binding of guanine nucleotide exchange factor (SOS) to GRB2. SOS activates RAS, which starts a cascade of activating phosphorylation mediated through RAF, MEK and ERK. ERK translocate into the nucleus and phosphorylates transcription factors (TF).

THE MYC/MAX/MXD/MNT NETWORK

MYC

MYC was first identified as an oncogene generated by viral integration (*v-gag-myc*) of the avian myelocytomatosis retrovirus MC29, which causes the leukemic disorder myelocytomatosis in birds (Sheiness and Bishop, 1979). The cellular homolog of *v-myc* was first identified in chicken (Vennstrom et al., 1982) followed by cloning and characterization of the human, mouse and rat *c-MYC* genes (Dalla-Favera et al., 1982; Hayashi et al., 1987; Stanton et al., 1984). Later, two additional members of the MYC family, the human *MYCN* and *MYCL* were identified by amplifications in neuroblastoma and small cell lung carcinoma, respectively (Nau et al., 1985; Schwab et al., 1983). c-MYC will be referred to as MYC whereas MYCL and MYCN will be specified.

Regulation and expression of MYC

The *MYC* gene located at chromosome 8 is represented by euchromatin with acetylated histones H3, H4 and non-methylated histone H3 Lysine 9 (H3K9) both in mitogen induced, *MYC* expressing and in resting cells that does not express *MYC* (Farris et al., 2005; Gombert et al., 2003). No difference in the levels of methylated H3K9 was observed but proliferating cells expressed higher levels of acetylated histone H3 compared to resting cells. The euchromatin is flanked on both sides by heterochromatin

characterized by hypoacetylated histones H3, H4 and methylated H3K9. The heterochromatin regions are anchored to the nuclear matrix by a matrix attachment region (MAR) on each side, which form a chromosomal loop structure that separate the *MYC* locus from neighboring genes and permits association of the *MYC* gene with transcriptionally active areas. Upstream of the promoter region the euchromatin and heterochromatin regions are separated by a MYC insulator element (MINE), which is composed of barrier and CTCF-binding elements. Binding of CTCF mediates blocking of enhancer activity and the recruitment of the SNF-2 like chromodomain helicase protein CHD8 by CTCF contributes to the barrier function. These two prevents the spread of adjacent transcriptionally inactive heterochromatin (Gombert et al., 2003).

The *MYC* promoter contains four different transcription start sites P0, P1, P2 and P3. The majority of transcription occurs from P2, which contain a TATA-box as well as two initiator (INR) elements. Promoter P1 has a less optimal TATA-box sequence and no INR elements and are the second most commonly used *MYC* promoter. Both P0 and P3 lack the TATA-box sequence (Battey et al., 1983; Wierstra and Alves, 2008). The *MYC* gene encodes for two proteins of 64 and 67 kDa each. Translation of p67 is initiated by a cryptic start codon in the end of the first exon whereas p64 is produced from the ATG start codon in second exon (Hann et al., 1988). The smaller MYC protein is the major product produced. MYC is translocated to the nucleus where it regulates gene expression. Cleavage of MYC creates a cytoplasmic protein, MYC-nick, which have been described to be involved during differentiation (Conacci-Sorrell et al., 2010).

Expression of *Myc* is relatively high during early embryonic development and deletion of either *c-Myc* or *N-Myc* is lethal at embryonic day 9-10 and 11, respectively (Davis et al., 1993; Stanton et al., 1992). Compared to the almost ubiquitously expression of *c-Myc,* expression of *N-Myc* and *L-Myc* are restricted to specific tissue and stage of development. *N-Myc* is highly expressed early in embryonic development in various tissues and its level decline later in development often when cells are induced to differentiate. *L-Myc* is embryonically expressed and decline after birth in most tissues except for the lung. In differentiated cells the expression of *Myc* is low or even undetectable. Whereas expression of *N-Myc* or *L-Myc* does not correlate with proliferation the expression of *c-Myc* does (reviewed in (Henriksson and Luscher, 1996)). Upon mitogen stimulation of quiescent cells the levels of *Myc* is rapidly induced whereas it declines to basal levels again throughout the cell cycle and in continuously proliferating cells (Hann et al., 1985; Rabbitts et al., 1985; Thompson et al., 1985). *MYC* expression is dependent on the presence of growth factors and is down regulated upon removal of growth factors, stimuli to differentiate or anti proliferative signals. MYC has an important role in regulation of cell growth and even small changes in the expression or activity of MYC have consequences for cell fate. Therefore MYC is tightly regulated at several levels including chromatin architecture across the *MYC* promoter, transcriptional initiation and elongation, translation, stability of mRNA and protein, posttranslational modifications and interacting proteins (reviewed in (Hooker and Hurlin, 2006; Wierstra and Alves, 2008)).

Nucleosomal mapping of the *MYC* promoter showed that the DNA in the upstream region, the promoter region, upstream of the P1 promoter and at the P1 promoter is organized in nucleosome structure in differentiated cells (Pullner et al., 1996). In contrast, the P2 promoter was free from nucleosomes in both active and inactive *MYC* promoters (Albert et al., 1997; Michelotti et al., 1996; Pullner et al., 1996). The *MYC* gene has been shown to be hypermethylated and silenced in terminally differentiated cells, induced to differentiate with DMSO. The *MYC* promoter is also regulated by chromatin remodeling factors such as the ATP-dependent SWI/SNF nucleosome remodeling complexes BAF and PBAF where the ATPase subunit BRG-1 are associated with the *MYC* promoter (reviewed in (Wierstra and Alves, 2008)). Several signaling pathways, such as Wnt, Notch, NF-κB and TGF-β, are known to be involved in regulation of *MYC* transcription (reviewed in (Liu and Levens, 2006)). For example, Wnt signaling regulates the *MYC* promoter by T cell factor-4 (TCF-4) binding (He et al., 1998). TCF-4 binding sites have also been reported in enhancer regions of the *MYC* gene (Hallikas et al., 2006; Mautner et al., 1995). The β-Catenin-TCF4 complex has been shown to bind to a single-nucleotide polymorphism (SNP) located 335 kb from the *MYC* gene and by chromatin looping this distal enhancer activity increases *MYC* expression in colon cancer cells (Wright et al., 2010). Furthermore, the *MYC* promoter is activated or repressed upon binding of a variety of transcription factors such as E2F, SMAD 2-4, STAT1, STAT3, NF-κB, ETS1-2, c-JUN/c-FOS. These factors modify chromatin architecture via recruitment of HATs or HDACs in order to regulate gene expression. For example, E2F-HDAC complexes repress the *MYC* promoter in quiescent cells whereas mitogen stimulation activates immediate early genes and *MYC* is activated by transcription factors like AP-1, ETS1/2, NF-κB and STATs. In addition, association of MLL/SET1-type histone methyltransferase such as MLL2 has been reported at the *MYC* promoter (reviewed in (Wierstra and Alves, 2008)).

MYC also auto-represses its own promoter in a concentration dependent manner. Independently of binding to the E-box sequence (which will be discussed in more detail below) a mechanism involving the INR elements and E2F binding sites are involved in repression of the P2 promoter. Binding of p107 to both Myc and E2F is also involved in repression of the *MYC* gene (reviewed in (Wierstra and Alves, 2008)). In addition, MYC binding sites has been identified in an intron the *MYC* gene by an unbiased whole genome mapping strategy (Zeller et al., 2006).

Deregulation of MYC in cancer

The expression of *MYC* is deregulated in the majority of human cancers. However, unlike other proto-oncogenes, such as *RAS* that is activated by mutations, *MYC* is more often de-regulated by insertional mutagenesis, chromosomal translocation and gene amplification. In addition, *MYC* can be de-regulated at the level of its expression, mRNA and protein stability (reviewed in (Meyer and Penn, 2008)). Translocations between the immunoglobulin (Ig) heavy and light chain genes and the *MYC* gene are observed in Burkitts lymphoma (reviewed in (Boxer and Dang, 2001)) and overexpression of *MYC* is often found in leukemias (reviewed in (Delgado and Leon, 2010)). Accurate expression of MYC is of importance for cellular activities and

differentiation requires downregulation of *Myc* (Coppola and Cole, 1986; Nguyen et al., 1995).

Studies using genetically modified mice with overexpression or deletions of *Myc* have shown the importance of MYC for self-renewal and differentiation of hematopoietic stem cells. Normally hematopoietic cells differentiate along different lineages resulting in distinct mature phenotypes of blood cells where pluripotent hematopoietic stem cell gives rise to plateles, erythrocytes, monocytes and granulocytes (neutrophils, eosinophils and basophils). Fully differentiated neutrophils are developed through differentiation of myeloid stem cells, progenitor cells, myeloblasts, promyelocytes and myelocytes. Enforced expression of MYC was shown to block differentiation of several leukemia derived cell lines whereas overexpression of MYC or MYCN in mature cells from the myeloid lineages was tumorigenic. Leukemia can be subdivided into several different types where for example the highly malignant leukemia subtype acute promyelocytic leukemia (APL) originates from the promyelocytic (reviewed in (Delgado and Leon, 2010)).

Amplifications of *MYC* or *MYCN* are often observed in solid tumors such as breast cancer, gastric cancer, prostate cancer and neuroblastoma (reviewed in (Vita and Henriksson, 2006)). As one of the most common solid tumors in children, neuroblastoma and is the cancer that result in most deaths in infants (reviewed in (Brodeur, 2003)). The most cases of neuroblastoma are diagnosed during the first year of life (Hogarty, 2003). Approximately, 800 new cases in the US, 100 new cases in the United Kingdom and 20 new cases in Sweden occur each year. Neuroblastoma tumors arise in the sympathetic nervous system from the developing neural crest and are most likely caused by aberrations in normal developmental processes (Seeger et al., 1985). The neural crest is an embryonic structure that arises from ectoderm during the closure of the neural tube. Proper interplay between Wnt and Hedgehog signaling is important for the formation of the neural crest (Fodde and Brabletz, 2007). Both these signaling pathways induce MYCN that stimulates proliferation and migration of neuroblasts whereas decreased levels of MYCN is associated with terminal differentiation. Amplification of *MYCN* occurs in neuroblastoma and is one of the predictors of poor clinical outcome (reviewed in (Brodeur, 2003)). High-risk tumors without *MYCN* amplification often express increased levels of c-MYC (Westermann et al., 2008). In addition to amplifications of *MYCN* neuroblastoma are often associated with chromosomal abnormalities such as deletion in chromosome 1q, 11q and 14q and gain of chromosome 13q (reviewed in (Bown, 2001)). The clinical outcome for neuroblastoma patients varies from low-grade tumors that often regress spontaneously, to differentiating tumors, tumors that are cured with chemotherapy to metastatic tumors that are currently not curable (reviewed in (Weinstein et al., 2003)).

Cellular activities regulated by MYC

As described *MYC* is often de-regulated in human tumors and as an oncogene *MYC* induces genomic instability, uncontrolled cell proliferation, independence of growth factors, immortalization and escape from immune surveillance (Figure 5). However, as a proto-oncoprotein MYC is involved in a several cellular processes regulating the normal cell such as proper regulation of cell growth and division, cell cycle progression, apoptosis, inhibition of cell differentiation, metabolism, angiogenesis, cell adhesion and motility (Figure 5) (reviewed in (Vita and Henriksson, 2006)). Some of these cellular activities mediated by MYC will be discussed below.

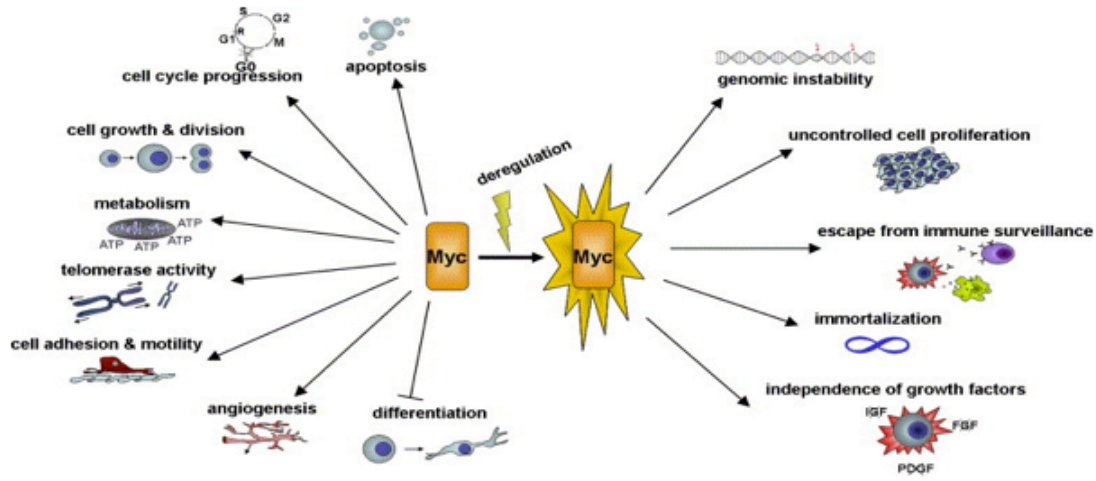

Figure 5. Cellular processes controlled by MYC in normal cells and during tumorigenesis. Re-printed from (Vita and Henriksson, 2006) with permission from the publisher.

MYC - cell proliferation and cell cycle progression

The cell cycle is divided into the following phases: G1, S, G2 and M. Resting, non-proliferating cells exit the cell cycle and enter G0 (quiescent state). G1 is the time from the last cell division until initiation of DNA synthesis. In the G1 phase the restriction point or the point of no return decides if cells are entering the cell cycle or growth arrest. During the S phase the DNA is replicated. Before and after DNA replication there are two additional checkpoints in the G1/S and G2/M transitions, respectively. During the G2 phase cells are prepared to enter mitosis and cell division. The M phase is when mitosis occurs and the cellular material is divided into the two daughter cells. Mitosis is divided in four sub-phases: prophase (condensation of the DNA), metaphase (alignment), anaphase (separation) and telophase (decondensation of the DNA) (reviewed in (Nurse, 2000)).

Cyclins, cyclin-dependent kinases (CDKs) and CDK inhibitors (CDKIs) are important factors for regulation of cell cycle progression. CDKs are serine/threonine kinases, which phosphorylates its substrate once bound by cyclins (Morgan, 1997). Upon mitogenic signals in early G1 Cyclin D1, D2 and D3 are up regulated and associate with CDK4 and CDK6, which phosphorylate pRb. This in turn inhibits the interaction

between pRb and E2F, which then can transcriptionally activate *Cyclin A*, *Cyclin E, CDK1/2*. Cyclin E/CDK2 complexes are active near the restriction point in late G1 and are required for S phase entry. Cyclin E/CDK2 is repressed by $p27^{Kip1}$, which also interact with Cyclin D/CDK4/6. The increased levels of Cyclin D during G1 phase increases the amount of $p27^{Kip1}$ to be sequestered by Cyclin D/CDK4/6 complexes, resulting in elevated levels of active Cyclin E/CDK2. Once cells have entered the S phase CDK2 associates with Cyclin A and allows progression through the S phase. Later in the S phase Cyclin A associates with CDK1 (Pagano et al., 1992). As cells enter G2 Cyclin B/Cdk1 triggers mitosis. In addition, CDK4 inhibitors (INK4) including $p16^{INK4A}$, $p15^{INK4B}$, $p18^{INK4C}$, $p19^{INK4D}$ that binds CDK4 and 6 and inhibits binding of D type Cyclins and Cip/Kip inhibitors including p21*Cip1*, p27*Kip1*, p57*Kip2* and targets CDK1/2 and inhibits binding of Cyclin E, A and B (Aprelikova et al., 1995; Toyoshima and Hunter, 1994).

MYC plays an important role during cell cycle progression and especially during G1/S transition when MYC levels are rapidly induced (Mateyak et al., 1997). It transcriptionally activates the expression of *E2F*, *Cyclin D1*, *Cyclin D2*, *Cyclin E*, *CDK1*, *CDK2* and *CDK4* (reviewed in (Pelengaris et al., 2002)). In addition, MYC induces the expression of miR-17-5p and miR-20a, which negatively regulate E2F expression. By this MYC tightly controls proliferation by both activating E2F transcription and regulating its translation (O'Donnell et al., 2005). Furthermore, MYC represses the transcriptional activation of CDK inhibitors $p15^{INK4B}$ and $p21^{Cip1}$ through interaction with Zinc finger protein MIZ-1, as will be discussed later (reviewed in (Adhikary and Eilers, 2005)).

MYC - apoptosis

Deregulation of *MYC* does not only drive cell proliferation and growth but does also sensitize cells for apoptosis or programmed cell death. Too high expression of MYC or lack of survival factors triggers apoptosis (Askew et al., 1991; Evan et al., 1992; Harrington et al., 1994). In addition, *Myc* null cells have been shown to be resistant to apoptosis stimuli (de Alboran et al., 2004; Soucie et al., 2001). A variety of different cellular pathways, such as ligation of the Fas death receptor, serum deprivation, hypoxia, and cytotoxic drugs sensitize cells to MYC induced apoptosis (reviewed in (Meyer and Penn, 2008; Nilsson and Cleveland, 2003)). Stressed cells thereby induce apoptosis as a response to MYC activation. Survival signaling via the PI3-kinase or NFκB pathways can rescue these cells from undergoing apoptosis. The pathways by which MYC mediates apoptosis are not completely uncovered but at least two major pathways have been proposed. First, MYC activates p53 directly (Hermeking and Eick, 1994; Wagner et al., 1994) or indirectly by up regulation the tumor suppressor protein p19ARF (p14ARF in humans) (Zindy et al., 1998), which in turn activates p53 by binding and sequestering the MDM2 E3 ligase, which normally targets p53 for degradation (reviewed in (Larsson and Henriksson, 2010)). The tumor suppressor p53 activates pro-apoptotic genes such as *BAX* and *PUMA* as well as genes encoding for proteins involved in cell cycle arrest including *p21CIP1*. However, MYC also counteracts p53-induced cell cycle arrest by repressing transcription of the CDK

inhibitor p21CIP1 through interaction with MIZ-1 (Herold et al., 2002; Seoane et al., 2002; Wu et al., 2003). Secondly, MYC alters the balance between pro- and anti-apoptotic factors where MYC indirectly suppresses expression of the anti-apoptotic proteins BCL-2 and BCL-XL (Eischen et al., 2001a; Eischen et al., 2001b; Maclean et al., 2003). These two proteins regulate BAX, which in turn controls release of cytochrome c from the mitochondria membrane (Dansen et al., 2006; Juin et al., 2002). MYC also regulates the conformational change that activates BAX (Annis et al., 2005; Soucie et al., 2001). In the cytoplasm, cytochrome c mediates the formation of the apoptosome that triggers the activation of downstream caspases, which eventually kills the cell (reviewed in (Larsson and Henriksson, 2010)).

MYC – differentiation

Inhibition of cell differentiation was one of the first cellular activities described to be regulated by MYC. MYC was shown to block the chemically induced erythroid differentiation of Friend murine erythroid leukemia cells (Coppola and Cole, 1986; Dmitrovsky et al., 1986; Prochownik and Kukowska, 1986). At the same time development of B lymphocytes was shown to be impaired prior to lymphoma progression in young Eμ-*MYC* transgenic mice (Langdon et al., 1986). Since then, expression of MYC has been shown to inhibit cellular differentiation in a number of primary cells and cell lines, including for example hematopoietic cells, adipocytes, neuronal and muscular cells (Leon et al., 2009). In addition, MYC was shown to inhibit differentiation *in vivo* (Bettess et al., 2005; Dubois et al., 2008) and premature differentiation in neuronal progenitor cells was inhibited by MYCN (Knoepfler et al., 2002). MYC also inhibits differentiation of murine embryonic stem cells (Cartwright et al., 2005). Interestingly, MYC was also shown to be one of the four transcription factors needed to reprogram differentiated murine and human cells into induced pluripotent stem cells (Lowry et al., 2008; Okita et al., 2007; Park et al., 2008; Takahashi et al., 2007; Wernig et al., 2007). In addition, results obtained from transgenic mice carrying conditional *Myc* alleles indicate that anti-differentiation activity mediated by MYC might be critical for MYC induced tumorigenesis. In most of these models inactivation of *Myc* result in tumor regression, which was suggested to be due to re-differentiation of tumor cells (Leon et al., 2009). However, MYC has also been shown to enhance differentiation in human keratinocytes (Gandarillas and Watt, 1997) and in several mouse transgenic models MYC stimulates differentiation of precursor cells from mammary tissue, epidermis and blood (reviewed in (Leon et al., 2009)).

Whereas MYC is efficiently translocated to the nucleus in proliferating cells, where it activates genes involved in proliferation and represses genes involved in differentiation cleavage of MYC creating MYC-nick was proposed to regulate MYC upon terminal differentiation. The increased levels of intracellular calcium and activated calpains cleave the MYC C-terminal domain upon differentiation. Cleavage of MYC together with down regulation of MYC reduces the level of nuclear MYC and genes involved in differentiation are de-repressed. In addition, MYC-nick has been shown to localize in the cytoplasm where it contributes to terminal differentiation by acetylation of α-

tubulin by recruitment of the HAT GCN5 to microtubules (Conacci-Sorrell and Eisenman, 2011). In addition, cells that are terminally differentiated usually correlates with permanent cell cycle exit. Since MYC regulates proliferation and cell cycle progression (as discussed above) MYC has been suggested to inhibit differentiation by preventing exit from the cell cycle to maintain cells in the proliferative state. However, it has also been shown that MYC inhibits differentiation by blocking induction of transcription factors, such as c-JUN and GATA-1, which are important to control neuronal and erythroid differentiation in cell lines, respectively. MYC might repress induction of these transcription factors directly or indirectly via for example induction of miRNAs. In addition, MYC could inhibit differentiation by antagonizing transcriptional activity of its antagonists MXD and MNT, which are active in differentiated cells and will be discussed below (reviewed in (Leon et al., 2009)).

