Part One
General Biological and Statistical Basics

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The Biology of *MYC* in Health and Disease: A High Altitude View

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1.1
Introduction

The *MYC* oncogene has been intensely studied over the past 25 years, in part due to its extensive involvement in many forms of human cancer. *MYC* appears to be critical for several cellular processes, including cell division (proliferation), cell survival, DNA-replication, transcriptional regulation, energy metabolism, and differentiation. Overall, *MYC* appears to be an important element in a cell's ability to sense and integrate extracellular signals in its environment. This seems to be a feature that arose along with a metazoan lifestyle of organisms. The extensive research that has focused on *MYC* has yet to provide a consensus on the functions of *MYC*, because of its involvement in a large number of cellular functions and the use of systems that provide divergent results. One additional level of complication in the study of *MYC* is that it serves as a regulator of many cellular processes while not being directly involved in those specific pathways. For instance, *MYC* regulates cell division or proliferation, but is not a part of the cell cycle machinery. Likewise, *MYC* has been shown to regulate cell survival, but is not involved in the cellular pathways that regulate apoptosis. Along these lines, *MYC* has also been shown to act as a weak transcriptional activator, but the nature and identity of its target genes are poorly defined. The current view on the role of *MYC* in gene regulation is that it affects the expression of a broad set of genes, perhaps by some greater effects on chromatin modification than a "traditional" transcription factor. In addition, *MYC* appears to be involved in facilitating DNA replication as a means to support cell division. Together, these new ideas may help explain some of the observations regarding roles for *MYC* in stem cell maintenance and differentiation, as well as in transformation. Some of these issues may be clarified with ongoing work in the field, as well as the involvement of additional disciplines in biology, that may be better suited to model some of these enigmas and provide testable hypotheses for experimental biologists. This chapter provides a brief summary of the involvement of *MYC* in many aspects of normal cell physiology, as well as in cancer, with an emphasis on the contributions of *MYC* to hematopoietic malignancies.

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The following nomenclature is used in this chapter:

Human gene and protein	*MYC*
Other human forms of myc	MYCN, L-MYC, S-MYC
	(genes in italics, proteins are not italicized)
Mouse gene	*c-myc*
Mouse protein	c-Myc

1.2 *MYC* and Normal Physiology

Pin-pointing precisely the "normal" function, or the mechanism of *MYC* function, would be a difficult if not impossible task. This is because *MYC* is involved in the regulation of metabolism, cell-cycle regulation and differentiation, cell adhesion, apoptosis, protein synthesis, and transcription of microRNAs. The involvement of *MYC* in so many aspects of normal cell physiology has yielded numerous publications concerning its function, but has precluded the formulation of a concerted view of *MYCs* function. *MYC* carries out these functions in the cell through various mechanisms, including transcriptional regulation (both activation and repression), control of DNA methylation, and chromatin remodeling [1]. The best way to impart an overall appreciation for the importance of *MYC* in a normal cells and tissues would be to present a brief overview of each of the areas mentioned above. A more detailed account of the functions of *MYC* in each of these fields of biology can be found in the various reviews or references cited in the text. An underlying principle that should become evident throughout this chapter is that *MYC's* powerful role is ultimately defined by the context of its expression (Figure 1.1). Importantly, the expression of *MYC* is tightly regulated and transient in normal settings. The *MYC* protein has a very short half-life (roughly 20–30 min) and is a member of a family of loosely associated genes (*MYCN, L-MYC, S-MYC*). These related genes are expressed at different times and in different tissues and there is some evidence that they have similar or redundant functions. The overexpression of *MYC* in disease states exaggerates its effects on a particular cellular pathway. These are some of the sources used to ascertain the role of *MYC* in normal physiology. When drawing conclusions about *MYC* is it important to consider the experimental design that is being used, keeping in mind the differences between cell lines and whole organisms and possible indirect influences of loss and gain of function.