MYC - cellular transformation

Deregulated expression of MYC induces both proliferation and apoptosis depending on the level of MYC and surrounding survival/growth factors. Suppression of MYC-induced apoptosis is crucial for cellular transformation. Co-expression of *MYC* and oncogenic *RAS* was shown to be enough to induce transformation of rodent primary cells (Land et al., 1983). By regulating MYC stability and degradation through MAPK signaling, RAS is suggested to inhibit MYC induced apoptosis (Kauffmann-Zeh et al., 1997). In addition, RAS induces the AKT pathway, which phosphorylates FOXO1 and FOXO3a that normally bind to many MYC target genes and targets these for proteasomal degradation. RAS also induce PI3K/Rho-dependent phosphorylation of MYCS71, which results in repression of Thrombospondin-1 and increased angiogenesis (reviewed in (Adhikary and Eilers, 2005)). Furthermore, cells are transformed by *MYC* and *BCL-2*, in which the latter abrogates MYC induced apoptosis (Bissonnette et al., 1992; Fanidi et al., 1992; Strasser et al., 1990).

In mice tumor growth is dependent on MYC as inactivation of *Myc* results in tumor regression in *Myc* transgenic mice models. *Myc* inactivation results in proliferative arrest, differentiation, senescence and/or apoptosis. However, many tumors re-appear once *Myc* is activated again (Arvanitis and Felsher, 2006). Inactivation of MYC by a dominant negative *Myc* (OmoMyc) results in tumor regression of *K-RAS* driven lung tumors with reversible or mild side effects. Tumors cells, which are dependent on MYC for their high metabolic rate undergoes apoptosis whereas normal cells exhibit reduced proliferation rate upon MYC inactivation (Sodir et al., 2011; Soucek et al., 2008). Recently, inactivation of MYC by OmoMyc in mice was shown not only to regress tumor growth but also to eradicate *RAS*-driven lung tumors (Soucek et al., 2013). Since MYC is deregulated in many human tumors an attractive target for therapy would be to inhibit MYC and/or MYC pathways.

Transcriptional regulation by the MYC/MAX/MXD/MNT network

MYC is part of a network of transcription factors, which includes MXD1, MXD2, MXD3, MXD4 (formerly MAD1, MXI1, MAD3, MAD4), MAX, MGA and MNT). These proteins all belong to the basic Helix-Loop-Helix Leucine Zipper (bHLHZip) family of transcription factors (Figure 6).

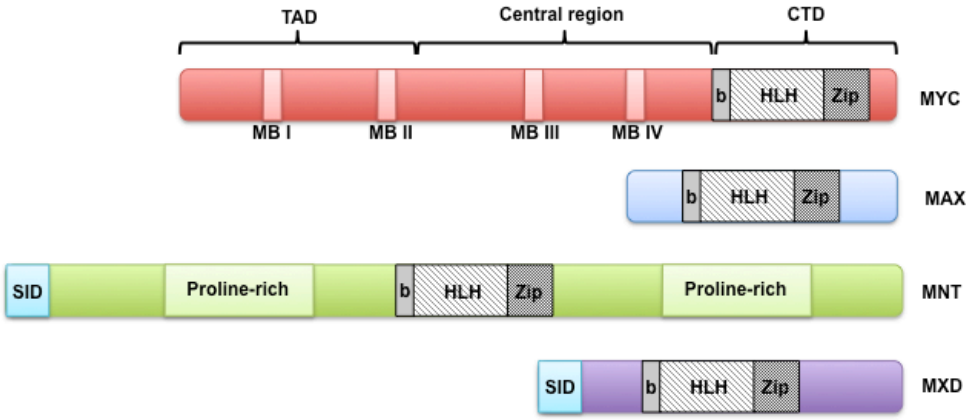

Figure 6. Protein domain structure of MYC, MAX, MNT and MXD. All proteins belong to basic helix-loop-helix-Leucine Zipper (bHLHZip) family of transcription factors and contain basic, HLH and Zip regions. MYC proteins also contain MYC boxes (MBI, MBII, MBIII, MBIV) whereas MXD and MNT have a SIN3 interacting domain (SID). The MYC protein consists of three regions, the N-terminal transactivation domain (TAD), the central region and the C-terminal domain (CTD).

To regulate transcription these proteins need to bind 5'-CACGTG-3' E-box sequences in the DNA of target promoters as dimers with the central player of the network MAX (Figure 7). Dimerization with MAX is mediated through the HLHZip motif and binding to DNA by the basic domain (Figure 6) (Ayer et al., 1993; Blackwell et al., 1990; Blackwood and Eisenman, 1991; Blackwood et al., 1992; Hurlin et al., 1997a; Hurlin et al., 1995b; Hurlin et al., 1999; Meroni et al., 1997; Murre et al., 1989; Prendergast et al., 1991; Zervos et al., 1993). MAX, which is also a bHLHZip protein was identified in a screen of a human cDNA library designed to identify proteins interacting with MYC (Blackwood and Eisenman, 1991; Prendergast et al., 1991). MAX is the most evolutionary conserved member of the network and is ubiquitously expressed with a relative long half-life (reviewed in (Hurlin and Huang, 2006)). Deficiency of *Max* result in severely altered phenotype and early embryonic lethality in mice (Gilladoga et al., 1992; Shen-Li et al., 2000).

Whereas MYC/MAX activates transcription by recruitment of co-factors containing HAT activity, MXD/MAX and MNT/MAX repress transcription by chromatin condensation mediated by associated HDAC activity (Figure 7) (reviewed in (Wahlstrom and Henriksson, 2007)). Transcriptional regulation by MYC and MNT will be discussed in more detail below.

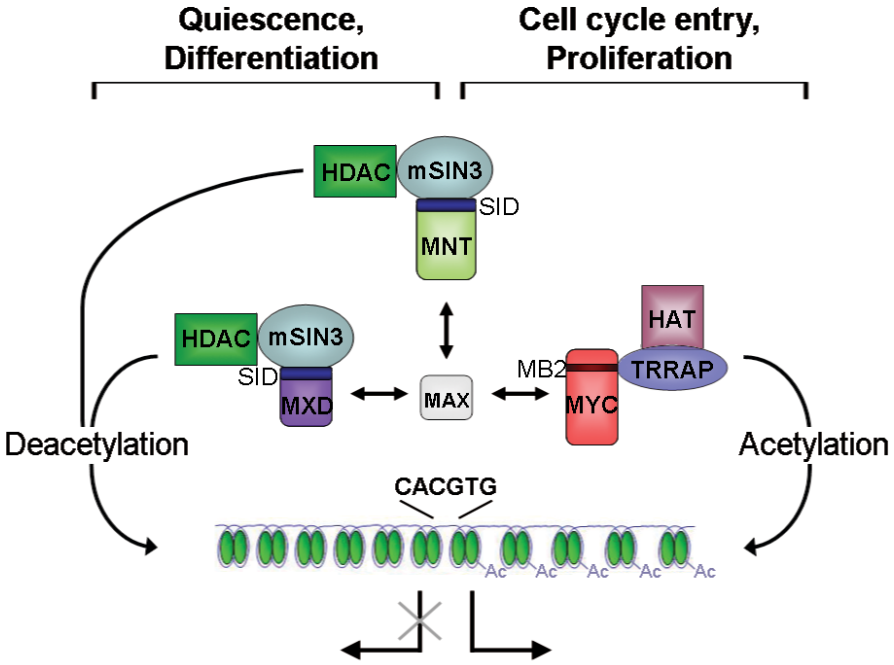

Figure 7. MYC/MAX, MXD/MAX and MNT/MAX heterodimers bind to E-boxes in promoters of target genes and activate or repress transcription by recruitment of co-factors, which contain either HAT or HDAC activity. MYC interacts with co-factors such as Transformation/Transcription domain-Associated Protein (TRRAP) through Myc box II (MB2) and MXD/MNT proteins interact with mSIN3 via the SIN3 interacting domain (SID). Adapted from (Wahlstrom and Henriksson, 2007) with permission from the publisher.

The MYC protein

The MYC protein consists of three regions: the N-terminal transactivation domain (TAD), a central region and the carboxy-terminal domain (CTD) (Figure 6). The bHLHZip domain needed for MAX heterodimerization and DNA binding is located in the CTD and the nuclear localization signal (NLS), needed for nuclear transport in the central region of the MYC protein (reviewed in (Henriksson and Luscher, 1996)). However, in order for MYC to regulate transcription the TAD domain is required. This region of MYC interacts with several proteins including the transcription factor TBP, which was shown to induce a more structured folding of the TAD domain (McEwan et al., 1996). The TAD domain contains two MYC boxes (MB I and MB II) (Figure 6). These were identified in a screen for domains in the MYC protein needed for MYC induced cellular transformation (Stone et al., 1987). MB I and II were shown to be necessary for transformation of REFs by *MYC* and *H-RAS* as well as for MYC to induce apoptosis and to block differentiation (Evan et al., 1992; Freytag et al., 1990). The MYC boxes are highly homologous regions that are evolutionary conserved between different species (Amati et al., 1992). Although MYCL is more distantly related compared to c-MYC and MYCN it shares several MYC boxes.

MB I contains phosphorylation sites (S62 and T58), which are important for regulation of MYC stability and degradation. RAS mediates phosphorylation of S62 through the RAF-MAPK pathway (Figure 4) and inhibits glycogen synthase kinase -3 (GSK3) through Phosphatidylinositol 3-kinase (PI3K) signaling resulting in increased protein stability. Once S62 is phosphorylated T58 can be phosphorylated by GSK3 (Henriksson et al., 1993). Phosphorylated T58 is recognized by prolyl isomerase (PIN1) which results in isomerization of Pro59 and Phosphatase-2A (PP2A) can de-phosphorylate S62. The ubiquitin ligase FBW7 poly-ubiquitinates MYC when phosphorylated at T58 and labels the MYC protein for degradation by the proteasome (reviewed in (Adhikary and Eilers, 2005)). MYC and proteasomes were suggested to accumulate at the nucleoli where downregulation of MYC occur (Arabi et al., 2003). In addition, the SKP2 ubiquitin ligase also regulates MYC degradation but was in contrast to FBW7 shown also to be involved in MYC induced transactivation (von der Lehr et al., 2003). The MYC protein normally has a short half-life of about 20 - 30 minutes. However, mutations in MB I affect the turnover rate (Gregory and Hann, 2000; Sears et al., 2000) and mutations within this region have been observed in some tumors (Bhatia et al., 1993; Lutterbach and Hann, 1994). It has also been shown that deletions and mutations of phosphorylation sites in this region affect MYC induced cellular transformation (Henriksson et al., 1993; Kim et al., 2003; Stone et al., 1987).

MB II is needed for transcriptional activities since most of the co-factors, which will be described later, bind to this region in the MYC protein. Deletion of MBII reduced MYC induced transactivation dramatically. Mutations in MBII has been shown to inhibit binding of co-factors and does also inhibit the corresponding phenotype (Conzen et al., 2000; Cowling and Cole, 2006; Evan et al., 1992; Freytag et al., 1990; Nikiforov et al., 2002; Oster et al., 2003).

Two additional MYC boxes have been identified, MBIII and MBIV, which are located in the central region (Figure 6). MBIII is involved in apoptosis, transformation, and tumorigenesis (Herbst et al., 2005) whereas MBIV is needed for MYC induced transformation and apoptosis. In addition, deletion of MB IV potentiates MYC induced G2-arrest (Cowling et al., 2006).

Transcriptional activation by MYC

In order to activate transcription MYC need to bind, as a heterodimer with MAX, to E-box sequences in the promoter regions of target promoters (Figure 6). However, MYC is a relatively weak transcriptional activator and activates transcription with approximately two-fold (reviewed in (Cowling and Cole, 2006)). Instead MYC/MAX binds to 10-15 % of the human genome (reviewed in (Luscher and Vervoorts, 2012)) and has been shown to bind preferentially to promoters within active chromatin displaying histone marks such as H3K4me2, H3K4me3, H3K79me3, H3K9ac, H3K18ac and H3K27ac (Guccione et al., 2006; Martinato et al., 2008). In addition, MYC binds to genomic regions containing CpG islands, which is often associated with transcriptionally active promoters (Fernandez et al., 2003). Furthermore, MYC was recently proposed to act as an amplifier of gene expression rather than mediating the

on-off switch since it accumulates at already active genes (Lin et al., 2012; Nie et al., 2012). MYC has also been suggested to be involved in regulating release of paused RNA Pol II, rather than recruiting the polymerase to target promoters (Rahl et al., 2010). Taken together, MYC is suggested to be involved in fine-tuning of gene expression.

To activate transcription MYC recruits co-factors containing HAT activity to the promoter region of its target gene (Figure 7). Several interacting proteins associate with the MYC N-terminus especially with the MYC boxes (Figure 6). TRRAP was identified to bind directly to the MYC TAD (Brown et al., 2001; McMahon et al., 1998; Park et al., 2001) and was shown to be an essential co-factor for MYC-mediated functions since deletions in MB I and II, which inhibits cellular transformation also inhibited the binding between TRRAP and MYC (McMahon et al., 1998; Nikiforov et al., 2002). In addition, TRRAP is recruited to MYC target genes upon serum stimulation (Bouchard et al., 1999; Frank et al., 2001). Transcriptional activation induced by MYC is associated with increased promoter histone acetylation mediated by recruited HAT activity through interaction with TRRAP, which is part of a large multi-protein complex containing HATs such as GCN5 (Liu et al., 2003; McMahon et al., 2000), TIP60 (Frank et al., 2003; Ikura et al., 2000) and the ATPase domain-containing co-factors TIP48 (RUVBL2) and TIP49 (RUVBL1) (Ikura et al., 2000), which also interact with MYC independently of TRRAP (Wood et al., 2000). In addition, MYC also binds the co-activators p300 and CREB binding protein (CBP), which also possesses HAT activity (Faiola et al., 2005; Vervoorts et al., 2003). Once HATs are recruited to the promoter, specific histone tails are acetylated at different positions creating the histone code. Acetylated H3K9, H3K14, H3K18, H4K5, and H4K12 are some examples of marks generated upon MYC binding at target promoters (Martinato et al., 2008).

MYC also activates transcription without mediating increased histone acetylation (Eberhardy et al., 2000). Deletion of MBII was shown to inhibit binding of TRRAP and reduces transcriptional activation of most target genes. However, some MYC target genes are still activated independently of TRRAP binding and recruited HAT activity (Cowling et al., 2006; Nikiforov et al., 2000). For example, MYC was shown to regulate RNA Pol II promoter clearance and efficient transcription elongation by recruiting the RNA Pol II CTD kinase TFIIH and the positive transcription elongation factor (P-TEFb) to target promoters with stalled RNA Pol II to promote phosphorylation of the RNA Pol II CTD (Cowling and Cole, 2006). Moreover, MYC stimulates gene expression by mediating enhanced mRNA 5’ cap methylation (Cole and Cowling, 2009). In addition, MYC regulates the chromatin structure by recruiting the ATP-dependent chromatin remodeling complex SWI/SNF (Cheng et al., 1999; Park et al., 2002).

MYC induced transcriptional repression

MYC is not only a transcriptional activator but can also repress transcription by blocking MIZ-1 induced transactivation (reviewed in (Adhikary and Eilers, 2005)). MIZ-1 binds to INR elements the core promoter of its target genes and activates transcription by recruitment of p300. MYC/MAX heterodimers was shown to block MIZ-1 induced activation of the genes encoding the CDK-inhibitors $p21^{Cip1}$ and $p15^{INK4B}$ by disrupting the interaction between MIZ-1 and p300 as well as by recruiting DNA methyltransferase DNMT3a (Brenner et al., 2005; Herold et al., 2002; Seoane et al., 2002; Staller et al., 2001; Wu et al., 2003). The ATPases TIP48 and TIP49 have also been suggested to be involved in MYC induced transcriptional repression (Bellosta et al., 2005; Etard et al., 2005; Wood et al., 2000). Apart from MIZ-1 there are additional transcription factors, such as Specificity protein-1 (SP1) that potentially recruit MYC to core promoters without E-box elements (Gartel et al., 2001).

MYC target genes

As was mentioned previously MYC binds to 10-15 % of the human genome and is regulating a large number of target genes. Many high-throughput screens based on microarray gene expression profiling, chromatin immunoprecipitation (ChIP) followed by genomic array analysis, serial analysis of gene expression (SAGE) and MYC methylase chimeric proteins have given information about the number and identity of genes regulated by MYC. Even though MYC is binding and regulating transcription globally target genes are over-represented by genes involved in cell cycle regulation, metabolism, ribosome biogenesis, protein synthesis and mitochondrial function. In addition, MYC represses genes involved in growth arrest and cell adhesion. Many MYC target genes have been described. Some examples are *Cyclin D2*, *CDK4*, *Cyclin E*, *p21*, *p15INK4A*, *human telomerase reverse transcriptase (hTERT)*, *Ornithine decarboxylase (ODC)* and *Carbamoylphosphate dihydroorotase (CAD)*. MYC also targets miRNAs, which in turn affect the stability of a large number of mRNAs (Dang et al., 2006). In this thesis two bona fide MYC target genes, *cyclin D2* and *hTERT* as well as the miR-17-92 cluster has been studied.

Cyclin D2

Cyclin D2 has an important role in the early G1 phase of the cell cycle. By regulating the expression of *cyclin D2* the MYC network plays a role in cell cycle progression. Whereas MYC/MAX bind to the E-boxes in the *cyclin D2* promoter and activates its transcription MXD/MAX or MNT/MAX represses the promoter by binding to the same binding sites (Bouchard et al., 2001; Bouchard et al., 1999; Popov et al., 2005).

hTERT

hTERT is the catalytic subunit of the enzyme telomerase, which extends chromosomal ends with telomeric repeats (Greider and Blackburn, 1987). Telomerase and *hTERT* is expressed during embryonic development and is repressed upon differentiation of embryonic cells (Forsyth et al., 2002; Rama et al., 2001; Ulaner et al., 1998). In contrast, *hTERT* and telomerase are not expressed in normal somatic cells, except germ cells and stem cells. Telomeres are shortened with every cell division, which limit the

lifespan of normal cells and tissues (Hayflick and Moorhead, 1961). By activating *hTERT* expression and telomerase activity immortal cells overcome telomeric loss and can divide unlimited and thereby avoid replicative senescence (Bodnar et al., 1998). However, in order to transform normal cells *hTERT* need to be expressed together with the simian virus 40 large T oncoprotein and H-RAS (Hahn et al., 1999).

The *hTERT* promoter is GC-rich and lacks TATA box and CAAT boxes but is rich in binding sites for a variety of transcription factors (Figure 8) (Cong et al., 1999; Horikawa et al., 1999; Kyo et al., 2008; Poole et al., 2001). Among these binding sites the *hTERT* promoter contains two E-boxes, bound by MYC/MAX, MXD/MAX and MNT/MAX heterodimers (Figure 8) (Oh et al., 2000; Wahlstrom et al., 2013; Wang et al., 1998; Xu et al., 2001).

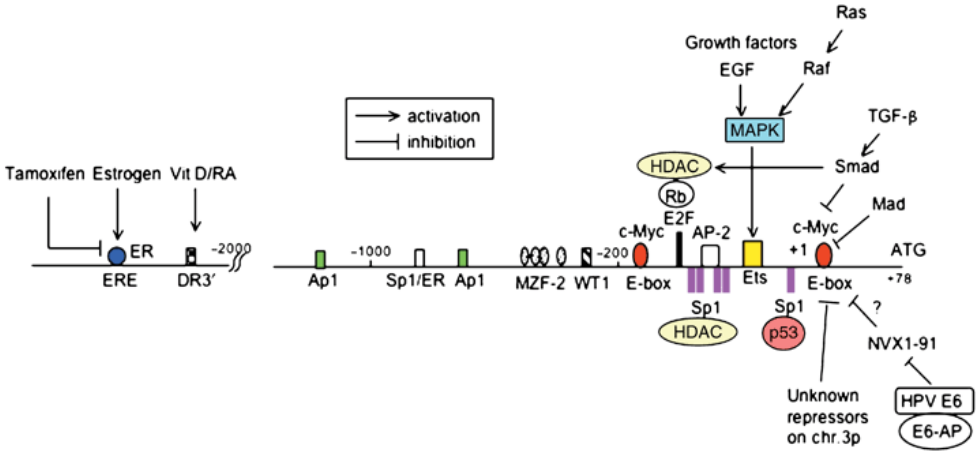

Figure 8. A schematic picture of the hTERT promoter, which contains several binding sites for various transcription factors including E-box elements bound by MYC/MAX/MXD/MNT network proteins. Adapted from (Kyo et al., 2008) with permission from the publisher.

Since telomerase is expressed in approximately 90 % of all tumors (Kim et al., 1994) several studies has suggested detection of telomerase activity as a prognostic marker and predictor of clinical outcome. For example, there is a strong correlation between high telomerase activity and poor prognosis in neuroblastoma, gastric cancer and non-small lung cancer (Hiyama and Hiyama, 2003). Furthermore, mutations in the *hTERT* promoter creating new binding motifs for ETS have been identified in human melanoma (Horn et al., 2013; Huang et al., 2013).

miR-17-92 cluster

The polycistronic miR-17-92 cluster encodes several miRNAs including miR-17, miR-18a, miR-19a, miR-19b, miR-20a and miR-92a. These miRNAs regulates the stability of a variety of different mRNAs involved in several cellular processes including cell proliferation and cell survival during normal development (Ventura et al., 2008). The miR-17-92 cluster is potent oncogenic miRNAs, which has been shown to be overexpressed in several tumor types (He et al., 2005; Volinia et al., 2006). In addition, miR-17-92 is located at chromosome 13q31, in a region that is frequently amplified in

Burkitts lymphoma, diffuse large B-cell lymphoma and lung cancer (Hayashita et al., 2005; Ota et al., 2004; Tagawa and Seto, 2005; Takakura et al., 2008). The miR-17-92 cluster is transcriptionally regulated by transcription factors including MYC (O'Donnell et al., 2005). In addition, we showed that miR-17-92 is upregulated in MYCN overexpressing neuroblastoma cells (Paper IV). The miR-17-92 cluster in turn interferes with the expression of several mRNAs including the tumor suppressors p21 and PTEN, the pro-apoptotic protein BIM, the nuclear hormone receptor estrogen receptor alpha (ERα) and the cell cycle regulator E2F1 (Fontana et al., 2008; Loven et al., 2010; Mu et al., 2009; O'Donnell et al., 2005; Olive et al., 2009; Ventura et al., 2008).