1.3 Regulation of Transcription and Gene Expression [2]

Transcription regulation and gene expression is a very complicated and highly regulated process. Although various mechanisms for *MYC* function have been described, the effects *MYC* exerts on various cell functions described below are

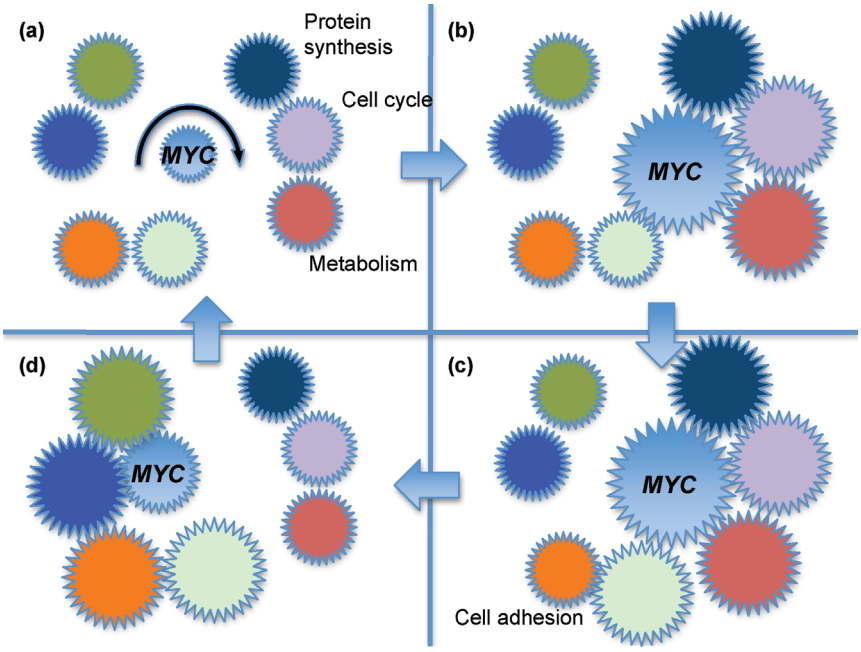

Figure 1.1 The master cog: *MYC* function is largely determined by the amount and context of gene expression. *MYC* levels are very low in a normal cell (a); *MYC* upregulation in a cell powers components of different aspects of the cell machinery (protein, synthesis, cell cycle, and metabolism pictured here) (b); *MYC* can have many secondary (tertiary, etc.) effects within a cell (cell adhesion pictured here) (c); a critical ability of a normal cell is the downregulation of *MYC* following its pleiotropic activity (d).

carried out by its ability to regulate transcription. The goal of many biochemical studies looking at *MYC*'s role in transcriptional regulation and gene expression is to discover how *MYC* controls so many processes and how deregulated *MYC* can be such a powerful oncogene.

To summarize briefly, *MYC* belongs to the family of helix-loop-helix leucine zipper proteins that form homo- or heterodimers and bind to DNA sequences. *In vitro* c-Myc can form homodimers or heterodimerize with either Max, Mad1, Mxi1, Mad3, Mad4, or Mnt. *In vivo*, however, c-Myc is only found as a heterodimer with Max. When c-Myc is bound to Max it can both activate and repress transcription of its target genes. Max itself can form homodimers *in vivo,* but neither activate nor repress transcription. Max can also bind Mad family of proteins and they repress transcription. In general, Myc-Max complexes are often the majority of heterodimers found in proliferating cells and Mad-Max/Mnt-Max complexes are the majority in cells that have differentiated or are in a resting phase [3]. Since Max is usually in excess in cells, it is the relative amounts of c-Myc and Mad that ultimately determine whether c-Myc can activate or repress its specific targets and this is probably a reason why the levels of c-Myc inside a cell are tightly regulated and the half-life of this powerful protein is so