Transcriptional repression by MXD and MNT

MXD1 and MNT represses MYC mediated cellular activities such as proliferation, apoptosis and MYC/RAS induced cellular transformation of REFs (Cerni et al., 2002; Chen et al., 1995; Foley et al., 1998; Hurlin et al., 1997a; Queva et al., 1999). MXD/MAX and MNT/MAX heterodimers antagonize MYC induced transcriptional activation. The N-terminal SID in MXD and MNT proteins allow interaction with SIN3A and SIN3B (Figure 6, 7) (Ayer et al., 1995; Hurlin et al., 1997a). As scaffold and co-repressor proteins SIN3A and SIN3B transcriptionally silence genes through recruitment of HDACs, which de-acetylates histone tails causing condensation of the chromatin structure (Gallinari et al., 2007).

MXD1, MXD2, MXD3 and MXD4 have highly homologous sequences and similar functions. These are small, relatively short-lived proteins of about 30-35 kDa in size that are expressed differently in a tissue-specific manner (Queva et al., 1998). The expression of MXD1 is very low in proliferating cells. However, upon differentiation the levels of MXD1 are up-regulation followed by down-regulation of MYC. In contrast, MXD2 is associated with differentiation especially in hematopoietic cells but is also expressed in proliferating cells (Larsson et al., 1994). The expression of MXD3 and MXD4 is however restricted to S phase and is almost undetectable in adult tissues. Even though MXD3 is expressed in S phase it inhibits proliferation and transformation (Hurlin et al., 1995a). In contrast, MGA and MNT are larger proteins and apart from the similarity of the bHLHZip domain, heterodimerization with MAX and binding to E-box sequences in the DNA, these two proteins differ from the other four members of the MXD family of transcription factors (Figure 6). MGA contains a T-box domain, which is a DNA binding domain found in the TBX family of proteins (Hurlin et al., 1999). The biological function of MGA is unknown but it has been suggested to be part of an E2F6 repression complex (Ogawa et al., 2002). MNT, which is ubiquitously expressed does however contain the SID domain and represses transcription in a similar manner as the MXD proteins (Figure 7). However, deletion of the SID domain in MNT protein turn MNT into a transcriptional activator, which in contrast to MNT wt does not inhibit MYC/RAS induced cellular transformation. MNT containing a deletion of the SID domain also transforms primary cells with RAS alone. MNT contains proline-rich structures containing multiple repeats of proline-alanine/leucine-proline sequences (Figure 6). This region resembles the activation domain of several different classes of

transcription factors including MB I of the MYC activation domain (Hurlin et al., 1997a).

Target genes of MXD and MNT overlap with MYC targets (Nikiforov et al., 2003). Two well characterized target genes regulated by MYC, MXD and MNT are *cyclin D2* and *hTERT*. MXD1 was shown to bind and repress transcription of both these promoters in differentiated cells whereas the promoters were occupied and transcriptionally activated by MYC in proliferating cells (Bouchard et al., 2001; Xu et al., 2001). In addition, to regulate the status of acetylation MXD1 was shown to recruit the histone demethylase Retinoblastoma binding protein 2 (RBP2) or JARID1A/KDM5A to the *hTERT* promoter resulting in reduced levels of H3K4me3 (Ge et al., 2010). Interestingly, MNT bind to the promoters in both proliferating and differentiating cells (Popov et al., 2005; Xu et al., 2001). The functions of MNT will be discussed below.

MNT

MNT was discovered in 1997 in two independent screens. Hurlin *et al.,* identified the murine *Mnt* in a yeast-two hybrid screen of a cDNA library from day 9.5 and 10.5 mouse embryos (Hurlin et al., 1997a) whereas Meroni *et al.,* made a search for transcribed sequences from the human chromosome 17p13.3 and found the human homologue of *MNT*, initially termed *ROX* (Meroni et al., 1997).

Key regulator of the MYC/MAX/MXD/MNT network

Whereas MYC is strongly induced upon mitogen stimulation and thereafter expressed at low levels in proliferating cells, MNT has been detected in quiescent cells, throughout the cell cycle and during differentiation (Hurlin et al., 1997a; Hurlin et al., 2003; Hurlin et al., 2004; Popov et al., 2005). Efficient translation of *Mnt* transcripts under conditions such as quiescence and terminal differentiation when cap-dependent translation initiation is reduced might be mediated through the internal ribosome entry segment (IRES) present in the *Mnt* promoter (Stoneley et al., 2001). MNT is thus co-expressed with MYC in proliferating cells although it possess the opposite function (Hurlin et al., 1997a; Hurlin et al., 1997b; Popov et al., 2005; Pulverer et al., 2000; Smith et al., 2004; Sommer et al., 1998; Sommer et al., 1999).

MNT is a short-lived protein that migrates as a doublet of 72 and 74 kDa in SDS-PAGE. The slower migrating form of MNT is a phospho-protein. Phosphorylation is mediated by kinases in the MAPK/ERK pathway and regulates the functional activity of MNT by inhibiting the interaction between MNT and mSIN3A or mSin3B (Popov et al., 2005). MNT protein stability is also regulated by ERK mediated phosphorylation (Paper II). Similarly, the stability of MYC, MNT and MXD4 were stabilized upon stimulation of T cells. Whereas up-regulation of MYC promotes T cell proliferation the up-regulated levels of MXD4 and MNT were suggested to inhibit MYC-dependent cell death (Vasilevsky et al., 2011). In addition, the *Mnt* promoter has been identified as a

target for MYC in a serial analysis of gene expression (SAGE) approach validated by ChIP showing *in vivo* binding of MYC to the *Mnt* promoter (Menssen and Hermeking, 2002).

MNT/MAX complexes have been shown to bind with similar affinity to the consensus E-box sequence CACGTG as MYC/MAX heterodimers (Hurlin et al., 1997a). In addition, MNT/MAX also bind to non-consensus E-box binding element and has been shown to bind with higher affinity to CACGCG sites (Meroni et al., 1997). Differential binding of MYC/MAX and MNT/MAX heterodimers to DNA might influence regulation of the transcriptional activity of target genes. The transcriptional outcome of common target genes also depends on the relative expression of MYC and MNT as well as their competition for MAX. Concomitant with the increase in MYC expression upon cell cycle entry a shift from MNT/MAX to more MYC/MAX binding was observed at common target genes even though the total level of MNT remained unchanged (Hooker and Hurlin, 2006; Walker et al., 2005). The ratio between MYC/MAX and MNT/MAX at shared target genes was shown to regulate transcription of genes encoding for proteins involved in cell cycle regulation. In support, deletion of *Mnt* was shown to accelerate the G1 to S transition whereas overexpression of *Mnt* suppressed cell cycle entry and proliferation (Hurlin et al., 2003; Walker et al., 2005). In addition, a switch from MNT/MAX to MYC/MAX was shown at the *p53* and *cyclin D1* promoters resulting in increased expression of p53 and Cyclin D1 as well as apoptosis during cholestasis in mouse and human hepatocytes (Yang et al., 2009). Furthermore, accelerated progression of cholangiocarcinoma induced by chronic cholestasis was mediated by a mechanism involving down-regulation of miR-34a, up-regulation of miR-210 and a switch from MNT/MAX to MYC/MAX at the *Cyclin D1* promoter (Yang et al., 2011). The up-regulated levels of miR-210 might influence the level of MNT since MNT was identified as a target of miR-210 (Zhang et al., 2009). MNT mediated transcriptional repression has therefore been suggested to be of greater importance than sole activation by MYC. In support, relief of MNT mediated transcriptional repression by *Mnt* targeting RNAi allowed activation of MYC target genes (Paper I). We have also shown that MNT acts as a strong transcriptional repressor that needs to be expressed at low levels in order for co-expressed MYC to activate transcription (Paper III). MNT has thus been suggested as the key regulator of the MYC/MAX/MXD/MNT network.

MNT – a potential tumor suppressor

MNT has been proposed as a potential tumor suppressor based on several findings. First, *MNT* is located at chromosome 17p13.3 in a region, which frequently undergoes loss of heterozygosity (LOH) in several malignancies such as breast cancer, ovarian cancer, astrocytoma, bladder cancer, medulloblastoma, neuroectodermal cancer and osteosarcoma (reviewed in (Wahlstrom and Henriksson, 2007)). The LOH at chromosome 17p13.3 was shown to involve a region telomeric to *p53* (Hirano et al., 2001) and an additional tumor suppressor was suggested to be present in this region. *Hypermethylated in cancer (HIC1)* was a potential candidate until heterozygous mutation of *HIC1* in mice was shown not to form tumors. MNT was suggested as a

potential candidate since deletion of *Mnt* or overexpression of *Myc* results in adenocarcinoma in mammary glands (Hurlin et al., 2003; Hutchinson and Muller, 2000). In addition, deletions in chromosome 17p were identified in 8% of the samples from patients with chronic lymphocytic leukemia (CLL) analyzed by single-nucleotide polymorphism-array analysis. All of these, except two cases, had a deletion in *TP53*. The two remaining cases were shown to carry small tumor specific deletions in 17p13.3 and MNT was suggested as one of 10 candidate genes (Edelmann et al., 2012).

Several studies have been performed in order to identify potential inactivation of *MNT* in human tumors. Whereas the expression of *MNT* was significantly reduced in 6 out of 14 medulloblastoma tumors analyzed (Cvekl et al., 2004) no alterations was found in the expression of *MNT* mRNA or protein in 44 medulloblastoma, breast or lung samples including 32 primary tumors, 3 recurrent tumors and 9 cell lines (Lo Nigro et al., 1998; Sommer et al., 1999; Takahashi et al., 1998). In addition, no mutations in the SID or the bHLHZip domain screened by single-strand conformation polymorphism analyses or effects on DNA binding were observed in neuroblastoma tumors (Sommer et al., 1999). In contrast, mutations were identified in *MNT* in human lymphoblastic leukemia (Guo et al., 2007) as well as gain of *MYC* (in 75%) and loss of *MNT* (in 40-55 %) in malignant T cells analyzed from patients with the aggressive T cell lymphoma/leukemia Sézary syndrome (Vermeer et al., 2008).

MNT as an antagonist and modulator of MYC function

MNT was suggested as a MYC antagonist since transgene expression of *Mnt* under the β-actin promoter in mice generated embryos with delay in development that died during mid-gestation, when c-MYC and MYCN are critical (Hurlin et al., 1997a). In contrast, dMnt does not seem to be essential for normal development in *Drosophila* since no significant defects were observed during embryogenesis or larval development upon deletion. *dMnt* null flies were characterized with increased weight, larger cells and decreased life span. Interestingly, dMnt has been proposed as the sole repressor and dMyc antagonist in *Drosophila* since no additional dMxd proteins has been identified (Loo et al., 2005; Orian et al., 2003).

Mice lacking *Mnt* dies within 24 hours after birth. Therefore *Mnt* knockout (KO) MEFs were generated and analyzed (Hurlin et al., 2003). These are characterized as cells mimicking *Myc* overexpressing cells with respect to their increased rate of proliferation, pre-mature S phase entry, increased sensitivity to apoptosis, more efficient escape from senescence and ability to be transformed with RAS alone. MNT antagonize MYC induced cellular functions by mediating the opposite transcriptional activity of common target genes. In addition, *Mnt* KO MEFs also has reduced requirement for MYC to proliferate (Hurlin et al., 2003; Hurlin et al., 2004). Similar results were obtained by down regulation of *Mnt* with RNA interference (Nilsson et al., 2004). In addition, mice injected with MEFs expressing *Mnt* targeting RNAi and oncogenic RAS developed rapidly growing fibrosarcomas (Nilsson et al., 2004), suggesting MNT to suppress tumor formation. *Mnt* KO MEFs transformed by RAS or MYC and RAS did however, grow poorly or senesced compared to *Mnt* wt cells. In

addition, primary *Mnt* KO MEFs were refractory to transformation by MYC and the pro-survival protein BCL-2, which in combination transform *Mnt* wt cells. Immortalized (by serial culturing using the 3T9 protocol) *Mnt* KO MEFs expressing MYC and RAS or RAS alone were capable to grow anchorage independent in soft agar however with few and small colonies. These cells showed increase apoptosis and did not form tumors in nude mice unlike *Mnt* wt cells (Link et al., 2012). As a MYC antagonist, MNT is thus suppressing both pro-proliferative and pro-apoptotic functions induced by MYC. In addition, the sensitivity to apoptosis was increased in *Mnt* deficient cells expressing ectopic MYC as well as upon mitogen-induced expression of endogenous MYC (Link et al., 2012). Whereas conditional deletion of *Mnt* in T cells resulted in tumor formation (Dezfouli et al., 2006) simultaneous deletion of *Mnt* and induced ectopic expression of MYC was also shown to prevent tumor formation (Link et al., 2012). The combined pro-apoptotic functions of *Mnt* deletion and MYC overexpression resulted in high levels of apoptosis. In addition, apoptosis induced by *Mnt* deletion in combination with MYC overexpression could not be inhibited by co-expression of oncogenic RAS or BCL-2. MNT was suggested to control production of reactive oxygen species (ROS) since high levels were detected in *Mnt* null cells, which are sensitive to drugs that inhibit antioxidant systems or depletion of serum and glutamine. MNT control, at least in part, ROS production by promoting efficient oxidative metabolism in the mitochondria. The physiological role of MNT was suggested to be suppression of apoptosis and MNT might be needed in order for MYC to induce tumor formation by increasing the threshold for MYC induced apoptosis (Link et al., 2012). The balance between MYC and MNT is thus of great importance to regulate cellular activities such as proliferation and apoptosis by fine-tuning of gene expression of common target genes.

2 MATERIALS AND METHODS

A number of different methods have been used in the studies discussed in this thesis. Firstly the *Xenopus* oocyte system used for microinjections of *hTERT* promoter DNA and mRNA or DNA expression vectors encoding for *c-MYC*, *MAX* and *MNT* will be described. Next, assays used to study DNA-protein interactions (Chromatin Immunoprecipitation (ChIP) and Dimethyl sulfate (DMS) methylation protection) and cellular transformation (Foci formation and Soft agar assay) will be discussed.

XENOPUS OOCYTE MODEL

To study *in vivo* chromatin dynamics in Paper III we used the *Xenopus* oocyte model. During maturation of the oocyte huge amount of histones, basal transcription factors, and other components needed for the transcription and chromatin remodeling machinery, tRNAs and rRNAs are accumulated in the cell. These factors are enough for up to approximately 12 cell divisions, i.e. until mid-blastula transition. Oocytes thereby have a great capacity to transcribe and translate genes from expression vectors and *in vitro* synthesized mRNAs introduced by nuclear or cytoplasmic micro-injection, respectively (Almouzni and Wolffe, 1993; Belikov et al., 2001). In addition, the promoter of interest can be reconstituted in the oocyte by nuclear injection of reporter DNA containing the promoter. Injected DNA undergoes chromatin assembly within a few hours using endogenous histones in the nucleus. The *Xenopus* oocyte thus allows studies of *in vivo* chromatin dynamics of a promoter upon binding of transcription factors as well as protein-DNA interactions and mediated transcriptional responses with high resolution and precision. Another advantage with this system is the low background mediated by endogenous DNA. This is due to the comparably high amounts of DNA injected in the oocytes. Whereas the oocyte contains 12 pg of chromosomal DNA 3-6 ng of the promoter DNA is routinely injected.

In our experiments oocytes were surgically isolated from *Xenopus leavis* frogs and defolliculated by collagenase treatment (Figure 9A, B) (Astrand et al., 2009). First, oocytes were injected with 0.5-5 ng of *in vitro* synthesized *c-MYC*, *MAX* or *Mnt* mRNA(s) in the cytoplasm. 5-7 hours later 3-6 ng of reporter DNA containing the *hTERT* promoter was injected in the nucleus. DNA expression vectors encoding *c-MYC*, *MAX* or *Mnt* was also used and were then co-injected with the *hTERT* promoter reporter DNA in the nucleus. 24 hours post-injection protein expression, protein-DNA interaction, histone modifications, chromatin structural changes and DNA accessibility were analyzed by a variety of methods (Figure 9C). The effect mediated by endogenous *tert, myc* or *mnt*, which are all present in oocytes are very low (Juergens et al., 2005; Kuramoto et al., 2001; Mantell and Greider, 1994; Taylor et al., 1986; Vriz et al., 1989).

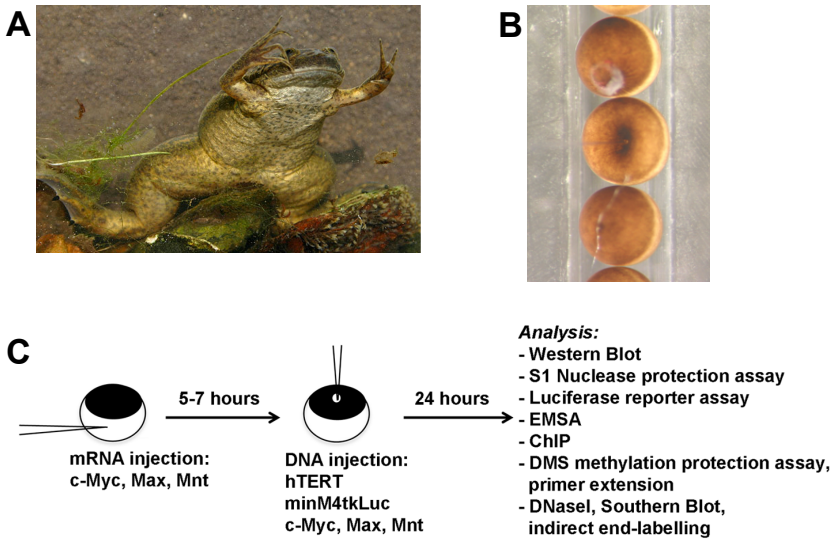

Figure 9. (A) Xenoupus leavis frog. (B) Oocytes visualized under the microscope ready to be injected. (C) Experimental outline of microinjection used to study protein-DNA interaction, protein expression, histone modifications, DNA accessibility and chromatin structural changes by a variety of different assays. Photographs in A and B, courtesy of Professor Örjan Wrange, Karolinska Institutet. The experimental outline in C is re-printed from (Wahlstrom et al., 2013) with permission from the publisher.

CHROMATIN IMMUNOPRECIPITATION AND DMS METHYLATION PROTECTION ASSAY

Both Chromatin Immunoprecipitation (ChIP) and DMS methylation protection assay are used to analyze *in vivo* binding of proteins to DNA.

In ChIP experiments proteins are cross-linked to the DNA by formaldehyde treatment of the living cells. After homogenization and cell lysis the DNA is fragmented by sonication to a length of 700-1000 bp. Specific antibodies are used to immunoprecipitate proteins together with cross-linked DNA. After reverse-crosslinking the immunoprecipitated DNA is purified and amplified using specific primers. By quantification increased or decreased binding of a specific protein to a certain binding site can be determined. In addition, ChIP can be used to analyze histone modifications associated with certain regions of the DNA using antibodies specific to histone marks (Orlando et al., 1997).

In contrast, DMS methylation protection assay (Belikov et al., 2001) is used to study sequence-specific protein-DNA interactions *in vivo*. DMS is a small molecule that penetrates the cell and methylate guanines at N7 position in the major groove of DNA. In addition, DMS methylates adenines at N3 position although with 10 times less efficiency. Protein-DNA interactions are thus detected as changes in DNA reactivity as a result of protein binding. Interestingly, DMS methylate guanines in DNA organized in nucleosomes with similar reactivity to naked DNA in solution (McGhee and Felsenfeld, 1979). By elevating the temperature and by addition of alkali or strong

amine methylated guanines are converted to DNA strand breaks (Maxam and Gilbert, 1977) and can be developed by ^{33}P-labeled primer extension. The products of linear amplification are separated using denaturing PAGE gels followed by analysis of the dried gel using phospho-imaging. Quantifications and scans are made by Image Gauge V3.3 software and are used to determine changes in intensity of the bands i.e. the reactivity of DMS caused by bound proteins. Decreased intensity of the band corresponds to more protein binding at this position i.e. methylation protection.

Both ChIP and DMS methylation protection assay was used in Paper III to show binding of c-MYC/MAX or MNT/MAX to the E-boxes in the *hTERT* promoter. However, we encountered different results using the two techniques when analyzing binding of MNT/MAX to the E-boxes in the *hTERT* promoter. By ChIP we detected binding to both the 5' and the 3' E-box whereas no DMS methylation protection was observed at guanines in the E-box analyzed by DMS methylation protection. However, by analyzing the overall pattern of DMS methylation protection of the E-box region we demonstrated that MNT/MAX induces a condensed chromatin structure since the observed pattern of methylation was virtually identical to the pattern of naked DNA i.e. DNA which is not bound by proteins. As a control, we used an artificial E-box containing promoter and could show sequence specific binding by MNT/MAX to all E-boxes in this promoter. This is most probably due to lack of additional binding sites in the artificial promoter, which are present in the *hTERT* promoter, needed in order to induce proper chromatin architecture. In summary, each of the two techniques have their advantages and specificities to provide information about protein-DNA interactions and chromatin architecture.

FOCI FORMATION AND SOFT AGAR ASSAY

When normal cells in culture grow too confluent proliferation will stop due to contact inhibition. However, upon transformation cells loose this mechanism and can start to grow on top of each other. Overexpression of two oncogenes (such as *MYC* and *RAS*) is enough to transform rodent primary cells (Land et al., 1983).

In Foci formation assays primary cells are transfected with plasmids encoding the oncogenes of interest, for example *MYC* and *RAS* in combination with selection marker. After transfection cells are cultured under selective conditions for approximately two weeks in order to select for transfected cells. Transformed cells will start to grow on top of each other forming foci. Giemsa staining is used to stain foci, which can be quantified by counting. The transformed cells forming foci can also be picked in order to create stable transfected cell lines.

Transformed cells are also characterized by their ability to grow anchorage independently. To test this cells are grown in soft agar, which prevent the surface and intercellular contacts. Cells are cultures for approximately two weeks and transformed cells, which are able to grow anchorage independent and will thus form colonies.

Transformed cells are stained with MTT and the number and sometimes size of colonies is quantified by counting.

In this thesis both Foci formation and Soft agar assay were used to investigate the effect of MNT and MNT phosphorylation sites specific mutants on MYC/RAS induced cellular transformation (Paper II).

ETHICAL STATEMENT

The work in this thesis includes studies performed in *Xenopus leavis* oocytes, primary rat embryo fibroblasts (REFs), breast carcinoma, neuroblastoma and fetal tissues, which are all under appropriate ethical approvals. These ethical permissions are specified in the respective papers (II, III, IV).

3 AIMS OF THIS THESIS

The overall aim was to investigate the interplay between MYC and MNT in regulation of transcription and chromatin dynamics. This thesis consist of four papers with the specific aims to:

- Investigate functional regulation of MNT as a transcriptional repressor during cell cycle progression of fibroblasts and upon differentiation of leukemia cells (Paper I).

- Characterize the role of phosphorylation in regulation of MNT and the interplay between MYC and MNT in cellular transformation (Paper II).

- Analyze chromatin dynamics upon transcriptional activation or repression by MYC and MNT (Paper III).

- Identify and elucidate the role of MYCN regulated miRNAs in neuroblastoma (Paper IV).

4 RESULTS AND DISCUSSION

PAPER I

MNT transcriptional repressor is functionally regulated during cell cycle progression

MYC is induced upon cell cycle entry and is thereafter expressed at low levels in proliferating cells. Interestingly, MNT is ubiquitously expressed and is thus co-expressed with MYC in proliferating cells although it exerts the opposite function (reviewed in (Wahlstrom and Henriksson, 2007)). The levels as well as regulation of MNT is therefore of great importance in order to understand how MYC regulates its target genes.

In paper I, we have studied regulation of MNT as a transcriptional repressor. First, we confirmed ubiquitous expression of MNT in quiescent, proliferating and in differentiating cells. To study cell cycle progression we synchronized NIH3T3 mouse fibroblasts by density arrest and serum starvation for 48 hours. Cells were stimulated with 10 % serum to re-enter into the cell cycle. Differentiation of human promyelocytic leukemia cells (HL60) was induced by treatment with dimethyl sulfoxide (DMSO). We observed a shift in Mnt protein mobility upon serum stimulation of quiescent cells as well as in proliferating HL60 cells. The upper 74 kDa MNT protein was identified as a phosphoprotein and by using kinase inhibitors we identified the MEK/ERK kinase as a potential candidate for phosphorylation of Mnt.

MNT represses transcription through interacting with mSIN3 and recruitment of HDAC activity (Hurlin et al., 1997a; Meroni et al., 1997). To investigate if phosphorylation of MNT upon cell cycle entry affects the ability for MNT to repress transcription we performed immunoprecipitation experiments and could show that the MNT-mSIN3 interaction was inhibited upon serum stimulation of quiescent cells. Similarly, the interaction between MNT and mSIN3B was inhibited in proliferating compared to differentiating HL60 cells. Furthermore, we could show that the interaction between MNT and mSIN3 was restored upon treatment with the MEK inhibitor of serum-stimulated cells. In addition, the recruitment of HDAC activity was decreased 5 hours after serum stimulation of quiescent cells. This suggests that MNT is functionally regulated as a transcriptional repressor upon phosphorylation.

Interestingly, we observed *in vitro* binding of MNT/MAX to the E-box sequence throughout the cell cycle by EMSA. To investigate binding of MNT as well as recruitment of SIN3 *in vivo* we performed ChIP assays using primers specific for the E-box area in the *cyclin D2* promoter. This gene is a well known MYC and MXD target gene important for the G1 to S transition during cell cycle progression (Bouchard et al., 1999). Although MNT binds to the *cyclin D2* promoter throughout the cell cycle, the recruitment of mSIN3B was inhibited 5 hours after serum stimulation of quiescent cells. In addition, MYC protein expression was induced early after serum stimulation

followed by acetylation of histone H4 and H3 and induction of Cyclin D2, CDK4, and Cyclin E. In support, these target genes was induced upon interference with *Mnt* using *Mnt*-targeting RNAi.

We suggested that proliferation-stimulatory signals mediated by growth factors induce phosphorylation of MNT through MAPK signaling as a response to serum stimulation. Disruption of MNT-mSIN3B interaction and decreased recruitment of HDAC activity upon phosphorylation mediates relief of MNT mediated transcriptional repression. However, since phosphorylated MNT still binds to the *cyclin D2* promoter the ratio as well as competition for interaction with MAX and E-box binding between MYC and MNT determines the transcriptional outcome. Since MYC levels are highly upregulated upon serum stimulation of quiescent cells common target genes are probably activated due to the increased levels of MYC. In addition, transcriptional repression will be inhibited at promoters bound by phosphorylated MNT/MAX. Perhaps also the two E-boxes in the *cyclin D2* promoter are differentially regulated by the two transcription factors (Figure 10).

In summary, we show that relief of MNT mediated repression concomitant with increased expression of MYC determines the transcriptional outcome of common target genes, suggesting MNT as the key regulator of the network.

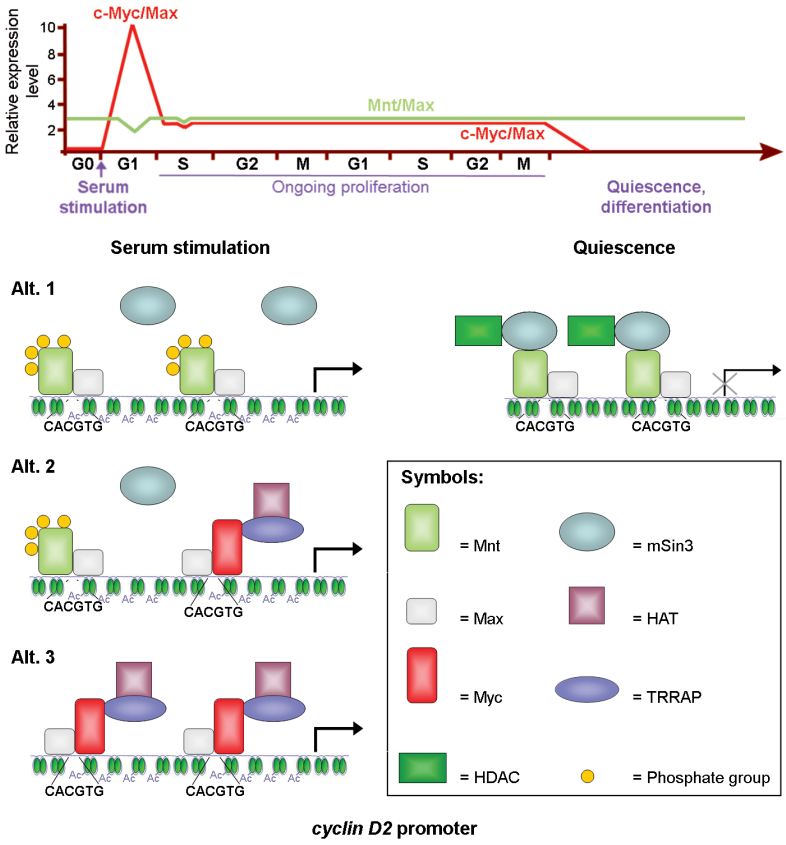

Figure 10. Model describing three possible alternatives for transcriptional activation of the MYC/MNT target gene cyclin D2 at cell cycle entry. Re-printed from (Wahlstrom and Henriksson, 2007) with permission from the publisher

PAPER II

Phosphorylation at serine 70 controls MNT accumulation and MYC-dependent cell transformation

We showed in Paper I that MNT is functionally regulated by phosphorylation during cell cycle progression (Paper I). In Paper II, we have further characterized phosphorylation of MNT. By *in vitro* kinase experiments we identified the region between amino acid 66 and 80 to be phosphorylated by ERK2. Using mutational analysis of the potential MAPK phosphorylation site in this region, S70, we generated Alanine (A) mutants that inhibit phosphorylation and Aspartic acid (D) and Glutamic acid (E) mutants mimicking constitutive phosphorylation of S70. By Western blot analysis of protein expression of the MNT S70 mutants we showed that phosphorylation of MNT S70 induced the shift in mobility, similar to which we observed previously during cell cycle entry. Next, we analyzed the ability of the mutants to interact with mSIN3B and to repress transcription of an E-box containing artificial promoter by co-immunoprecipitation experiments and Luciferase reporter assays. Surprisingly, we showed that all MNT S70 mutants interacted with mSIN3B and repressed transcription. Thus, phosphorylation of MNT S70 is not enough to inhibit the ability of MNT to repress transcription. By a more detailed analysis of the proteins expressed by the MNT S70 constructs we identified several MNT phospho-species. Probably phosphorylation at more sites is needed in order to abrogate MNT as a transcriptional repressor. In support, phosphorylation of MNT by a constitutively active MEK1 plasmid still represses transcription.

When analyzing protein expression of the S70 mutants we observed differences in protein accumulation between the mutants. By cyclohexamide experiments the S70 site was identified to regulate the stability of MNT. Furthermore, MYC and MNT protein stability and degradation was co-regulated via MAPK signaling, suggesting that MNT levels are stabilized concomitantly with the increase in MYC expression upon serum stimulation of quiescent cells.

Whereas MYC activates tumor-suppressive failsafe mechanisms resulting in apoptosis, RAS promotes cellular senescence in normal cells. By evading these two key barriers to cancer development, MYC and RAS transforms rodent cells (Land et al., 1983). To analyze the role of MNT in MYC/RAS induced cellular transformation we transfected primary rat embryo fibroblasts (REFs) with *MYC*, *RAS* and the different *Mnt* S70 mutants. Whereas MNT wt and MNT S70A inhibits MNT S70D promotes MYC/RAS induced transformation. To analyze this finding further we picked foci and generated stably transfected cell lines. The ability of these cells to grow anchorage independent was tested in soft agar experiments. Similar to the data obtained in foci formation experiments, cells expressing MYC/RAS/MNTS70D grew and formed large colonies whereas cells expressing MYC/RAS and MNT wt or S70A formed few and very small colonies. Protein levels were analyzed in the cell lines and cells expressing MntS70D were shown to express elevated levels of MNT compared to cells expressing

endogenous, wt or S70A MNT proteins. Furthermore, we analyzed the rate of proliferation and cells expressing MNT S70D grew slower compared to cells expressing MNT S70A and wt, supporting our hypothesis that MNT antagonizes MYC induced proliferation.

The mechanism underlying MYC/RAS induced transformation was studied by Tsuneoka *et al.,* in transformed cell lines with independently controllable *MYC* and *RAS*. The RAS/RAF/MEK/ERK pathway was suggested to be of importance for suppression of MYC-induced apoptosis in cellular transformation since removal of RAS or inhibition of MEK was shown to promote MYC induced apoptosis. In contrast, activated MEK1 blocked MYC mediated apoptosis. Furthermore, separate mechanisms were suggested for suppression of MYC induced apoptosis in serum starved and stimulated cells, where activated RAS was not enough to suppress MYC dependent apoptosis under serum-starved conditions (Tsuneoka and Mekada, 2000). Furthermore, Link *et al.,* showed recently that oncogenic transformation induced by MYC and RAS is dependent on MNT since transformation was inhibited in *Mnt* KO MEFs due to increased apoptosis (Link et al., 2012)

Collectively, we have shown that RAS mediated MAPK signaling stabilizes MNT through phosphorylation of S70 concurrent with MYC accumulation upon serum stimulation to control MYC induced activities (Figure 11).

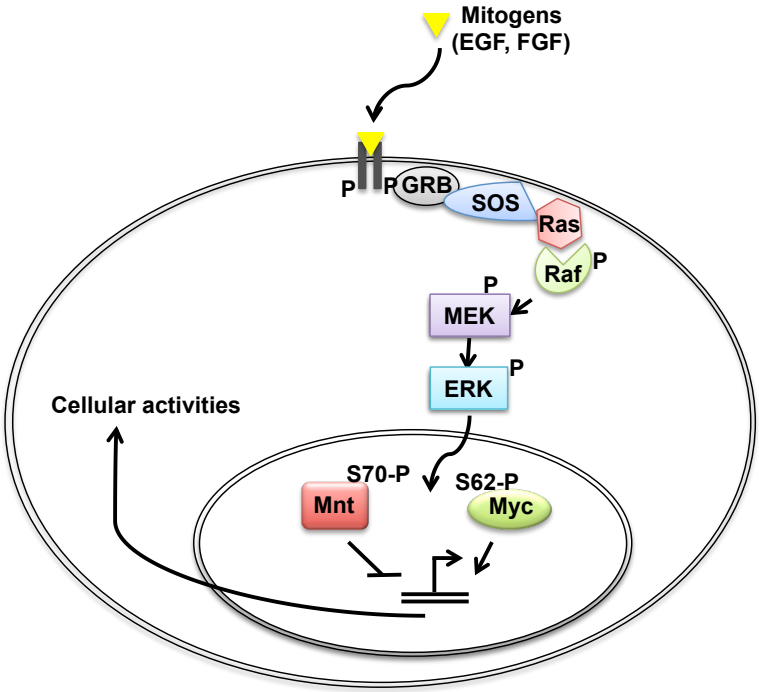

Figure 11. Schematic model of co-regulated protein stability of MNT and MYC upon stimulation with mitogens such as Fibroblast growth factor (FGF) of Epidermal growth factor (EGF) through MEK/ERK induced phosphorylation. The balance between MYC and MNT regulates the biological outcome by regulation of target genes encoding proteins involved in cellular activities.

PAPER III

Chromatin dynamics at the hTERT promoter during transcriptional activation and repression by c-MYC and MNT in Xenopus leavis oocytes

MYC and MNT regulate gene expression by binding to E-box elements in the promoter region of target promoters as heterodimers with MAX. Whereas MYC activate transcription by recruitment of co-factors containing HAT activity, MNT represses transcription by interaction with SIN3 and recruitment of HDACs. HATs and HDACs acetylate and deacetylate histone tails respectively and thereby regulate the accessibility to DNA by modifying chromatin dynamics (reviewed in (Wahlstrom and Henriksson, 2007)). In paper III, we were interested to understand how c-MYC and MNT regulate transcription by changing the chromatin structure of the *hTERT* promoter. In order to perform *in vivo* studies of chromatin dynamics we used the *Xenopus leavis* oocyte model (Belikov et al., 2001). Oocytes from the African clawed frog are large cells, which allows cytoplasmic microinjection of *in vitro* synthesized *c-MYC*, *MAX* or *Mnt* mRNA and nuclear injections of the corresponding expression vectors and/or reporter DNA containing the *hTERT* promoter. The DNA will be assembled into chromatin within a few hours by using endogenous factors in the oocyte (Almouzni and Wolffe, 1993). This system allows studies of the interaction between c-MYC/MAX or MNT/MAX and newly assembled chromatin of the *hTERT* promoter as well as transcriptional response with high precision.

First protein expression of *in vitro* synthesized *c-MYC*, *MAX* or *Mnt* mRNA or the corresponding expression vector in oocytes was confirmed by ^{14}C-Lysine incorporation detected by radio autography/phosphoimaging or Western blot analysis. Next, dimer formation with MAX and binding *in vitro* to the CACGTG E-box sequence was analyzed by electrophoretic mobility shift assay (EMSA). Interestingly, we observed a novel MAX-containing complex upon expression of MAX alone. Due to the migration of this band, *Xenopus* Myc is a potential member of this complex (Vriz et al., 1989). Unfortunately, we did not have access to a *Xenopus* specific Myc antibody and used therefore several different antibodies against human and mouse c-MYC and MYCN but none of these shifted the band. To verify binding of c-MYC/MAX and MNT/MAX to the E-boxes in the *hTERT* promoter *in vivo* we used ChIP and could show specific binding to both the 5' and the 3' E-box. In addition, we observed transcriptional activation and repression upon binding of c-MYC/MAX and MNT/MAX, respectively.

To analyze the accessibility to DNA in the E-box region of the *hTERT* promoter upon binding of c-MYC/MAX and MNT/MAX we used DNase I hypersensitivity assay. We confirmed previously published findings showing c-MYC/MAX binding to already active chromatin (Guccione et al., 2006; Lin et al., 2012; Martinato et al., 2008; Nie et al., 2012). Furthermore, we detected increased DNase I hypersensitivity upon binding of c-MYC/MAX. By using ChIP assays with antibodies specific for active histone marks (H3K9ac and K4K16ac) we showed acetylation across the E-box region in the *hTERT* promoter in oocytes as well as increased acetylation upon binding of c-

MYC/MAX. In contrast, DNase I hypersensitivity as well as acetylation of histone H3K9 and H4K16 across the E-box region decreased upon binding of MNT/MAX.

Furthermore, we used DMS methylation protection assay, which allows studies of sequence-specific interactions between proteins and DNA. DMS methylates DNA at the N7 position of Guanine in the major groove of DNA in the living cell. Methylated Guanines are converted to DNA strands breaks and the pattern of methylation protection is developed by ^{33}P-labeled primer extension. However, when bound by proteins the DNA is protected from DMS methylation. We showed specific binding of c-MYC/MAX to the 3' E-box in the *hTERT* promoter whereas no DMS methylation protection of the guanines in the 3' E-box was observed upon co-expression of MNT/MAX. Unfortunately, we could not analyze the pattern of DMS methylation protection at the 5' E-box due to technical difficulties with the very GC-rich sequence of that region of the *hTERT* core promoter. However, using ChIP we showed binding of c-MYC/MAX and MNT/MAX to both E-boxes in the *hTERT* promoter. Interestingly, the pattern of methylation protection across the E-box region in oocytes expressing MNT/MAX differed substantially from the pattern detected in oocytes injected with the *hTERT* reporter DNA alone. It appeared very similar to the naked DNA control, suggesting that MNT/MAX induces a chromatin structure of the *hTERT* promoter that is similar to chromatin, which is not bound by proteins. As a control we used the artificial minM4tkLuc promoter, which contains four repetitive E-boxes upstream of the minimal thymidine kinase (tk) promoter and observed specific binding of both c-MYC/MAX and MNT/MAX to all E-boxes by DMS methylation protection assay. This suggests that MNT/MAX does not repress the minM4tkLuc reporter in the same manner as it does with *hTERT*. Most probably the artificial promoter lack binding sites for co-factors needed to mediate proper chromatin structure. MNT/MAX is thus repressing transcription by de-acetylation of histones, condensation of chromatin and induction of a chromatin structure that prevents interaction of other transcription factors with DNA. In support, we found that MNT needs to be expressed at very low levels in order for co-injected MYC to activate *hTERT* transcription, suggesting MNT to act as a strong transcriptional repressor. Furthermore, Wang *et al.,* showed that the nucleosome-free regions in the *hTERT* core promoter, which are present in proliferating cells disappear once cells are induced to differentiate and transcription is repressed (Wang and Zhu, 2004). Interestingly, the DNA covering the 3' E-box was still sensitive to DNase I activity in oocytes expressing MNT/MAX. Similar results has been shown for gene silencing by Groucho (Gro/TLE/Grg) co-repressors, which upon recruitment by transcription factors mediates higher order condensed complexes of polynucleosome arrays which also are in an open exposed configuration accessible to DNase I (Sekiya and Zaret, 2007). This might suggest that MNT/MAX represses transcription by similar mechanisms.

In summary, c-MYC activates transcription by binding to already active chromatin resulting in local hyper-acetylation and increased DNA accessibility. In contrast, MNT/MAX act as a strong transcriptional repressor, which represses transcription by

de-acetylation of histone tails and complete condensation of the chromatin structure across the E-box region in the *hTERT* promoter (Figure 12).

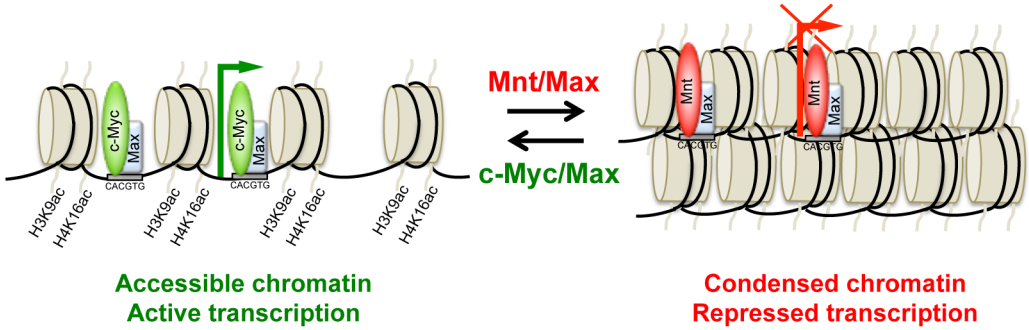

Figure 12. Schematic illustration describing transcriptional activation and repression of the hTERT promoter by MYC/MAX and MNT/MAX, respectively. Re-printed from graphical abstract (Wahlstrom et al., 2013) with permission from the publisher.

PAPER IV

MYCN-regulated microRNAs repress estrogen receptor-α (ESR1) expression and neuronal differentiation in human neuroblastoma

Neuroblastoma is a heterogeneous childhood cancer with a clinical outcome ranging from spontaneous regression to rapid progression and death. These tumors are derived from the sympathetic nervous system in the developing neural crest and are most likely caused by aberrations in normal developmental processes. Amplification of *MYCN* in neuroblastoma is associated with poor clinical outcome (Johnsen et al., 2009). The mechanism for tumor development in *MYCN* overexpressing neuroblastoma is however poorly understood. Abnormal expression of miRNAs has been observed in tumors suggesting these small non-coding RNAs to be involved in tumor development (Deng et al., 2008; Kent and Mendell, 2006). In Paper IV we explored MYCN regulated miRNAs in MYCN overexpressing neuroblastoma cells.