short. The continuous degradation of MYC and its short half-life suggest that *MYC* binding to a given site on DNA is transient. The rate of *MYC* production required to maintain steady-state levels would ensure that all *MYC* target sequences in a genome may become occupied at some point during the surge of *MYC* expression. Probably not all of the targets are bound at any one point in time, due to the transient nature of the heterodimers. An additional level of complication is the ability of the complexes that involve *MYC* to recruit transcriptional coregulators, further increasing the complexity of the transcriptional profile that is affected by *MYC*. Among these are the TRRAP coactivator, the Tip60 complex, the Pim1-kinase, the Lid/Rpb2 H3-K4 demethylase, and the HectH9 ubiquitin ligase. These factors are involved in histone modification and alteration of chromatin states and nucleosome instability. The absence of a discrete set of gene targets for *MYC* may come as a result of its ability to induce significant changes at the chromatin level. In addition, the contributions of *MYC* to reprogramming of somatic cells into induce pluripotent stem cells (iPSs) may result from its ability to induce an open chromatin structure.

1.4 Metabolism [4]

Cell metabolism can be defined as a complex set of chemical reactions that allow the cell to live in a given environment. The types of reactions that take place are set in motion from cues that the cell receives from that particular environment. *MYC* plays an important role in cell metabolism because it can regulate metabolic processes that enable cells to grow in suboptimal conditions such as hypoxia. Under normal circumstances this is critical for mounting an immune response as specific antigen dependent cells need to hyperproliferate to combat the microbial invader. During the development (and maintenance) of cancer *MYC* can function to supply the energy for tumor growth when the environment would otherwise tell the cells to stop proliferating in such crowded conditions.

MYC influences cell metabolism by participating in several metabolic pathways, including glucose uptake and glycolysis, which makes sense because a cell that is growing and proliferating needs energy to carry out these activities [5]. *MYC* specifically upregulates transcripts of important enzymes of glucose metabolism, including glucose transporter, enolase A, lactate dehydrogenase A, phosphofructokinase, and hexokinase II [6–8]. During the process of transformation, *MYC* has been shown to induce glutaminolysis and glutamine addiction through the upregulation transcripts of glutamine transporters, glutaminase, and lactate dehydrogenase A (LDH-A) [9]. Iron metabolism is also an important cellular function that is driven by *MYC* as enzymes that catalyze energy metabolism and DNA synthesis require iron. Reports have shown coordinated regulation of iron-controlling gene transcripts by *MYC,* including cell surface receptors such as the transferring receptor (TFRC1) [10]. *MYC* also affects transcription of genes involved in generating the building blocks for DNA synthesis, called nucleotides, such as ornithine decarboxylase that functions in the synthesis of polyamines required for nucleotide biosynthesis enzymes [11].

1.5
Cell-Cycle Regulation and Differentiation [12]

The cell-cycle is the process by which one cell divides symmetrically into two daughter cells. This involves a highly-regulated series of events with many checks and balances. It is critical that new cells have all the necessary information (DNA) and machinery (proteins, etc.) to survive as a new cell, respond to physiological cues, and divide as needed.

Expression analysis of *MYC* and cell cycle and growth genes demonstrates that [13] *MYC* influences the transcription of a large number of cell cycle genes and gene products. *MYC* positively regulates expression of proteins that push the cell cycle forward: G1-specific cyclin-dependent kinases (CDKs) by inactivating inhibitors of these kinases. *MYC* also induces activators of specific CDKs. The net result of these activities is that *MYC* prevents cell cycle arrest in the presence of growth-inhibitory signals or after withdrawal of activating signals or under signals to differentiate. If *MYC* is activated, or dysregulated, the cell will be pushing to divide and not differentiate. Hence, many differentiation programs require the downregulation of *MYC* to accomplish terminal differentiation. One recent observation that supports this notion relates to the need for ectopic overexpression of *MYC*, along with three other transcription factors (Oct4, Sox2, and Klf4), to reprogram fibroblasts into iPS cells.

1.6
Protein Synthesis [14]

Protein synthesis is closely tied to cell-cycle because cells need to produce new proteins in order to divide, as one of the check-points in the cell cycle is the determination of whether the cell has reached a large enough mass. As with other processes in a cell, protein synthesis occurs as a result of cues the cell receives from its environment. One of the final effector proteins in this chain of events is *MYC*.