We performed genome wide miRCURY Locked Nucleic Acid (LNA) miRNA expression array in order to identify miRNAs regulated by MYCN in Tet21N neuroblastoma cells with inducible MYCN expression. Several miRNA genes were shown to be up or downregulated by MYCN. Among the upregulated miRNAs were miR-17, miR-18a, miR19a, which belong to the polycistronic miR-17-92 cluster. These oncogenic miRNAs were previously identified as targets of MYC (He et al., 2005; O'Donnell et al., 2005). To verify up regulation of these miRNAs in MYCN expressing neuroblastoma cells we used Northern blotting and quantitative PCR. Importantly, we also showed binding of MYCN/MAX together with acetylated histone H4 to E-box elements upstream of miR-17-92 by ChIP supporting direct transcriptional regulation of these miRNAs by MYCN.

Next we analyzed the function of the identified miRNAs. The miR-17-92 cluster was previously shown to correlate with neuroblastoma tumorigenesis by overexpression of the cluster both *in vitro* and *in vivo* (Fontana et al., 2008). This study described the role of miR-17 and miR-20a in neuroblastoma and we therefore wanted to investigate the role of miR-18a and miR-19a from the same polycistronic cluster. Upon interference with the expression of miR-18a and miR-19a using LNA knockdown oligonucleotides a decline in cell cycle progression was observed by fluorescence-activated cell sorting (FACS). We also showed that miR-18a, and to a lesser extend miR19a, inhibit differentiation upon long-term inhibition of miR-18a and miR-19a using lentiviral vectors encoding the specific anti-sense miRNA sequence. Several target genes downstream of miR-18a and miR-19a were predicted using PicTar and Targetscan. Among these we chose to study the ligand-inducible transcription factor estrogen receptor-α (ESR1), a nuclear hormone receptor important in breast cancer proliferation and progression. ESR1 was previously shown to, upon ligand activation, induce growth arrest in the *MYCN*-amplified neuroblastoma cell line SK-N-BE (Ma et al., 1993). To explore the potential role of MYCN induced miR-18a and miR-19a mediated repression of ESR1 for inhibition of neuroblast differentiation we first used luciferase reporter assays and could show that miR-18a and miR-19a indeed represses the expression of ESR1. The decrease in expression of ESR1 mRNA and protein was also confirmed in MCF-7 breast carcinoma cells, which express high levels of endogenous ESR1, transfected with miR-18a and miR-19a mimic oligonucleotides. Furthermore, we also showed the role of ESR1 in induction of cell cycle arrest and differentiation by transducing SK-N-BE(2) neuroblastoma cells with ESR1 lentiviral construct. In addition, we could demonstrate ESR1 expression in human fetal sympathetic ganglia by immunohistochemical staining, suggesting a role for ESR1 during the development of the sympathetic nervous system. The abnormal expression of ESR1 in sympathetic cells induced by MYCN-driven miRNAs might inhibit normal neuroblast differentiation and promote tumorigenesis and development of neuroblastoma. In support, bio-informatic analysis of microarray data from neuroblastoma tumors showed a correlation between high expression of ESR1 and increased event-free survival in patients. In addition, MYCN is expressed in the proliferating and migrating neuroblasts of the neural crest. Upon terminal differentiation of these cells the levels of MYCN are normally decreasing. However, overexpression of MYCN in migrating neural crest was shown to correlate with neuroblastoma formation in transgenic mice (Johnsen et al., 2009; Weiss et al., 1997).

In summary, we have identified a mechanism by which MYCN via induction of miR-18a and miR-19a interferes with expression of ESR1, which will prevent normal differentiation of neuroblasts and instead promote tumorigenesis and development of neuroblastoma (Figure 13).

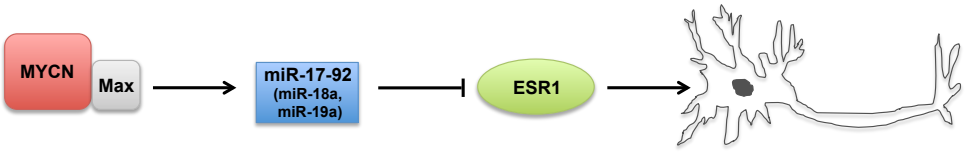

Figure 13. Model describing the role of MYCN in regulation of the miR-17-92 cluster and especially miR-18a and miR-19a, which in turn interferes with the expression of ESR1 and neuronal differentiation.

5 CONCLUDING REMARKS

The overall conclusion from the work presented in this thesis is that accurate balance between MYC and its antagonist and modulator MNT is important to maintain normal cellular activities. When either of these two transcription factors is de-regulated the biological outcome is affected. More specifically the conclusions are:

- Relief of MNT mediated transcriptional repression is important for activation of MYC target genes.

- MNT is functionally regulated as a transcription factor by phosphorylation.

- Phosphorylation of S70 increases the MNT protein stability and MYC/RAS induced cellular transformation.

- The stability of MNT and MYC is co regulated through the MAPK pathway.

- MYC is involved in fine-tuning of already active genes by local hyper-acetylation and increased chromatin accessibility.

- MNT is a strong transcriptional repressor that represses transcription by complete chromatin condensation.

- MYCN transcriptionally activates miR-18a and miR-19a, which in turn interferes with several targets including expression of ESR1 resulting in inhibition of neuroblast differentiation and neuroblastoma development.

6 FUTURE PERSPECTIVES

The results presented in this thesis highlight the importance of the interplay between MYC and its antagonist MNT for cellular activities. The expression of *MYC* is de-regulated in the majority of human cancers (reviewed in (Vita and Henriksson, 2006)). However, less is known about the expression and regulation of MNT during tumorigenesis. *MNT* has been suggested as a potential tumor suppressor first due to its chromosomal location close to *p53* on human chromosome 17p13.3, a region that frequently undergoes LOH in several malignancies. In addition, reduced expression, point mutations and deletions of *MNT* have been observed in some tumors. In contrast, there are also studies where no mutation or deletions in *MNT* were described. However, since deletion of *Mnt* was shown to mimic several hallmarks of *Myc* overexpression and deficiency of *Mnt* or overexpression of *Myc* results in tumor formation in mammary glands (reviewed in (Wahlstrom and Henriksson, 2007)) it would therefore be interesting to further explore MNT expression in human tumors.

To understand more about the interplay between MYC and MNT in tumorigenesis we want to investigate the role of MNT in MYC/RAS induced cellular transformation in more detail. The cells lines generated in Paper II will be used to analyze cells expressing MYC, RAS and different levels of MNT. More specifically, we will analyze differences in induction of apoptosis and cellular senescence between the cell lines. Our hypothesis is that phosphorylation of MNT S70 stabilizes the MNT protein, which will antagonize MYC induced apoptosis resulting in the increased foci formation we observe in cells expressing MYC/RAS/MNTS70D. Potentially, increased levels of MNT in these cells will counteract MYC mediated inhibition of senescence.

To explore the role of MNT in development and tumorigenesis of MYCN amplified neuroblastoma, regulation of MYCN regulated miRNAs and neuroblast differentiation would be of interest. Importantly, transgene overexpression of *Mnt* is lethal and embryos die between embryonic day E8.5 and E10.5, when c-MYC and MYCN are critical, suggesting the balance between MYC and MNT to be important during development (Hurlin et al., 1997a). Analysis of the expression and regulation of MNT in neuroblastoma cell lines and tumors would also be of interest. Our group has previously shown that changes in the expression of MYCN but not in MXD1 or MNT are essential for neuroblastoma cell differentiation. However, overexpression of MXD1 or MNT induced differentiation of neuroblastoma cells (Smith et al., 2004).

Phosphorylation of MNT S70 did not abrogate the function of MNT as a transcriptional repressor even though this mutant generated the shift in MNT protein mobility, which we observed upon serum stimulation of quiescent cells in Paper I. This suggests that phosphorylation at other sites and/or other modifications are needed to inhibit the interaction with mSIN3 and recruitment of HDAC activity. In addition, we identified several MNT phospho-proteins in Paper II. Further characterization of MNT

phosphorylation in order to identify more kinases and phosphorylation sites is therefore of interest.

Furthermore, we have also shown that MNT is a strong transcriptional repressor, which need to be expressed at low levels in order for co-expressed MYC to activate transcription (Paper III). We demonstrated that MNT repress transcription of the *hTERT* promoter by de-acetylation of histone tails as well as by induction of a very compact chromatin structure. This suggests that MNT closes the chromatin structure and by that prevents binding of other transcription factors. ChIP experiments analyzing binding of MYC and other factors to the *hTERT* promoter in oocytes expressing MNT could be performed to study this. The fact that we observed DNase I hypersensitivity at the 3' E-box in the *hTERT* promoter upon binding of MNT/MAX suggests that MNT might repress transcription in a similar manner as Groucho co-repressors, which mediates higher order condensed complexes of polynucleosomes that are in an open exposed configuration accessible to DNase I (Sekiya and Zaret, 2007). In order to explain in more detail how MNT/MAX represses transcription higher order chromatin confirmations, possible loop structures, co-factors and histone modifications mediated by MNT/MAX could be investigated.

Since MYC is activated in the majority of human cancers targeting MYC and/or MYC pathways is of great interest in design of new treatment regimens for cancer. Several conditional transgenic mice model have shown that inactivation of MYC result in tumor regression. In addition, tumor regression of RAS driven tumors was observed upon inactivation of MYC (reviewed in (Larsson and Henriksson, 2010)). MYC activity could be targeted by interference with the MYC/MAX interaction or DNA binding, by inhibition of co-factors/HATs or by MB II specific molecules, which will inhibit the interaction between MYC and co-factors. Antagonizing MYC by stimulating the expression or stability of MNT could be another potential target for therapy. However, MNT has not only been suggested as an MYC antagonist but also as a modulator of MYC function. Whereas overexpression of MNT inhibits MYC/RAS driven cell transformation (Dezfouli et al., 2006; Hurlin et al., 2003; Toyo-oka et al., 2006) by antagonizing the pro-proliferative function of MYC, certain amounts of MNT are needed in order to inhibit MYC induced apoptosis. Link *et al.,* showed that MNT is needed for oncogenic transformation both in cell culture models and in mice. MNT therefore acts as a pro-survival protein especially in cells that overexpress MYC (Link et al., 2012). The balance between MYC and MNT is thus of importance since MNT counteracts both pro-proliferative and pro-apoptotic functions of MYC. More knowledge is therefore needed in order to determine whether or not MNT could serve as a target for therapy.

Furthermore, we showed in Paper IV that MYCN inhibits normal neuroblast differentiation and instead promotes neuroblastoma tumorigenesis by upregulation of miR-18a and miR-19a, which in turn interferes with the expression of ESR1. Inhibition of miR-18a induced growth arrest and differentiation and targeting miR-18a could therefore be a potential target for therapy to explore.

7 POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA

Kroppens alla organ är uppbyggda av celler som utför olika funktioner. Cellerna i magen tar t.ex. hand om matsmältningen medan blodcellerna transporterar syre till kroppens alla delar och skyddar mot infektioner. Vissa celler producerar hormoner och andra utvecklas till nervceller. Genom celldelning bildas kontinuerligt nya celler, dock med olika hastighet i olika organ. Celldelningen är snabbast i magen och tarmens slemhinnor samt i benmärgen. Normal celldelning är mycket noggrant kontrollerad av speciella gener i vår arvsmassa som signalerar till cellen när den ska börja och sluta dela sig. Fel som uppstår i cellernas genetiska information (DNA), som finns förvarad i vår arvsmassa, repareras vanligtvis av cellen själv. Om detta inte fungerar genomgår cellen en mekanism som leder till celldöd. Detta är en säkerhetsåtgärd för att förhindra att celler med felaktig genetisk information ska dela sig och sprida denna information vidare. Om den normala celldelningen rubbas kan celler börja växa och dela sig ohämmat vilket leder till en ansamling av celler som kallas tumör. Det finns både godartade och elakartade tumörer varav de godartade inte har förmågan att växa igenom andra vävnader samt sprida sig till andra organ. Elakartade tumörer (cancer) däremot delar sig okontrollerat och växer in i närliggande vävnader, når så småningom blod- och lymfkärl med vilka de sprider sig till andra delar av kroppen där de kan bilda nya dottertumörer. Cancerceller har gemensamt att de delar sig obegränsat, har en oförmåga att dö, stimulerar sin egen tillväxt, de reagerar inte på utifrån kommande tillväxthämmande signaler, stimulerar tillväxten av blodkärl till tumören samt har en förmåga att sprida sig och bilda dottertumörer. Cancer är många olika sjukdomar och beroende på den typ av cell som tumören uppstår ifrån utvecklas olika typer av cancer. En cancer som har sitt ursprung i en levercell bildar levercancer medan cancer som utvecklats i bröstceller bildar bröstcancer. Till skillnad från dessa solida tumörer kan cancer även utvecklas i blodceller. Denna form av cancer kallad leukemi bildar ingen klump av celler utan dessa cancerceller flyter omkring tillsammans med de normala blodcellerna, breder ut sig och tar plats från de normala cellerna samt att de inte kan de uppgifter som de normala cellerna har.

Hos vuxna utvecklas cancer ofta under en lång tid. Från att den första sjuka cellen växt till en tumör kan ta upp till 20 år eller mer. Uppkomsten av tumörer påverkas av ärftliga faktorer men kan även påskyndas eller framkallas av cancerframkallande ämnen som finns i bl.a. tobaksrök, snus och kost. Vissa typer av strålning och virus kan även orsaka cancer. Hos barn däremot bildas cancer under en kortare tidsperiod. Orsaken till cancer hos barn är okänd. En möjlighet kan vara att slumpmässiga förändringar sker i för-stadie cellernas arvsanlag och att tumörer uppstår från dessa celler. Efter leukemi och hjärntumör är neuroblastom den vanligaste cancer sjukdomen hos barn. Neuroblastom uppkommer ur omogna neuroblaster som är för-stadieceller till nervceller och bildar tumörer i nervvävnad invid ryggraden. I Sverige diagnostiseras ett tjugotal barn varje år med neuroblastom. Av dessa har hälften en sjukdom med god prognos medan den andra hälften har elakartade tumörer och en mycket dålig överlevnadsfrekvens.

MYC är en gen som har stor betydelse för både normal celldelning och bildande av tumörer. I normala celler reglerar MYC flera cellulära funktioner såsom celldelning, celltillväxt och kontrollerad celldöd. I majoriteten av de cancersjukdomar som drabbar människor är MYC-genen felaktigt kontrollerad vilket leder till att mer MYC uttrycks från den genetiska arvsmassan. Detta felaktiga uttryck av MYC ökar celldelningen, och minskar förmågan för självdöd. Överaktivitet hos MYC är starkt kopplat till tumörens svårighetsgrad och innebär en dålig prognos i de flesta typer av cancer. Elakartade tumörer av barncancerformen neuroblastom har t.ex. ofta överuttryck av MYCN genen.

MYC genen kodar för proteiner som kallas transkriptionsfaktorer, vilka utför sina funktioner genom att reglera uttryck av specifika målgener. MYC reglerar ungefär 10-15 % av den humana arvsmassan och kan på detta sätt utföra alla de cellulära funktioner beskrivna ovan genom uttryck av ett stort antal olika proteiner. För att reglera genuttryck måste MYC bilda specifika DNA bindande komplex tillsammans med proteinet MAX. MYC/MAX komplexet aktiverar sedan sina målgener genom att rekrytera ytterligare proteiner som i sin tur påverkar strukturen mellan DNA och de proteiner (histoner) som DNA:t är lindat runt. Medan MYC/MAX öppnar upp denna struktur, även kallad kromatin, och tillåter avläsning av den genetiska informationen motverkar proteinet MNT detta genom att som komplex med MAX binda till samma sekvens i DNA och istället rekrytera proteiner som stänger denna struktur. Balansen mellan Myc och dess motståndare MNT är därför mycket viktig för reglering av deras gemensamma målgener.

Denna avhandling beskriver fyra delarbeten i vilka vi har studerat MYC och MNT i reglering av genuttryck. För att förstå mer om hur MYC antagonisten MNT fungerar har vi i delarbete I studerat hur MNT regleras i olika stadier av cellcykeln. Under denna process delas cellen efter att den har dubblerat sin genetiska arvsmassa. Genom att stimulera vilande celler att genomgå cellcykeln skickas signaler via tillväxtfaktorer på utsidan av cellen via ett signaleringssystem kallat MAPK signalering till proteiner inne i cellen. Vi har visat att MNT proteinet modifieras med fosfat-grupper via denna signaleringsväg och att dessa fosforyleringar påverkar MNTs förmåga att stänga av gener (delarbete I). I delarbete II analyserade vi fosforylering av MNT ytterligare och identifierade ett specifikt ställe kallat S70 på MNT proteinet som modifieras med fosforylering. Fosforylering av S70 är dock inte tillräcklig för att blockera MNTs förmåga att stänga av gener men visade sig vara viktig för stabilisering av MNT proteinet. Vidare har vi även visat att fosforylering av MYC och MNT via denna signalväg stabiliserar de båda proteinerna samtidigt. Ökade nivåer av MNT motverkar då den kraftiga ökning av MYC som sker då celler påbörjar en ny cell cykel (delarbete II).

I delarbete III har vi studerat hur MYC och MNT reglerar genuttryck på kromatinnivå, d.v.s. hur strukturen som innefattar DNA och histoner ändras. Vi har visat att MYC/MAX binder till DNA i kromatin som redan är aktivt för att sedan rekrytera ytterligare proteiner som lokalt ytterligare öppnar strukturen och tillåter den genetiska informationen att bli översatt till proteiner. MNT däremot binder till samma ställen i

DNA men rekryterar andra proteiner som stänger strukturen och därmed förhindrar den genetiska informationen att bli avläst. Vi har visat att MNT mycket kraftfullt reglerar avstängning av gener samt att MNT inducerar en struktur mellan DNA och histoner som är helt stängd och därmed hindrar andra protein från att binda (delarbete III).

I det fjärde delarbetet har vi identifierat små icke-kodande RNA molekyler (mikroRNA) som aktiveras i neuroblastomceller som överuttrycker MYCN. Vi har visat att MYCN bl.a. aktiverar två mikroRNA, miR18a och miR-19a, vilka i sin tur blockerar uttryck av estrogen receptor-α, en transkriptionsfaktor som påverkar utveckling av neuronala celler. Genom att förhindra normal utveckling av neuroblaster och att istället aktivera celltillväxt tros MYCN medverka till bildande av tumörer och utveckling av barncancer sjukdomen neuroblastom (delarbete IV).

Sammanfattningsvis har arbetet i denna avhandling visat att samspelet mellan MYC och MNT är viktigt för kontroll av cellulära funktioner genom reglering av gemensamma gener.

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9 REFERENCES

Adhikary, S., and M. Eilers. 2005. Transcriptional regulation and transformation by Myc proteins. *Nature reviews. Molecular cell biology* 6:635-645.

Albert, T., J. Mautner, J.O. Funk, K. Hortnagel, A. Pullner, and D. Eick. 1997. Nucleosomal structures of c-myc promoters with transcriptionally engaged RNA polymerase II. *Molecular and cellular biology* 17:4363-4371.

Allfrey, V.G., R. Faulkner, and A.E. Mirsky. 1964. Acetylation and Methylation of Histones and Their Possible Role in the Regulation of Rna Synthesis. *Proceedings of the National Academy of Sciences of the United States of America* 51:786-794.

Almouzni, G., and A.P. Wolffe. 1993. Replication-coupled chromatin assembly is required for the repression of basal transcription in vivo. *Genes & development* 7:2033-2047.

Amati, B., S. Dalton, M.W. Brooks, T.D. Littlewood, G.I. Evan, and H. Land. 1992. Transcriptional activation by the human c-Myc oncoprotein in yeast requires

Annis, M.G., E.L. Soucie, P.J. Dlugosz, J.A. Cruz-Aguado, L.Z. Penn, B. Leber, and D.W. Andrews. 2005. Bax forms multispanning monomers that oligomerize to permeabilize membranes during apoptosis. *The EMBO journal* 24:2096-2103.

Aprelikova, O., Y. Xiong, and E.T. Liu. 1995. Both p16 and p21 families of cyclin-dependent kinase (CDK) inhibitors block the phosphorylation of cyclin-dependent kinases by the CDK-activating kinase. *The Journal of biological chemistry* 270:18195-18197.

Arabi, A., C. Rustum, E. Hallberg, and A.P. Wright. 2003. Accumulation of c-Myc and proteasomes at the nucleoli of cells containing elevated c-Myc protein levels. *Journal of cell science* 116:1707-1717.

Arvanitis, C., and D.W. Felsher. 2006. Conditional transgenic models define how MYC initiates and maintains tumorigenesis. *Seminars in cancer biology* 16:313-317.

Askew, D.S., R.A. Ashmun, B.C. Simmons, and J.L. Cleveland. 1991. Constitutive c-myc expression in an IL-3-dependent myeloid cell line suppresses cell cycle arrest and accelerates apoptosis. *Oncogene* 6:1915-1922.

Astrand, C., S. Belikov, and O. Wrange. 2009. Histone acetylation characterizes chromatin presetting by NF1 and Oct1 and enhances glucocorticoid receptor binding to the MMTV promoter. *Experimental cell research* 315:2604-2615.

Ayer, D.E., L. Kretzner, and R.N. Eisenman. 1993. Mad: a heterodimeric partner for Max that antagonizes Myc transcriptional activity. *Cell* 72:211-222.

Ayer, D.E., Q.A. Lawrence, and R.N. Eisenman. 1995. Mad-Max transcriptional repression is mediated by ternary complex formation with mammalian homologs of yeast repressor Sin3. *Cell* 80:767-776.

Ballestar, E., and A.P. Wolffe. 2001. Methyl-CpG-binding proteins. Targeting specific gene repression. *European journal of biochemistry / FEBS* 268:1-6.

Bartel, D.P. 2009. MicroRNAs: target recognition and regulatory functions. *Cell* 136:215-233.

Battey, J., C. Moulding, R. Taub, W. Murphy, T. Stewart, H. Potter, G. Lenoir, and P. Leder. 1983. The human c-myc oncogene: structural consequences of translocation into the IgH locus in Burkitt lymphoma. *Cell* 34:779-787.

Belikov, S., B. Gelius, and O. Wrange. 2001. Hormone-induced nucleosome positioning in the MMTV promoter is reversible. *The EMBO journal* 20:2802-2811.

Bell, O., V.K. Tiwari, N.H. Thoma, and D. Schubeler. 2011. Determinants and dynamics of genome accessibility. *Nature reviews. Genetics* 12:554-564.

Bellosta, P., T. Hulf, S. Balla Diop, F. Usseglio, J. Pradel, D. Aragnol, and P. Gallant. 2005. Myc interacts genetically with Tip48/Reptin and Tip49/Pontin to control growth and proliferation during Drosophila development. *Proceedings of the National Academy of Sciences of the United States of America* 102:11799-11804.

Bettess, M.D., N. Dubois, M.J. Murphy, C. Dubey, C. Roger, S. Robine, and A. Trumpp. 2005. c-Myc is required for the formation of intestinal crypts but dispensable for homeostasis of the adult intestinal epithelium. *Molecular and cellular biology* 25:7868-7878.

Bhatia, K., K. Huppi, G. Spangler, D. Siwarski, R. Iyer, and I. Magrath. 1993. Point mutations in the c-Myc transactivation domain are common in Burkitt's lymphoma and mouse plasmacytomas. *Nature genetics* 5:56-61.

Bissonnette, R.P., F. Echeverri, A. Mahboubi, and D.R. Green. 1992. Apoptotic cell death induced by c-myc is inhibited by bcl-2. *Nature* 359:552-554.

Blackwell, T.K., L. Kretzner, E.M. Blackwood, R.N. Eisenman, and H. Weintraub. 1990. Sequence-specific DNA binding by the c-Myc protein. *Science* 250:1149-1151.

Blackwood, E.M., and R.N. Eisenman. 1991. Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. *Science* 251:1211-1217.

Blackwood, E.M., B. Luscher, and R.N. Eisenman. 1992. Myc and Max associate in vivo. *Genes & development* 6:71-80.

Bodnar, A.G., M. Ouellette, M. Frolkis, S.E. Holt, C.P. Chiu, G.B. Morin, C.B. Harley, J.W. Shay, S. Lichtsteiner, and W.E. Wright. 1998. Extension of life-span by introduction of telomerase into normal human cells. *Science* 279:349-352.

Bos, J.L. 1989. ras oncogenes in human cancer: a review. *Cancer research* 49:4682-4689.

Bouchard, C., O. Dittrich, A. Kiermaier, K. Dohmann, A. Menkel, M. Eilers, and B. Luscher. 2001. Regulation of cyclin D2 gene expression by the Myc/Max/Mad network: Myc-dependent TRRAP recruitment and histone acetylation at the cyclin D2 promoter. *Genes & development* 15:2042-2047.

Bouchard, C., K. Thieke, A. Maier, R. Saffrich, J. Hanley-Hyde, W. Ansorge, S. Reed, P. Sicinski, J. Bartek, and M. Eilers. 1999. Direct induction of cyclin D2 by Myc contributes to cell cycle progression and sequestration of p27. *The EMBO journal* 18:5321-5333.

Bown, N. 2001. Neuroblastoma tumour genetics: clinical and biological aspects. *Journal of clinical pathology* 54:897-910.

Boxer, L.M., and C.V. Dang. 2001. Translocations involving c-myc and c-myc function. *Oncogene* 20:5595-5610.

Brenner, C., R. Deplus, C. Didelot, A. Loriot, E. Vire, C. De Smet, A. Gutierrez, D. Danovi, D. Bernard, T. Boon, P.G. Pelicci, B. Amati, T. Kouzarides, Y. de Launoit, L. Di Croce, and F. Fuks. 2005. Myc represses transcription through recruitment of DNA methyltransferase corepressor. *The EMBO journal* 24:336-346.

Brodeur, G.M. 2003. Neuroblastoma: biological insights into a clinical enigma. *Nature reviews. Cancer* 3:203-216.

Brown, C.E., L. Howe, K. Sousa, S.C. Alley, M.J. Carrozza, S. Tan, and J.L. Workman. 2001. Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit. *Science* 292:2333-2337.

Brownell, J.E., J. Zhou, T. Ranalli, R. Kobayashi, D.G. Edmondson, S.Y. Roth, and C.D. Allis. 1996. Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. *Cell* 84:843-851.

Cartwright, P., C. McLean, A. Sheppard, D. Rivett, K. Jones, and S. Dalton. 2005. LIF/STAT3 controls ES cell self-renewal and pluripotency by a Myc-dependent mechanism. *Development* 132:885-896.

Cerni, C., B. Skrzypek, N. Popov, S. Sasgary, G. Schmidt, L.G. Larsson, B. Luscher, and M. Henriksson. 2002. Repression of in vivo growth of Myc/Ras transformed tumor cells by Mad1. *Oncogene* 21:447-459.

Chen, J., T. Willingham, L.R. Margraf, N. Schreiber-Agus, R.A. DePinho, and P.D. Nisen. 1995. Effects of the MYC oncogene antagonist, MAD, on proliferation, cell cycling and the malignant phenotype of human brain tumour cells. *Nature medicine* 1:638-643.

Cheng, S.W., K.P. Davies, E. Yung, R.J. Beltran, J. Yu, and G.V. Kalpana. 1999. c-MYC interacts with INI1/hSNF5 and requires the SWI/SNF complex for transactivation function. *Nature genetics* 22:102-105.

Choy, M.K., M. Movassagh, H.G. Goh, M.R. Bennett, T.A. Down, and R.S. Foo. 2010. Genome-wide conserved consensus transcription factor binding motifs are hyper-methylated. *BMC genomics* 11:519.

Chu, E.C., and A.S. Tarnawski. 2004. PTEN regulatory functions in tumor suppression and cell biology. *Medical science monitor : international medical journal of experimental and clinical research* 10:RA235-241.

Cole, M.D., and V.H. Cowling. 2009. Specific regulation of mRNA cap methylation by the c-Myc and E2F1 transcription factors. *Oncogene* 28:1169-1175.

Conacci-Sorrell, M., and R.N. Eisenman. 2011. Post-translational control of Myc function during differentiation. *Cell Cycle* 10:604-610.

Conacci-Sorrell, M., C. Ngouenet, and R.N. Eisenman. 2010. Myc-nick: a cytoplasmic cleavage product of Myc that promotes alpha-tubulin acetylation and cell differentiation. *Cell* 142:480-493.

Cong, Y.S., J. Wen, and S. Bacchetti. 1999. The human telomerase catalytic subunit hTERT: organization of the gene and characterization of the promoter. *Human molecular genetics* 8:137-142.

Conzen, S.D., K. Gottlob, E.S. Kandel, P. Khanduri, A.J. Wagner, M. O'Leary, and N. Hay. 2000. Induction of cell cycle progression and acceleration of apoptosis are two separable functions of c-Myc: transrepression correlates with acceleration of apoptosis. *Molecular and cellular biology* 20:6008-6018.

Coppola, J.A., and M.D. Cole. 1986. Constitutive c-myc oncogene expression blocks mouse erythroleukaemia cell differentiation but not commitment. *Nature* 320:760-763.

Cowling, V.H., S. Chandriani, M.L. Whitfield, and M.D. Cole. 2006. A conserved Myc protein domain, MBIV, regulates DNA binding, apoptosis, transformation, and G2 arrest. *Molecular and cellular biology* 26:4226-4239.

Cowling, V.H., and M.D. Cole. 2006. Mechanism of transcriptional activation by the Myc oncoproteins. *Seminars in cancer biology* 16:242-252.

Cvekl, A., Jr., J. Zavadil, B.K. Birshtein, M.A. Grotzer, and A. Cvekl. 2004. Analysis of transcripts from 17p13.3 in medulloblastoma suggests ROX/MNT as a potential tumour suppressor gene. *Eur J Cancer* 40:2525-2532.

Dalla-Favera, R., M. Bregni, J. Erikson, D. Patterson, R.C. Gallo, and C.M. Croce. 1982. Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. *Proceedings of the National Academy of Sciences of the United States of America* 79:7824-7827.

Dang, C.V., K.A. O'Donnell, K.I. Zeller, T. Nguyen, R.C. Osthus, and F. Li. 2006. The c-Myc target gene network. *Seminars in cancer biology* 16:253-264.

Dansen, T.B., J. Whitfield, F. Rostker, L. Brown-Swigart, and G.I. Evan. 2006. Specific requirement for Bax, not Bak, in Myc-induced apoptosis and tumor suppression in vivo. *The Journal of biological chemistry* 281:10890-10895.

Davis, A.C., M. Wims, G.D. Spotts, S.R. Hann, and A. Bradley. 1993. A null c-myc mutation causes lethality before 10.5 days of gestation in homozygotes and reduced fertility in heterozygous female mice. *Genes & development* 7:671-682.

de Alboran, I.M., E. Baena, and A.C. Martinez. 2004. c-Myc-deficient B lymphocytes are resistant to spontaneous and induced cell death. *Cell death and differentiation* 11:61-68.

Delgado, M.D., and J. Leon. 2006. Gene expression regulation and cancer. *Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico* 8:780-787.

Delgado, M.D., and J. Leon. 2010. Myc roles in hematopoiesis and leukemia. *Genes & cancer* 1:605-616.

Deng, S., G.A. Calin, C.M. Croce, G. Coukos, and L. Zhang. 2008. Mechanisms of microRNA deregulation in human cancer. *Cell Cycle* 7:2643-2646.

Dezfouli, S., A. Bakke, J. Huang, A. Wynshaw-Boris, and P.J. Hurlin. 2006. Inflammatory disease and lymphomagenesis caused by deletion of the Myc antagonist Mnt in T cells. *Molecular and cellular biology* 26:2080-2092.

Dmitrovsky, E., W.M. Kuehl, G.F. Hollis, I.R. Kirsch, T.P. Bender, and S. Segal. 1986. Expression of a transfected human c-myc oncogene inhibits differentiation of a mouse erythroleukaemia cell line. *Nature* 322:748-750.

Dubois, N.C., C. Adolphe, A. Ehninger, R.A. Wang, E.J. Robertson, and A. Trumpp. 2008. Placental rescue reveals a sole requirement for c-Myc in embryonic erythroblast survival and hematopoietic stem cell function. *Development* 135:2455-2465.

Eberhardy, S.R., C.A. D'Cunha, and P.J. Farnham. 2000. Direct examination of histone acetylation on Myc target genes using chromatin immunoprecipitation. *The Journal of biological chemistry* 275:33798-33805.

Edelmann, J., K. Holzmann, F. Miller, D. Winkler, A. Buhler, T. Zenz, L. Bullinger, M.W. Kuhn, A. Gerhardinger, J. Bloehdorn, I. Radtke, X. Su, J. Ma, S. Pounds, M. Hallek, P. Lichter, J. Korbel, R. Busch, D. Mertens, J.R. Downing, S. Stilgenbauer, and H. Dohner. 2012. High-resolution genomic profiling of chronic lymphocytic leukemia reveals new recurrent genomic alterations. *Blood* 120:4783-4794.

Eischen, C.M., G. Packham, J. Nip, B.E. Fee, S.W. Hiebert, G.P. Zambetti, and J.L. Cleveland. 2001a. Bcl-2 is an apoptotic target suppressed by both c-Myc and E2F-1. *Oncogene* 20:6983-6993.

Eischen, C.M., D. Woo, M.F. Roussel, and J.L. Cleveland. 2001b. Apoptosis triggered by Myc-induced suppression of Bcl-X(L) or Bcl-2 is bypassed during lymphomagenesis. *Molecular and cellular biology* 21:5063-5070.

Etard, C., D. Gradl, M. Kunz, M. Eilers, and D. Wedlich. 2005. Pontin and Reptin regulate cell proliferation in early Xenopus embryos in collaboration with c-Myc and Miz-1. *Mechanisms of development* 122:545-556.

Evan, G.I., A.H. Wyllie, C.S. Gilbert, T.D. Littlewood, H. Land, M. Brooks, C.M. Waters, L.Z. Penn, and D.C. Hancock. 1992. Induction of apoptosis in fibroblasts by c-myc protein. *Cell* 69:119-128.

Faiola, F., X. Liu, S. Lo, S. Pan, K. Zhang, E. Lymar, A. Farina, and E. Martinez. 2005. Dual regulation of c-Myc by p300 via acetylation-dependent control of Myc

protein turnover and coactivation of Myc-induced transcription. *Molecular and cellular biology* 25:10220-10234.

Fanidi, A., E.A. Harrington, and G.I. Evan. 1992. Cooperative interaction between c-myc and bcl-2 proto-oncogenes. *Nature* 359:554-556.

Farris, S.D., E.D. Rubio, J.J. Moon, W.M. Gombert, B.H. Nelson, and A. Krumm. 2005. Transcription-induced chromatin remodeling at the c-myc gene involves the local exchange of histone H2A.Z. *The Journal of biological chemistry* 280:25298-25303.

Fearon, E.R., and B. Vogelstein. 1990. A genetic model for colorectal tumorigenesis. *Cell* 61:759-767.

Fernandez, P.C., S.R. Frank, L. Wang, M. Schroeder, S. Liu, J. Greene, A. Cocito, and B. Amati. 2003. Genomic targets of the human c-Myc protein. *Genes & development* 17:1115-1129.

Fero, M.L., E. Randel, K.E. Gurley, J.M. Roberts, and C.J. Kemp. 1998. The murine gene p27Kip1 is haplo-insufficient for tumour suppression. *Nature* 396:177-180.

Flynt, A.S., and E.C. Lai. 2008. Biological principles of microRNA-mediated regulation: shared themes amid diversity. *Nature reviews. Genetics* 9:831-842.

Fodde, R., and T. Brabletz. 2007. Wnt/beta-catenin signaling in cancer stemness and malignant behavior. *Current opinion in cell biology* 19:150-158.

Foley, K.P., G.A. McArthur, C. Queva, P.J. Hurlin, P. Soriano, and R.N. Eisenman. 1998. Targeted disruption of the MYC antagonist MAD1 inhibits cell cycle exit during granulocyte differentiation. *The EMBO journal* 17:774-785.

Fontana, L., M.E. Fiori, S. Albini, L. Cifaldi, S. Giovinazzi, M. Forloni, R. Boldrini, A. Donfrancesco, V. Federici, P. Giacomini, C. Peschle, and D. Fruci. 2008. Antagomir-17-5p abolishes the growth of therapy-resistant neuroblastoma through p21 and BIM. *PloS one* 3:e2236.

Forsyth, N.R., W.E. Wright, and J.W. Shay. 2002. Telomerase and differentiation in multicellular organisms: turn it off, turn it on, and turn it off again. *Differentiation; research in biological diversity* 69:188-197.

Frank, S.R., T. Parisi, S. Taubert, P. Fernandez, M. Fuchs, H.M. Chan, D.M. Livingston, and B. Amati. 2003. MYC recruits the TIP60 histone acetyltransferase complex to chromatin. *EMBO reports* 4:575-580.

Frank, S.R., M. Schroeder, P. Fernandez, S. Taubert, and B. Amati. 2001. Binding of c-Myc to chromatin mediates mitogen-induced acetylation of histone H4 and gene activation. *Genes & development* 15:2069-2082.

Freytag, S.O., C.V. Dang, and W.M. Lee. 1990. Definition of the activities and properties of c-myc required to inhibit cell differentiation. *Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research* 1:339-343.

Friedrich, R., V.L. Morris, H.M. Goodman, J.M. Bishop, and H.E. Varmus. 1976. Differences between genomes of two strains of mouse mammary tumor virus as shown by partial RNA sequence analysis. *Virology* 72:330-340.

Gallinari, P., S. Di Marco, P. Jones, M. Pallaoro, and C. Steinkuhler. 2007. HDACs, histone deacetylation and gene transcription: from molecular biology to cancer therapeutics. *Cell research* 17:195-211.

Gandarillas, A., and F.M. Watt. 1997. c-Myc promotes differentiation of human epidermal stem cells. *Genes & development* 11:2869-2882.

Gartel, A.L., X. Ye, E. Goufman, P. Shianov, N. Hay, F. Najmabadi, and A.L. Tyner. 2001. Myc represses the p21(WAF1/CIP1) promoter and interacts with Sp1/Sp3. *Proceedings of the National Academy of Sciences of the United States of America* 98:4510-4515.

Ge, Z., W. Li, N. Wang, C. Liu, Q. Zhu, M. Bjorkholm, A. Gruber, and D. Xu. 2010. Chromatin remodeling: recruitment of histone demethylase RBP2 by Mad1 for transcriptional repression of a Myc target gene, telomerase reverse transcriptase. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 24:579-586.

Gilladoga, A.D., S. Edelhoff, E.M. Blackwood, R.N. Eisenman, and C.M. Disteche. 1992. Mapping of MAX to human chromosome 14 and mouse chromosome 12 by in situ hybridization. *Oncogene* 7:1249-1251.

Gombert, W.M., S.D. Farris, E.D. Rubio, K.M. Morey-Rosler, W.H. Schubach, and A. Krumm. 2003. The c-myc insulator element and matrix attachment regions define the c-myc chromosomal domain. *Molecular and cellular biology* 23:9338-9348.

Gregory, M.A., and S.R. Hann. 2000. c-Myc proteolysis by the ubiquitin-proteasome pathway: stabilization of c-Myc in Burkitt's lymphoma cells. *Molecular and cellular biology* 20:2423-2435.

Greider, C.W., and E.H. Blackburn. 1987. The telomere terminal transferase of Tetrahymena is a ribonucleoprotein enzyme with two kinds of primer specificity. *Cell* 51:887-898.

Grinkevich, V.V., F. Nikulenkov, Y. Shi, M. Enge, W. Bao, A. Maljukova, A. Gluch, A. Kel, O. Sangfelt, and G. Selivanova. 2009. Ablation of key oncogenic pathways by RITA-reactivated p53 is required for efficient apoptosis. *Cancer cell* 15:441-453.

Guccione, E., F. Martinato, G. Finocchiaro, L. Luzi, L. Tizzoni, V. Dall' Olio, G. Zardo, C. Nervi, L. Bernard, and B. Amati. 2006. Myc-binding-site recognition in the human genome is determined by chromatin context. *Nature cell biology* 8:764-770.

Guo, X.L., L. Pan, X.J. Zhang, X.H. Suo, Z.Y. Niu, J.Y. Zhang, F. Wang, Z.R. Dong, W. Da, and R. Ohno. 2007. Expression and mutation analysis of genes that encode the Myc antagonists Mad1, Mxi1 and Rox in acute leukaemia. *Leukemia & lymphoma* 48:1200-1207.

Hahn, W.C., C.M. Counter, A.S. Lundberg, R.L. Beijersbergen, M.W. Brooks, and R.A. Weinberg. 1999. Creation of human tumour cells with defined genetic elements. *Nature* 400:464-468.

Hallikas, O., K. Palin, N. Sinjushina, R. Rautiainen, J. Partanen, E. Ukkonen, and J. Taipale. 2006. Genome-wide prediction of mammalian enhancers based on analysis of transcription-factor binding affinity. *Cell* 124:47-59.

Hanahan, D., and R.A. Weinberg. 2000. The hallmarks of cancer. *Cell* 100:57-70.

Hanahan, D., and R.A. Weinberg. 2011. Hallmarks of cancer: the next generation. *Cell* 144:646-674.

Hann, S.R., M.W. King, D.L. Bentley, C.W. Anderson, and R.N. Eisenman. 1988. A non-AUG translational initiation in c-myc exon 1 generates an N-terminally distinct protein whose synthesis is disrupted in Burkitt's lymphomas. *Cell* 52:185-195.

Hann, S.R., C.B. Thompson, and R.N. Eisenman. 1985. c-myc oncogene protein synthesis is independent of the cell cycle in human and avian cells. *Nature* 314:366-369.

Harrington, E.A., M.R. Bennett, A. Fanidi, and G.I. Evan. 1994. c-Myc-induced apoptosis in fibroblasts is inhibited by specific cytokines. *The EMBO journal* 13:3286-3295.

Hayashi, K., R. Makino, H. Kawamura, A. Arisawa, and K. Yoneda. 1987. Characterization of rat c-myc and adjacent regions. *Nucleic acids research* 15:6419-6436.

Hayashita, Y., H. Osada, Y. Tatematsu, H. Yamada, K. Yanagisawa, S. Tomida, Y. Yatabe, K. Kawahara, Y. Sekido, and T. Takahashi. 2005. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer research* 65:9628-9632.

Hayflick, L., and P.S. Moorhead. 1961. The serial cultivation of human diploid cell strains. *Experimental cell research* 25:585-621.

He, L., J.M. Thomson, M.T. Hemann, E. Hernando-Monge, D. Mu, S. Goodson, S. Powers, C. Cordon-Cardo, S.W. Lowe, G.J. Hannon, and S.M. Hammond. 2005. A microRNA polycistron as a potential human oncogene. *Nature* 435:828-833.

He, T.C., A.B. Sparks, C. Rago, H. Hermeking, L. Zawel, L.T. da Costa, P.J. Morin, B. Vogelstein, and K.W. Kinzler. 1998. Identification of c-MYC as a target of the APC pathway. *Science* 281:1509-1512.

Henriksson, M., A. Bakardjiev, G. Klein, and B. Luscher. 1993. Phosphorylation sites mapping in the N-terminal domain of c-myc modulate its transforming potential. *Oncogene* 8:3199-3209.

Henriksson, M., and B. Luscher. 1996. Proteins of the Myc network: essential regulators of cell growth and differentiation. *Advances in cancer research* 68:109-182.

Herbst, A., M.T. Hemann, K.A. Tworkowski, S.E. Salghetti, S.W. Lowe, and W.P. Tansey. 2005. A conserved element in Myc that negatively regulates its proapoptotic activity. *EMBO reports* 6:177-183.

Hermeking, H., and D. Eick. 1994. Mediation of c-Myc-induced apoptosis by p53. *Science* 265:2091-2093.

Herold, S., M. Wanzel, V. Beuger, C. Frohme, D. Beul, T. Hillukkala, J. Syvaoja, H.P. Saluz, F. Haenel, and M. Eilers. 2002. Negative regulation of the mammalian UV response by Myc through association with Miz-1. *Molecular cell* 10:509-521.