Unsurprisingly, various studies have identified translation initiation (protein synthesis) factors as targets of *c-myc* [14]. As mentioned above, translation initiation directly affects both growth and division in a cell. Ribosomal content and ribosomal genes are affected by *MYC*. In one gene screen with N-myc, it was shown to enhance the expression of a large set of genes functioning in ribosome biogenesis and protein synthesis in neuroblastoma cells [15]. Specific studies have shown the influence of *MYC* on RNA pol I, II, and III activation [15–20].

1.7
Cell Adhesion [4]

Cell adhesion is important for tissues and organisms because for efficient functioning of organs, like heart, lung, and brain, these cells need to remain together. In

"organs" such as the immune system, cell adhesion is turned on and off many times during a cell's life because the immune system must patrol the entire body. Cells of the immune system travel through the blood stream and lymph system to receive signals and carry out their effector functions such as antibody production or cell-killing.

Out of 218 differentially expressed genes in keratinocytes from *MYC* transgenic mouse, 30% are downregulated cellular adhesion genes and 11% are cytoskeletal related genes. Specifically, expression analysis in primary human fibroblasts *MYC* was found to repress genes encoding the extracellular matrix proteins fibronectin and collagen, and the cytoskeletal protein tropomyosin [13]. More recently *MYC* has been shown to regulate cell surface adhesion molecules such as N-cadherin on hematopoietic stem cells. Upregulation of *MYC* was associated with downregulation of N-cadherin and mobilization away from the stem cell niche [21].

1.8 Apoptosis [22]

Apoptosis is defined as programmed cell death and is crucial to the homeostasis of many organisms. New cells are created all the time and it is important to have a defined system to remove old cells. Apoptosis is also highly regulated because if cells die when they are not supposed to, or when cells do not die and they are supposed to, there is disease, as in the case of cancer. As mentioned in the introduction *MYC* function is highly dependent on the context of MYC expression. This point is quite clear when the role of *MYC* is considered in the cellular function of apoptosis, or programmed cell death.

c-Myc normally serves as a survival signal under physiological conditions but can contribute to apoptosis under conditions of stress (such as chemotoxic agents, transcription factor inhibitors, etc.). For example, under normal physiological conditions c-Myc can upregulate the glycolysis pathway as a mechanism to regulate cell survival. This is a critical function for the immune system because even if the environment is telling the cell that nutrients are low, an activating signal through c-Myc can supply the energy required to mount an effective immune response. Also, c-Myc has been shown to be critical in the response of activated B-cells to cytokines.

c-Myc is also necessary and sufficient, under stress conditions, for efficient response to transcription/translation inhibitors, hypoxia glucose deprival, chemotoxins, DNA damage, heat shock, and chemotherapeutic agents. This has been shown in work demonstrating the requirement of c-Myc and the function of death receptors [23–25]. Its somewhat paradoxical function of controlling cell fate has been put into a "dual function model" where c-Myc is seen as the ultimate co-ordinate activator of cell proliferation or apoptosis. For example, c-Myc overexpression in cells that are exposed to some form of stress results in continued proliferation until the cells die through the standard p53-dependent forms of apoptosis in the context of genotoxic stress. c-Myc itself, however, is not involved in the death program. In a

similar fashion, c-Myc can coordinate proliferation, but is not itself involved in the cell cycle machinery.

1.9
MicroRNAs

MicroRNAs (miRNAs) were only discovered recently and have added another degree of complexity to the study of how cells regulate the content of their particular make-up. Originally, it was believed that proteins were responsible for determining how much of another protein was around, through what are called feedback mechanisms. With the finding of miRNA, it has been shown that nucleic acid sequences can also regulate the amount of protein by directly controlling the amount of message for a particular protein.

Although *MYC*'s role in regulation of MicroRNA is a relatively new field, studies have shown that *MYC* activates a cluster of six microRNAs on human chromosome 13, two of which negatively regulate an important transcription factor E2F1. *MYC* is involved in regulating miRNA transcription, as opposed to their processing or stability. When specific miRNAs that are repressed by c-Myc are forcibly expressed, investigators can reduce tumorigenicity for lymphoma cells [26, 27]. Because the knowledge base of how miRNAs function, how many there are, and what governs their specificities is just beginning, the future will certainly be interesting and challenging for biostatisticians, mathematical modelers, and systems biologists when trying to figure out how a master regulator like *MYC* regulates regulators.

1.10
Physiological Effects of Loss and Gain of c-myc Function in Mice

1.10.1
Loss of Function

Apart from gene array studies in established cell lines, the most informative studies, to date, on the normal function of c-Myc probably come from genetic modification studies where the specific gene of interest can be turned on or off and in specific tissues. Early attempts at reducing the levels of c-Myc showed that there was an increase in cell doubling time in rat fibroblasts that were missing both alleles of *c-myc*. Notably, the rat fibroblasts were generated by chemical mutagenesis and it is unclear what other mutations they might harbor [28]. In addition, the investigators observed decreased cell mass, total mRNA, and protein levels demonstrating the importance of *c-myc* in these processes [29].

To circumvent the problem of embryonic lethality of conventional gene knock-out mice, investigators have injected *c-myc*$-/-$ and *c-myc*$+/-$ cells into blastocysts of wild-type mice to study the requirement of c-Myc in mature immune cells. This approach demonstrated that lymphocytes in *c-myc*$-/-$ have difficulty maturing and

they fail to grow and proliferate normally [30]. Importantly, the myc ko animals dies at E14 from severe anemia, implicating c-Myc in Epo signaling. Analysis of cytokines that rely on the gc of the IL-2 receptor in B-cells have shown a critical role for c-Myc in mediating cytokine-dependent signals related to proliferation and survival of activated B-cells [31].

More sophisticated genetic modification methods have been developed for targeted disruption of *c-myc* in mice. One of these systems allows incremental reduction of expression. Because a complete knock out is lethal before birth, fibroblasts from these different mice have been used to show reduced cell proliferation as c-Myc levels are decreased and that cells will exit the cell cycle when no c-Myc is expressed. Reduction of c-Myc levels in the whole organism results in smaller organisms because c-Myc ultimately controls the decision to divide or not to divide. Also, while most organs in the *c-myc* targeted mice were proportionally decreased in size along with the size of the whole animal, the hematopoietic compartment was disproportionally affected. The cellularity of the bone marrow, thymus, spleen, and lymph nodes is highly dependent on endogenous *c-myc* for its homeostasis and maintenance. Such results confirm the role of c-Myc as a critical survival factor in hematopoietic cells.

1.10.2
Gain of Function: Inducible Transgenic Animals

Techniques to study the normal function of c-Myc in specific cell types or tissues often involve the overexpression of *c-myc* by way of tailoring its expression behind a specific tissues promoter. For example, transgenic E beta-myc mice were shown to have abnormal T cell development when c-Myc was overexpressed in thymocytes [32]. Traditional cell lines involving the use of transformed cells that stably overexpress c-Myc have been plagued with problems and shown to provide results that have not held up with more physiologically relevant systems. Two possible reasons for these problems could be the use of already transformed cells as the background for the experiments, and the unregulated, continuous, and high overexpression of a gene whose expression is very transient and tightly regulated and yields a protein with a short half-life. These novel approaches using genetically engineered mice have begun to yield some important information regarding the effects of overexpressing c-Myc in particular cell types and contexts.

Methods to study the effects of turning on *c-myc* at specific time-points in specific tissues began in 1999 with several versions of the tetracycline-transactivating system. One mouse would express *c-myc* in all tissues when a specific drug was given to the mice (doxycycline) and one mouse would express *c-myc* in cells that used the immunoglobulin enhancer element-T-cells and B-cells [33]. Variations on this theme produced results from tissue-specific expression of c-Myc in pancreas and skin. Islet beta-cells that overexpressed c-Myc would proliferate and undergo apoptosis unless exogenous survival signal like Bcl-XL could protect them [34]. In addition, the same group showed that activation of c-Myc in skin causes, proliferation, disruption of differentiation, hyperplasia/dysplasia, and, surprisingly, angiogenesis.