Hirano, A., M. Emi, M. Tsuneizumi, Y. Utada, M. Yoshimoto, F. Kasumi, F. Akiyama, G. Sakamoto, S. Haga, T. Kajiwara, and Y. Nakamura. 2001. Allelic losses of loci at 3p25.1, 8p22, 13q12, 17p13.3, and 22q13 correlate with postoperative recurrence in breast cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* 7:876-882.

Hiyama, E., and K. Hiyama. 2003. Telomerase as tumor marker. *Cancer letters* 194:221-233.

Hogarty, M.D. 2003. The requirement for evasion of programmed cell death in neuroblastomas with MYCN amplification. *Cancer letters* 197:173-179.

Hooker, C.W., and P.J. Hurlin. 2006. Of Myc and Mnt. *Journal of cell science* 119:208-216.

Horikawa, I., P.L. Cable, C. Afshari, and J.C. Barrett. 1999. Cloning and characterization of the promoter region of human telomerase reverse transcriptase gene. *Cancer research* 59:826-830.

Horn, S., A. Figl, P.S. Rachakonda, C. Fischer, A. Sucker, A. Gast, S. Kadel, I. Moll, E. Nagore, K. Hemminki, D. Schadendorf, and R. Kumar. 2013. TERT promoter mutations in familial and sporadic melanoma. *Science* 339:959-961.

Huang, F.W., E. Hodis, M.J. Xu, G.V. Kryukov, L. Chin, and L.A. Garraway. 2013. Highly recurrent TERT promoter mutations in human melanoma. *Science* 339:957-959.

Hurlin, P.J., K.P. Foley, D.E. Ayer, R.N. Eisenman, D. Hanahan, and J.M. Arbeit. 1995a. Regulation of Myc and Mad during epidermal differentiation and HPV-associated tumorigenesis. *Oncogene* 11:2487-2501.

Hurlin, P.J., and J. Huang. 2006. The MAX-interacting transcription factor network. *Seminars in cancer biology* 16:265-274.

Hurlin, P.J., C. Queva, and R.N. Eisenman. 1997a. Mnt, a novel Max-interacting protein is coexpressed with Myc in proliferating cells and mediates repression at Myc binding sites. *Genes & development* 11:44-58.

Hurlin, P.J., C. Queva, and R.N. Eisenman. 1997b. Mnt: a novel Max-interacting protein and Myc antagonist. *Current topics in microbiology and immunology* 224:115-121.

Hurlin, P.J., C. Queva, P.J. Koskinen, E. Steingrimsson, D.E. Ayer, N.G. Copeland, N.A. Jenkins, and R.N. Eisenman. 1995b. Mad3 and Mad4: novel Max-interacting transcriptional repressors that suppress c-myc dependent transformation and are expressed during neural and epidermal differentiation. *The EMBO journal* 14:5646-5659.

Hurlin, P.J., E. Steingrimsson, N.G. Copeland, N.A. Jenkins, and R.N. Eisenman. 1999. Mga, a dual-specificity transcription factor that interacts with Max and contains a T-domain DNA-binding motif. *The EMBO journal* 18:7019-7028.

Hurlin, P.J., Z.Q. Zhou, K. Toyo-oka, S. Ota, W.L. Walker, S. Hirotsune, and A. Wynshaw-Boris. 2003. Deletion of Mnt leads to disrupted cell cycle control and tumorigenesis. *The EMBO journal* 22:4584-4596.

Hurlin, P.J., Z.Q. Zhou, K. Toyo-Oka, S. Ota, W.L. Walker, S. Hirotsune, and A. Wynshaw-Boris. 2004. Evidence of mnt-myc antagonism revealed by mnt gene deletion. *Cell Cycle* 3:97-99.

Hutchinson, J.N., and W.J. Muller. 2000. Transgenic mouse models of human breast cancer. *Oncogene* 19:6130-6137.

Ikura, T., V.V. Ogryzko, M. Grigoriev, R. Groisman, J. Wang, M. Horikoshi, R. Scully, J. Qin, and Y. Nakatani. 2000. Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis. *Cell* 102:463-473.

Issaeva, N., P. Bozko, M. Enge, M. Protopopova, L.G. Verhoef, M. Masucci, A. Pramanik, and G. Selivanova. 2004. Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates p53 function in tumors. *Nature medicine* 10:1321-1328.

Jaenisch, R., and A. Bird. 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nature genetics* 33 Suppl:245-254.

Jakopovic, M., A. Thomas, S. Balasubramaniam, D. Schrump, G. Giaccone, and S.E. Bates. 2013. Targeting the Epigenome in Lung Cancer: Expanding Approaches to Epigenetic Therapy. *Frontiers in oncology* 3:261.

Johnsen, J.I., P. Kogner, A. Albihn, and M.A. Henriksson. 2009. Embryonal neural tumours and cell death. *Apoptosis : an international journal on programmed cell death* 14:424-438.

Johnson, S.M., H. Grosshans, J. Shingara, M. Byrom, R. Jarvis, A. Cheng, E. Labourier, K.L. Reinert, D. Brown, and F.J. Slack. 2005. RAS is regulated by the let-7 microRNA family. *Cell* 120:635-647.

Juergens, K., B. Rust, T. Pieler, and K.A. Henningfeld. 2005. Isolation and comparative expression analysis of the Myc-regulatory proteins Mad1, Mad3, and Mnt during Xenopus development. *Developmental dynamics : an official publication of the American Association of Anatomists* 233:1554-1559.

Juin, P., A. Hunt, T. Littlewood, B. Griffiths, L.B. Swigart, S. Korsmeyer, and G. Evan. 2002. c-Myc functionally cooperates with Bax to induce apoptosis. *Molecular and cellular biology* 22:6158-6169.

Karnoub, A.E., and R.A. Weinberg. 2008. Ras oncogenes: split personalities. *Nature reviews. Molecular cell biology* 9:517-531.

Katz, M., I. Amit, and Y. Yarden. 2007. Regulation of MAPKs by growth factors and receptor tyrosine kinases. *Biochimica et biophysica acta* 1773:1161-1176.

Kauffmann-Zeh, A., P. Rodriguez-Viciana, E. Ulrich, C. Gilbert, P. Coffer, J. Downward, and G. Evan. 1997. Suppression of c-Myc-induced apoptosis by Ras signalling through PI(3)K and PKB. *Nature* 385:544-548.

Kent, O.A., and J.T. Mendell. 2006. A small piece in the cancer puzzle: microRNAs as tumor suppressors and oncogenes. *Oncogene* 25:6188-6196.

Kim, N.W., M.A. Piatyszek, K.R. Prowse, C.B. Harley, M.D. West, P.L. Ho, G.M. Coviello, W.E. Wright, S.L. Weinrich, and J.W. Shay. 1994. Specific association of human telomerase activity with immortal cells and cancer. *Science* 266:2011-2015.

Kim, S.Y., A. Herbst, K.A. Tworkowski, S.E. Salghetti, and W.P. Tansey. 2003. Skp2 regulates Myc protein stability and activity. *Molecular cell* 11:1177-1188.

Knoepfler, P.S., P.F. Cheng, and R.N. Eisenman. 2002. N-myc is essential during neurogenesis for the rapid expansion of progenitor cell populations and the inhibition of neuronal differentiation. *Genes & development* 16:2699-2712.

Knudson, A.G., Jr. 1971. Mutation and cancer: statistical study of retinoblastoma. *Proceedings of the National Academy of Sciences of the United States of America* 68:820-823.

Kornberg, R.D. 1974. Chromatin structure: a repeating unit of histones and DNA. *Science* 184:868-871.

Kumar, M.S., S.J. Erkeland, R.E. Pester, C.Y. Chen, M.S. Ebert, P.A. Sharp, and T. Jacks. 2008. Suppression of non-small cell lung tumor development by the let-7 microRNA family. *Proceedings of the National Academy of Sciences of the United States of America* 105:3903-3908.

Kuramoto, M., K. Ohsumi, T. Kishimoto, and F. Ishikawa. 2001. Identification and analyses of the Xenopus TERT gene that encodes the catalytic subunit of telomerase. *Gene* 277:101-110.

Kyo, S., M. Takakura, T. Fujiwara, and M. Inoue. 2008. Understanding and exploiting hTERT promoter regulation for diagnosis and treatment of human cancers. *Cancer science* 99:1528-1538.

Land, H., L.F. Parada, and R.A. Weinberg. 1983. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature* 304:596-602.

Lane, D.P. 1992. Cancer. p53, guardian of the genome. *Nature* 358:15-16.

Langdon, W.Y., A.W. Harris, S. Cory, and J.M. Adams. 1986. The c-myc oncogene perturbs B lymphocyte development in E-mu-myc transgenic mice. *Cell* 47:11-18.

Larsson, L.G., and M.A. Henriksson. 2010. The Yin and Yang functions of the Myc oncoprotein in cancer development and as targets for therapy. *Experimental cell research* 316:1429-1437.

Larsson, L.G., M. Pettersson, F. Oberg, K. Nilsson, and B. Luscher. 1994. Expression of mad, mxi1, max and c-myc during induced differentiation of hematopoietic cells: opposite regulation of mad and c-myc. *Oncogene* 9:1247-1252.

Leon, J., N. Ferrandiz, J.C. Acosta, and M.D. Delgado. 2009. Inhibition of cell differentiation: a critical mechanism for MYC-mediated carcinogenesis? *Cell Cycle* 8:1148-1157.

Lin, C.Y., J. Loven, P.B. Rahl, R.M. Paranal, C.B. Burge, J.E. Bradner, T.I. Lee, and R.A. Young. 2012. Transcriptional Amplification in Tumor Cells with Elevated c-Myc. *Cell* 151:56-67.

Link, J.M., S. Ota, Z.Q. Zhou, C.J. Daniel, R.C. Sears, and P.J. Hurlin. 2012. A critical role for Mnt in Myc-driven T-cell proliferation and oncogenesis. *Proceedings of*

the National Academy of Sciences of the United States of America 109:19685-19690.

Liu, J., and D. Levens. 2006. Making myc. *Current topics in microbiology and immunology* 302:1-32.

Liu, X., J. Tesfai, Y.A. Evrard, S.Y. Dent, and E. Martinez. 2003. c-Myc transformation domain recruits the human STAGA complex and requires TRRAP and GCN5 acetylase activity for transcription activation. *The Journal of biological chemistry* 278:20405-20412.

Lo Nigro, C., T. Venesio, A. Reymond, G. Meroni, P. Alberici, S. Cainarca, F. Enrico, M. Stack, D.H. Ledbetter, D.S. Liscia, A. Ballabio, and R. Carrozzo. 1998. The human ROX gene: genomic structure and mutation analysis in human breast tumors. *Genomics* 49:275-282.

Loo, L.W., J. Secombe, J.T. Little, L.S. Carlos, C. Yost, P.F. Cheng, E.M. Flynn, B.A. Edgar, and R.N. Eisenman. 2005. The transcriptional repressor dMnt is a regulator of growth in Drosophila melanogaster. *Molecular and cellular biology* 25:7078-7091.

Loven, J., N. Zinin, T. Wahlstrom, I. Muller, P. Brodin, E. Fredlund, U. Ribacke, A. Pivarcsi, S. Pahlman, and M. Henriksson. 2010. MYCN-regulated microRNAs repress estrogen receptor-alpha (ESR1) expression and neuronal differentiation in human neuroblastoma. *Proceedings of the National Academy of Sciences of the United States of America* 107:1553-1558.

Lowe, S.W., E. Cepero, and G. Evan. 2004. Intrinsic tumour suppression. *Nature* 432:307-315.

Lowry, W.E., L. Richter, R. Yachechko, A.D. Pyle, J. Tchieu, R. Sridharan, A.T. Clark, and K. Plath. 2008. Generation of human induced pluripotent stem cells from dermal fibroblasts. *Proceedings of the National Academy of Sciences of the United States of America* 105:2883-2888.

Luger, K., A.W. Mader, R.K. Richmond, D.F. Sargent, and T.J. Richmond. 1997. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* 389:251-260.

Luscher, B., and J. Vervoorts. 2012. Regulation of gene transcription by the oncoprotein MYC. *Gene* 494:145-160.

Lutterbach, B., and S.R. Hann. 1994. Hierarchical phosphorylation at N-terminal transformation-sensitive sites in c-Myc protein is regulated by mitogens and in mitosis. *Molecular and cellular biology* 14:5510-5522.

Ma, Z.Q., G.P. Bondiolotti, M. Olasmaa, E. Violani, C. Patrone, G.B. Picotti, and A. Maggi. 1993. Estrogen modulation of catecholamine synthesis and monoamine oxidase A activity in the human neuroblastoma cell line SK-ER3. *The Journal of steroid biochemistry and molecular biology* 47:207-211.

Maclean, K.H., U.B. Keller, C. Rodriguez-Galindo, J.A. Nilsson, and J.L. Cleveland. 2003. c-Myc augments gamma irradiation-induced apoptosis by suppressing Bcl-XL. *Molecular and cellular biology* 23:7256-7270.

Mantell, L.L., and C.W. Greider. 1994. Telomerase activity in germline and embryonic cells of Xenopus. *The EMBO journal* 13:3211-3217.

Martin, G.S. 1970. Rous sarcoma virus: a function required for the maintenance of the transformed state. *Nature* 227:1021-1023.

Martinato, F., M. Cesaroni, B. Amati, and E. Guccione. 2008. Analysis of Myc-induced histone modifications on target chromatin. *PloS one* 3:e3650.

Mateyak, M.K., A.J. Obaya, S. Adachi, and J.M. Sedivy. 1997. Phenotypes of c-Myc-deficient rat fibroblasts isolated by targeted homologous recombination. *Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research* 8:1039-1048.

Mautner, J., S. Joos, T. Werner, D. Eick, G.W. Bornkamm, and A. Polack. 1995. Identification of two enhancer elements downstream of the human c-myc gene. *Nucleic acids research* 23:72-80.

Maxam, A.M., and W. Gilbert. 1977. A new method for sequencing DNA. *Proceedings of the National Academy of Sciences of the United States of America* 74:560-564.

McEwan, I.J., K. Dahlman-Wright, J. Ford, and A.P. Wright. 1996. Functional interaction of the c-Myc transactivation domain with the TATA binding protein: evidence for an induced fit model of transactivation domain folding. *Biochemistry* 35:9584-9593.

McGhee, J.D., and G. Felsenfeld. 1979. Reaction of nucleosome DNA with dimethyl sulfate. *Proceedings of the National Academy of Sciences of the United States of America* 76:2133-2137.

McLaughlin, F., P. Finn, and N.B. La Thangue. 2003. The cell cycle, chromatin and cancer: mechanism-based therapeutics come of age. *Drug discovery today* 8:793-802.

McMahon, S.B., H.A. Van Buskirk, K.A. Dugan, T.D. Copeland, and M.D. Cole. 1998. The novel ATM-related protein TRRAP is an essential cofactor for the c-Myc and E2F oncoproteins. *Cell* 94:363-374.

McMahon, S.B., M.A. Wood, and M.D. Cole. 2000. The essential cofactor TRRAP recruits the histone acetyltransferase hGCN5 to c-Myc. *Molecular and cellular biology* 20:556-562.

Menssen, A., and H. Hermeking. 2002. Characterization of the c-MYC-regulated transcriptome by SAGE: identification and analysis of c-MYC target genes. *Proceedings of the National Academy of Sciences of the United States of America* 99:6274-6279.

Meroni, G., A. Reymond, M. Alcalay, G. Borsani, A. Tanigami, R. Tonlorenzi, C. Lo Nigro, S. Messali, M. Zollo, D.H. Ledbetter, R. Brent, A. Ballabio, and R. Carrozzo. 1997. Rox, a novel bHLHZip protein expressed in quiescent cells that heterodimerizes with Max, binds a non-canonical E box and acts as a transcriptional repressor. *The EMBO journal* 16:2892-2906.

Meyer, N., and L.Z. Penn. 2008. Reflecting on 25 years with MYC. *Nature reviews. Cancer* 8:976-990.

Michelotti, G.A., E.F. Michelotti, A. Pullner, R.C. Duncan, D. Eick, and D. Levens. 1996. Multiple single-stranded cis elements are associated with activated chromatin of the human c-myc gene in vivo. *Molecular and cellular biology* 16:2656-2669.

Morgan, D.O. 1997. Cyclin-dependent kinases: engines, clocks, and microprocessors. *Annual review of cell and developmental biology* 13:261-291.

Mu, P., Y.C. Han, D. Betel, E. Yao, M. Squatrito, P. Ogrodowski, E. de Stanchina, A. D'Andrea, C. Sander, and A. Ventura. 2009. Genetic dissection of the miR-17~92 cluster of microRNAs in Myc-induced B-cell lymphomas. *Genes & development* 23:2806-2811.

Murre, C., P.S. McCaw, H. Vaessin, M. Caudy, L.Y. Jan, Y.N. Jan, C.V. Cabrera, J.N. Buskin, S.D. Hauschka, A.B. Lassar, and et al. 1989. Interactions between heterologous helix-loop-helix proteins generate complexes that bind specifically to a common DNA sequence. *Cell* 58:537-544.

Nau, M.M., B.J. Brooks, J. Battey, E. Sausville, A.F. Gazdar, I.R. Kirsch, O.W. McBride, V. Bertness, G.F. Hollis, and J.D. Minna. 1985. L-myc, a new myc-related gene amplified and expressed in human small cell lung cancer. *Nature* 318:69-73.

Nguyen, H.Q., M. Selvakumaran, D.A. Liebermann, and B. Hoffman. 1995. Blocking c-Myc and Max expression inhibits proliferation and induces differentiation of normal and leukemic myeloid cells. *Oncogene* 11:2439-2444.

Nie, Z., G. Hu, G. Wei, K. Cui, A. Yamane, W. Resch, R. Wang, D.R. Green, L. Tessarollo, R. Casellas, K. Zhao, and D. Levens. 2012. c-Myc Is a Universal Amplifier of Expressed Genes in Lymphocytes and Embryonic Stem Cells. *Cell* 151:68-79.

Nikiforov, M.A., S. Chandriani, J. Park, I. Kotenko, D. Matheos, A. Johnsson, S.B. McMahon, and M.D. Cole. 2002. TRRAP-dependent and TRRAP-independent transcriptional activation by Myc family oncoproteins. *Molecular and cellular biology* 22:5054-5063.

Nikiforov, M.A., I. Kotenko, O. Petrenko, A. Beavis, L. Valenick, I. Lemischka, and M.D. Cole. 2000. Complementation of Myc-dependent cell proliferation by cDNA expression library screening. *Oncogene* 19:4828-4831.

Nikiforov, M.A., N. Popov, I. Kotenko, M. Henriksson, and M.D. Cole. 2003. The Mad and Myc basic domains are functionally equivalent. *The Journal of biological chemistry* 278:11094-11099.

Nilsson, J.A., and J.L. Cleveland. 2003. Myc pathways provoking cell suicide and cancer. *Oncogene* 22:9007-9021.

Nilsson, J.A., K.H. Maclean, U.B. Keller, H. Pendeville, T.A. Baudino, and J.L. Cleveland. 2004. Mnt loss triggers Myc transcription targets, proliferation, apoptosis, and transformation. *Molecular and cellular biology* 24:1560-1569.

Nowell, P.C., and D.A. Hungerford. 1960. Chromosome studies on normal and leukemic human leukocytes. *Journal of the National Cancer Institute* 25:85-109.

Nurse, P. 2000. A long twentieth century of the cell cycle and beyond. *Cell* 100:71-78.

O'Donnell, K.A., E.A. Wentzel, K.I. Zeller, C.V. Dang, and J.T. Mendell. 2005. c-Myc-regulated microRNAs modulate E2F1 expression. *Nature* 435:839-843.

Ogawa, H., K. Ishiguro, S. Gaubatz, D.M. Livingston, and Y. Nakatani. 2002. A complex with chromatin modifiers that occupies E2F- and Myc-responsive genes in G0 cells. *Science* 296:1132-1136.

Oh, S., Y.H. Song, J. Yim, and T.K. Kim. 2000. Identification of Mad as a repressor of the human telomerase (hTERT) gene. *Oncogene* 19:1485-1490.

Okita, K., T. Ichisaka, and S. Yamanaka. 2007. Generation of germline-competent induced pluripotent stem cells. *Nature* 448:313-317.

Olive, V., M.J. Bennett, J.C. Walker, C. Ma, I. Jiang, C. Cordon-Cardo, Q.J. Li, S.W. Lowe, G.J. Hannon, and L. He. 2009. miR-19 is a key oncogenic component of mir-17-92. *Genes & development* 23:2839-2849.

Orian, A., B. van Steensel, J. Delrow, H.J. Bussemaker, L. Li, T. Sawado, E. Williams, L.W. Loo, S.M. Cowley, C. Yost, S. Pierce, B.A. Edgar, S.M. Parkhurst, and R.N. Eisenman. 2003. Genomic binding by the Drosophila Myc, Max, Mad/Mnt transcription factor network. *Genes & development* 17:1101-1114.

Orlando, V., H. Strutt, and R. Paro. 1997. Analysis of chromatin structure by in vivo formaldehyde cross-linking. *Methods* 11:205-214.

Oster, S.K., D.Y. Mao, J. Kennedy, and L.Z. Penn. 2003. Functional analysis of the N-terminal domain of the Myc oncoprotein. *Oncogene* 22:1998-2010.

Ota, A., H. Tagawa, S. Karnan, S. Tsuzuki, A. Karpas, S. Kira, Y. Yoshida, and M. Seto. 2004. Identification and characterization of a novel gene, C13orf25, as a target for 13q31-q32 amplification in malignant lymphoma. *Cancer research* 64:3087-3095.

Pagano, M., R. Pepperkok, F. Verde, W. Ansorge, and G. Draetta. 1992. Cyclin A is required at two points in the human cell cycle. *The EMBO journal* 11:961-971.

Papantonis, A., and P.R. Cook. 2013. Transcription factories: genome organization and gene regulation. *Chemical reviews* 113:8683-8705.

Park, I.H., R. Zhao, J.A. West, A. Yabuuchi, H. Huo, T.A. Ince, P.H. Lerou, M.W. Lensch, and G.Q. Daley. 2008. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 451:141-146.

Park, J., S. Kunjibettu, S.B. McMahon, and M.D. Cole. 2001. The ATM-related domain of TRRAP is required for histone acetyltransferase recruitment and Myc-dependent oncogenesis. *Genes & development* 15:1619-1624.

Park, J., M.A. Wood, and M.D. Cole. 2002. BAF53 forms distinct nuclear complexes and functions as a critical c-Myc-interacting nuclear cofactor for oncogenic transformation. *Molecular and cellular biology* 22:1307-1316.

Pelengaris, S., M. Khan, and G. Evan. 2002. c-MYC: more than just a matter of life and death. *Nature reviews. Cancer* 2:764-776.

Peterlin, B.M., and D.H. Price. 2006. Controlling the elongation phase of transcription with P-TEFb. *Molecular cell* 23:297-305.

Peterson, C.L., and M.A. Laniel. 2004. Histones and histone modifications. *Current biology : CB* 14:R546-551.

Poole, J.C., L.G. Andrews, and T.O. Tollefsbol. 2001. Activity, function, and gene regulation of the catalytic subunit of telomerase (hTERT). *Gene* 269:1-12.

Popov, N., T. Wahlstrom, P.J. Hurlin, and M. Henriksson. 2005. Mnt transcriptional repressor is functionally regulated during cell cycle progression. *Oncogene* 24:8326-8337.

Prendergast, G.C., D. Lawe, and E.B. Ziff. 1991. Association of Myn, the murine homolog of max, with c-Myc stimulates methylation-sensitive DNA binding and ras cotransformation. *Cell* 65:395-407.

Prochownik, E.V., and J. Kukowska. 1986. Deregulated expression of c-myc by murine erythroleukaemia cells prevents differentiation. *Nature* 322:848-850.

Pullner, A., J. Mautner, T. Albert, and D. Eick. 1996. Nucleosomal structure of active and inactive c-myc genes. *The Journal of biological chemistry* 271:31452-31457.

Pulverer, B., A. Sommer, G.A. McArthur, R.N. Eisenman, and B. Luscher. 2000. Analysis of Myc/Max/Mad network members in adipogenesis: inhibition of the proliferative burst and differentiation by ectopically expressed Mad1. *Journal of cellular physiology* 183:399-410.

Queva, C., P.J. Hurlin, K.P. Foley, and R.N. Eisenman. 1998. Sequential expression of the MAD family of transcriptional repressors during differentiation and development. *Oncogene* 16:967-977.

Queva, C., G.A. McArthur, L.S. Ramos, and R.N. Eisenman. 1999. Dwarfism and dysregulated proliferation in mice overexpressing the MYC antagonist MAD1. *Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research* 10:785-796.

Rabbitts, P.H., J.V. Watson, A. Lamond, A. Forster, M.A. Stinson, G. Evan, W. Fischer, E. Atherton, R. Sheppard, and T.H. Rabbitts. 1985. Metabolism of c-myc gene products: c-myc mRNA and protein expression in the cell cycle. *The EMBO journal* 4:2009-2015.

Rahl, P.B., C.Y. Lin, A.C. Seila, R.A. Flynn, S. McCuine, C.B. Burge, P.A. Sharp, and R.A. Young. 2010. c-Myc regulates transcriptional pause release. *Cell* 141:432-445.

Rama, S., Y. Suresh, and A.J. Rao. 2001. Regulation of telomerase during human placental differentiation: a role for TGFbeta1. *Molecular and cellular endocrinology* 182:233-248.

Schwab, M., K. Alitalo, K.H. Klempnauer, H.E. Varmus, J.M. Bishop, F. Gilbert, G. Brodeur, M. Goldstein, and J. Trent. 1983. Amplified DNA with limited homology to myc cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. *Nature* 305:245-248.

Scotting, P.J., D.A. Walker, and G. Perilongo. 2005. Childhood solid tumours: a developmental disorder. *Nature reviews. Cancer* 5:481-488.

Sears, R., F. Nuckolls, E. Haura, Y. Taya, K. Tamai, and J.R. Nevins. 2000. Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. *Genes & development* 14:2501-2514.

Seeger, R.C., G.M. Brodeur, H. Sather, A. Dalton, S.E. Siegel, K.Y. Wong, and D. Hammond. 1985. Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. *The New England journal of medicine* 313:1111-1116.

Sekiya, T., and K.S. Zaret. 2007. Repression by Groucho/TLE/Grg proteins: genomic site recruitment generates compacted chromatin in vitro and impairs activator binding in vivo. *Molecular cell* 28:291-303.

Seoane, J., H.V. Le, and J. Massague. 2002. Myc suppression of the p21(Cip1) Cdk inhibitor influences the outcome of the p53 response to DNA damage. *Nature* 419:729-734.

Sheiness, D., and J.M. Bishop. 1979. DNA and RNA from uninfected vertebrate cells contain nucleotide sequences related to the putative transforming gene of avian myelocytomatosis virus. *Journal of virology* 31:514-521.

Shen-Li, H., R.C. O'Hagan, H. Hou, Jr., J.W. Horner, 2nd, H.W. Lee, and R.A. DePinho. 2000. Essential role for Max in early embryonic growth and development. *Genes & development* 14:17-22.

Sherr, C.J. 2004. Principles of tumor suppression. *Cell* 116:235-246.

Smith, A.G., N. Popov, M. Imreh, H. Axelson, and M. Henriksson. 2004. Expression and DNA-binding activity of MYCN/Max and Mnt/Max during induced differentiation of human neuroblastoma cells. *Journal of cellular biochemistry* 92:1282-1295.

Sodir, N.M., L.B. Swigart, A.N. Karnezis, D. Hanahan, G.I. Evan, and L. Soucek. 2011. Endogenous Myc maintains the tumor microenvironment. *Genes & development* 25:907-916.

Sommer, A., K. Bousset, E. Kremmer, M. Austen, and B. Luscher. 1998. Identification and characterization of specific DNA-binding complexes containing members of the Myc/Max/Mad network of transcriptional regulators. *The Journal of biological chemistry* 273:6632-6642.

Sommer, A., A. Waha, J. Tonn, N. Sorensen, P.J. Hurlin, R.N. Eisenman, B. Luscher, and T. Pietsch. 1999. Analysis of the Max-binding protein MNT in human medulloblastomas. *International journal of cancer. Journal international du cancer* 82:810-816.

Soucek, L., J. Whitfield, C.P. Martins, A.J. Finch, D.J. Murphy, N.M. Sodir, A.N. Karnezis, L.B. Swigart, S. Nasi, and G.I. Evan. 2008. Modelling Myc inhibition as a cancer therapy. *Nature* 455:679-683.

Soucek, L., J.R. Whitfield, N.M. Sodir, D. Masso-Valles, E. Serrano, A.N. Karnezis, L.B. Swigart, and G.I. Evan. 2013. Inhibition of Myc family proteins eradicates KRas-driven lung cancer in mice. *Genes & development* 27:504-513.

Soucie, E.L., M.G. Annis, J. Sedivy, J. Filmus, B. Leber, D.W. Andrews, and L.Z. Penn. 2001. Myc potentiates apoptosis by stimulating Bax activity at the mitochondria. *Molecular and cellular biology* 21:4725-4736.

Staller, P., K. Peukert, A. Kiermaier, J. Seoane, J. Lukas, H. Karsunky, T. Moroy, J. Bartek, J. Massague, F. Hanel, and M. Eilers. 2001. Repression of p15INK4b

expression by Myc through association with Miz-1. *Nature cell biology* 3:392-399.

Stanton, B.R., A.S. Perkins, L. Tessarollo, D.A. Sassoon, and L.F. Parada. 1992. Loss of N-myc function results in embryonic lethality and failure of the epithelial component of the embryo to develop. *Genes & development* 6:2235-2247.

Stanton, L.W., P.D. Fahrlander, P.M. Tesser, and K.B. Marcu. 1984. Nucleotide sequence comparison of normal and translocated murine c-myc genes. *Nature* 310:423-425.

Stehelin, D., H.E. Varmus, J.M. Bishop, and P.K. Vogt. 1976. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature* 260:170-173.

Stone, J., T. de Lange, G. Ramsay, E. Jakobovits, J.M. Bishop, H. Varmus, and W. Lee. 1987. Definition of regions in human c-myc that are involved in transformation and nuclear localization. *Molecular and cellular biology* 7:1697-1709.

Stoneley, M., J.P. Spencer, and S.C. Wright. 2001. An internal ribosome entry segment in the 5' untranslated region of the mnt gene. *Oncogene* 20:893-897.

Strahl, B.D., and C.D. Allis. 2000. The language of covalent histone modifications. *Nature* 403:41-45.

Strasser, A., A.W. Harris, M.L. Bath, and S. Cory. 1990. Novel primitive lymphoid tumours induced in transgenic mice by cooperation between myc and bcl-2. *Nature* 348:331-333.

Tagawa, H., and M. Seto. 2005. A microRNA cluster as a target of genomic amplification in malignant lymphoma. *Leukemia* 19:2013-2016.

Takahashi, K., K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, and S. Yamanaka. 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131:861-872.

Takahashi, T., H. Konishi, K. Kozaki, H. Osada, S. Saji, T. Takahashi, and T. Takahashi. 1998. Molecular analysis of a Myc antagonist, ROX/Mnt, at 17p13.3 in human lung cancers. *Japanese journal of cancer research : Gann* 89:347-351.

Takakura, S., N. Mitsutake, M. Nakashima, H. Namba, V.A. Saenko, T.I. Rogounovitch, Y. Nakazawa, T. Hayashi, A. Ohtsuru, and S. Yamashita. 2008. Oncogenic role of miR-17-92 cluster in anaplastic thyroid cancer cells. *Cancer science* 99:1147-1154.

Taylor, M.V., M. Gusse, G.I. Evan, N. Dathan, and M. Mechali. 1986. Xenopus myc proto-oncogene during development: expression as a stable maternal mRNA uncoupled from cell division. *The EMBO journal* 5:3563-3570.

Thompson, C.B., P.B. Challoner, P.E. Neiman, and M. Groudine. 1985. Levels of c-myc oncogene mRNA are invariant throughout the cell cycle. *Nature* 314:363-366.

Todd, R., and D.T. Wong. 1999. Oncogenes. *Anticancer research* 19:4729-4746.

Tonna, S., A. El-Osta, M.E. Cooper, and C. Tikellis. 2010. Metabolic memory and diabetic nephropathy: potential role for epigenetic mechanisms. *Nature reviews. Nephrology* 6:332-341.

Toyo-oka, K., T.J. Bowen, S. Hirotsune, Z. Li, S. Jain, S. Ota, L. Escoubet-Lozach, I. Garcia-Bassets, J. Lozach, M.G. Rosenfeld, C.K. Glass, R. Eisenman, B. Ren, P. Hurlin, and A. Wynshaw-Boris. 2006. Mnt-deficient mammary glands exhibit impaired involution and tumors with characteristics of myc overexpression. *Cancer research* 66:5565-5573.

Toyoshima, H., and T. Hunter. 1994. p27, a novel inhibitor of G1 cyclin-Cdk protein kinase activity, is related to p21. *Cell* 78:67-74.

Tsuneoka, M., and E. Mekada. 2000. Ras/MEK signaling suppresses Myc-dependent apoptosis in cells transformed by c-myc and activated ras. *Oncogene* 19:115-123.

Tyagi, A., J. Ryme, D. Brodin, A.K. Ostlund Farrants, and N. Visa. 2009. SWI/SNF associates with nascent pre-mRNPs and regulates alternative pre-mRNA processing. *PLoS genetics* 5:e1000470.

Ulaner, G.A., J.F. Hu, T.H. Vu, L.C. Giudice, and A.R. Hoffman. 1998. Telomerase activity in human development is regulated by human telomerase reverse transcriptase (hTERT) transcription and by alternate splicing of hTERT transcripts. *Cancer research* 58:4168-4172.

Vasilevsky, N.A., C.E. Ruby, P.J. Hurlin, and A.D. Weinberg. 2011. OX40 engagement stabilizes Mxd4 and Mnt protein levels in antigen-stimulated T cells leading to an increase in cell survival. *European journal of immunology* 41:1024-1034.

Vassilev, L.T., B.T. Vu, B. Graves, D. Carvajal, F. Podlaski, Z. Filipovic, N. Kong, U. Kammlott, C. Lukacs, C. Klein, N. Fotouhi, and E.A. Liu. 2004. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* 303:844-848.

Vennstrom, B., D. Sheiness, J. Zabielski, and J.M. Bishop. 1982. Isolation and characterization of c-myc, a cellular homolog of the oncogene (v-myc) of avian myelocytomatosis virus strain 29. *Journal of virology* 42:773-779.

Ventura, A., A.G. Young, M.M. Winslow, L. Lintault, A. Meissner, S.J. Erkeland, J. Newman, R.T. Bronson, D. Crowley, J.R. Stone, R. Jaenisch, P.A. Sharp, and T. Jacks. 2008. Targeted deletion reveals essential and overlapping functions of the miR-17 through 92 family of miRNA clusters. *Cell* 132:875-886.

Vermeer, M.H., R. van Doorn, R. Dijkman, X. Mao, S. Whittaker, P.C. van Voorst Vader, M.J. Gerritsen, M.L. Geerts, S. Gellrich, O. Soderberg, K.J. Leuchowius, U. Landegren, J.J. Out-Luiting, J. Knijnenburg, M. Ijszenga, K. Szuhai, R. Willemze, and C.P. Tensen. 2008. Novel and highly recurrent chromosomal alterations in Sezary syndrome. *Cancer research* 68:2689-2698.

Vervoorts, J., J.M. Luscher-Firzlaff, S. Rottmann, R. Lilischkis, G. Walsemann, K. Dohmann, M. Austen, and B. Luscher. 2003. Stimulation of c-MYC transcriptional activity and acetylation by recruitment of the cofactor CBP. *EMBO reports* 4:484-490.

Vita, M., and M. Henriksson. 2006. The Myc oncoprotein as a therapeutic target for human cancer. *Seminars in cancer biology* 16:318-330.

Vivanco, I., and C.L. Sawyers. 2002. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nature reviews. Cancer* 2:489-501.

Vogelstein, B., and K.W. Kinzler. 1993. The multistep nature of cancer. *Trends in genetics : TIG* 9:138-141.

Volinia, S., G.A. Calin, C.G. Liu, S. Ambs, A. Cimmino, F. Petrocca, R. Visone, M. Iorio, C. Roldo, M. Ferracin, R.L. Prueitt, N. Yanaihara, G. Lanza, A. Scarpa, A. Vecchione, M. Negrini, C.C. Harris, and C.M. Croce. 2006. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proceedings of the National Academy of Sciences of the United States of America* 103:2257-2261.

von der Lehr, N., S. Johansson, S. Wu, F. Bahram, A. Castell, C. Cetinkaya, P. Hydbring, I. Weidung, K. Nakayama, K.I. Nakayama, O. Soderberg, T.K. Kerppola, and L.G. Larsson. 2003. The F-box protein Skp2 participates in c-Myc proteosomal degradation and acts as a cofactor for c-Myc-regulated transcription. *Molecular cell* 11:1189-1200.

Vriz, S., M. Taylor, and M. Mechali. 1989. Differential expression of two Xenopus c-myc proto-oncogenes during development. *The EMBO journal* 8:4091-4097.

Wagner, A.J., J.M. Kokontis, and N. Hay. 1994. Myc-mediated apoptosis requires wild-type p53 in a manner independent of cell cycle arrest and the ability of p53 to induce p21waf1/cip1. *Genes & development* 8:2817-2830.

Wahlstrom, T., S. Belikov, and M. Arsenian Henriksson. 2013. Chromatin dynamics at the hTERT promoter during transcriptional activation and repression by c-Myc and Mnt in Xenopus leavis oocytes. *Experimental cell research* 319:3160-3169.

Wahlstrom, T., and M. Henriksson. 2007. Mnt takes control as key regulator of the myc/max/mxd network. *Advances in cancer research* 97:61-80.

Walker, W., Z.Q. Zhou, S. Ota, A. Wynshaw-Boris, and P.J. Hurlin. 2005. Mnt-Max to Myc-Max complex switching regulates cell cycle entry. *The Journal of cell biology* 169:405-413.

Wang, G.G., C.D. Allis, and P. Chi. 2007a. Chromatin remodeling and cancer, Part I: Covalent histone modifications. *Trends in molecular medicine* 13:363-372.

Wang, G.G., C.D. Allis, and P. Chi. 2007b. Chromatin remodeling and cancer, Part II: ATP-dependent chromatin remodeling. *Trends in molecular medicine* 13:373-380.

Wang, J., L.Y. Xie, S. Allan, D. Beach, and G.J. Hannon. 1998. Myc activates telomerase. *Genes & development* 12:1769-1774.

Wang, S., and J. Zhu. 2004. The hTERT gene is embedded in a nuclease-resistant chromatin domain. *The Journal of biological chemistry* 279:55401-55410.

Weber, J.D., L.J. Taylor, M.F. Roussel, C.J. Sherr, and D. Bar-Sagi. 1999. Nucleolar Arf sequesters Mdm2 and activates p53. *Nature cell biology* 1:20-26.

Weinberg, R.A. 1995. The retinoblastoma protein and cell cycle control. *Cell* 81:323-330.

Weinstein, J.L., H.M. Katzenstein, and S.L. Cohn. 2003. Advances in the diagnosis and treatment of neuroblastoma. *The oncologist* 8:278-292.

Weiss, W.A., K. Aldape, G. Mohapatra, B.G. Feuerstein, and J.M. Bishop. 1997. Targeted expression of MYCN causes neuroblastoma in transgenic mice. *The EMBO journal* 16:2985-2995.

Wernig, M., A. Meissner, R. Foreman, T. Brambrink, M. Ku, K. Hochedlinger, B.E. Bernstein, and R. Jaenisch. 2007. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* 448:318-324.

Westermann, F., D. Muth, A. Benner, T. Bauer, K.O. Henrich, A. Oberthuer, B. Brors, T. Beissbarth, J. Vandesompele, F. Pattyn, B. Hero, R. Konig, M. Fischer, and M. Schwab. 2008. Distinct transcriptional MYCN/c-MYC activities are associated with spontaneous regression or malignant progression in neuroblastomas. *Genome biology* 9:R150.

Wierstra, I., and J. Alves. 2008. The c-myc promoter: still MysterY and challenge. *Advances in cancer research* 99:113-333.

Wolffe, A.P., and J.J. Hayes. 1999. Chromatin disruption and modification. *Nucleic acids research* 27:711-720.

Wood, M.A., S.B. McMahon, and M.D. Cole. 2000. An ATPase/helicase complex is an essential cofactor for oncogenic transformation by c-Myc. *Molecular cell* 5:321-330.

Wright, J.B., S.J. Brown, and M.D. Cole. 2010. Upregulation of c-MYC in cis through a large chromatin loop linked to a cancer risk-associated single-nucleotide polymorphism in colorectal cancer cells. *Molecular and cellular biology* 30:1411-1420.

Wu, S., C. Cetinkaya, M.J. Munoz-Alonso, N. von der Lehr, F. Bahram, V. Beuger, M. Eilers, J. Leon, and L.G. Larsson. 2003. Myc represses differentiation-induced

p21CIP1 expression via Miz-1-dependent interaction with the p21 core promoter. *Oncogene* 22:351-360.

Xu, D., N. Popov, M. Hou, Q. Wang, M. Bjorkholm, A. Gruber, A.R. Menkel, and M. Henriksson. 2001. Switch from Myc/Max to Mad1/Max binding and decrease in histone acetylation at the telomerase reverse transcriptase promoter during differentiation of HL60 cells. *Proceedings of the National Academy of Sciences of the United States of America* 98:3826-3831.

Yang, H., T.W. Li, K.S. Ko, M. Xia, and S.C. Lu. 2009. Switch from Mnt-Max to Myc-Max induces p53 and cyclin D1 expression and apoptosis during cholestasis in mouse and human hepatocytes. *Hepatology* 49:860-870.

Yang, H., T.W. Li, J. Peng, X. Tang, K.S. Ko, M. Xia, and M.A. Aller. 2011. A mouse model of cholestasis-associated cholangiocarcinoma and transcription factors involved in progression. *Gastroenterology* 141:378-388, 388 e371-374.

Zeller, K.I., X. Zhao, C.W. Lee, K.P. Chiu, F. Yao, J.T. Yustein, H.S. Ooi, Y.L. Orlov, A. Shahab, H.C. Yong, Y. Fu, Z. Weng, V.A. Kuznetsov, W.K. Sung, Y. Ruan, C.V. Dang, and C.L. Wei. 2006. Global mapping of c-Myc binding sites and target gene networks in human B cells. *Proceedings of the National Academy of Sciences of the United States of America* 103:17834-17839.

Zervos, A.S., J. Gyuris, and R. Brent. 1993. Mxi1, a protein that specifically interacts with Max to bind Myc-Max recognition sites. *Cell* 72:223-232.

Zhang, Z., H. Sun, H. Dai, R.M. Walsh, M. Imakura, J. Schelter, J. Burchard, X. Dai, A.N. Chang, R.L. Diaz, J.R. Marszalek, S.R. Bartz, M. Carleton, M.A. Cleary, P.S. Linsley, and C. Grandori. 2009. MicroRNA miR-210 modulates cellular response to hypoxia through the MYC antagonist MNT. *Cell Cycle* 8:2756-2768.

Zhu, J., J. Blenis, and J. Yuan. 2008. Activation of PI3K/Akt and MAPK pathways regulates Myc-mediated transcription by phosphorylating and promoting the degradation of Mad1. *Proceedings of the National Academy of Sciences of the United States of America* 105:6584-6589.

Zindy, F., C.M. Eischen, D.H. Randle, T. Kamijo, J.L. Cleveland, C.J. Sherr, and M.F. Roussel. 1998. Myc signaling via the ARF tumor suppressor regulates p53-dependent apoptosis and immortalization. *Genes & development* 12:2424-2433.

Zwart, R., F. Sleutels, A. Wutz, A.H. Schinkel, and D.P. Barlow. 2001. Bidirectional action of the Igf2r imprint control element on upstream and downstream imprinted genes. *Genes & development* 15:2361-2366.

10 PUBLICATIONS AND MANUSCRIPT