More recently, groups have demonstrated that overexpression of c-Myc in anergic B cells (immune cells that do not respond to antigen) breaks this state of non-responsiveness. Importantly, this work shows c-Myc is downstream of important activating signals and that c-Myc alone can replace for the absence of these signals.

By genetically modifying the context of c-Myc overexpression by way of transgenes, this paper demonstrates that various B cell malignancies can be modeled very precisely in mice when additional signals are provided [35]. The paper demonstrates that if bona fide cooperating transforming events can be determined then a host of new targets suddenly become available for the development of new cancer therapeutics.

1.11
Contributions of *MYC* to Tumor Biology

Deregulation of *MYC* expression is one of the most common features in most forms of cancer. The presence of a surfeit of *MYC* is common in many solid tumors, in addition to hematopoietic malignancies. *MYC* has been implicated in most breast, gynecological, prostate, and gastrointestinal cancers, among others [36–40]. The role of *MYC* in these cancers is not fully understood because the actions of *MYC* are notoriously pleiotropic. Like in hematopoietic malignancies, the deregulation of *MYC* alone is insufficient for tumorigenesis, but rather the deregulation of *MYC* must accompany other changes in cell to form a tumor. In fact, *MYC* overexpression is usually associated with activating mutations in Ras genes, other members of the MAPK signaling pathways, Akt genes, loss of PTEN, or loss of BRCA1. Most of the genetic alterations discussed in this section result in the overexpression of MYC. It is thought that the continuous presence of elevated levels of MYC in the cell alter its physiology by enabling the cell to operate despite physiological control mechanisms. We will next review the various genetic alterations that result in overexpressed MYC that have been reported in hematological malignancies.

Some of these genetic changes are conserved across both hematopoietic cancers and solid tumors, while others are specific to solid tumors, or even certain types of solid tumors. *MYC* expression promotes progression through the cell cycle and enhances cellular growth in both hematopoietic and non-hematopoietic tumors (discussed above) [36–40]. In addition, *MYC* promotes increased cell adhesion, metastasis, and vasculogenesis in solid tumors [36, 41]. These are important characteristics of solid tumors, but are dispensable for lymphoid malignancies that are already circulating throughout the body. Finally, in both hematopoietic and non-hematopoietic tumors, increased *MYC* expression generally correlates to more aggressive tumors and poor patient outcomes.

Deregulation of *MYC* family genes can occur through several mechanisms. Chromosomal translocations involving *MYC* figure prominently in lymphoid malignancies, but are uncommon in solid tumors. Instead, *MYC* overexpression is achieved through either gene amplification, mutations that result in the stabilization

of RNA or protein products of *MYC* family genes, or an increase in *MYC* transcription through an aberrantly activated signaling pathway and mutations in the transcriptional regulatory sequences of *MYC* [76]. We focus this part of the chapter on the contributions of *MYC* to hematopoietic malignancies.

1.12 Introduction of Hematopoietic Malignancies

The cellular components that make up the blood are derived from pluripotent hematopoietic stem cells (HSCs). These HSCs differentiate into mature red and white blood cells through various intermediate cell types before becoming terminally differentiated. HSCs are a heterogonous pool of long-term self-renewing HSC (LT-HSC), transiently self-renewing HSC (short-term HSC), and non-self-renewing multipotent HSC. LT-HSCs have the capacity to develop into lymphoid and myeloid precursors. Lymphoid precursors further differentiate into natural killer cells, B-lymphocytes, and T-lymphocytes whereas myeloid precursors give rise to erythroid (red blood cells), megakaryocytic, or granulocytic/monocytic lineages [42]. The genetic mutations that give rise to cancer can occur at any stage of development and lead to the clonal expansion of cells of a particular developmental stage.

Hematological malignancies are those affecting the blood, bone marrow, and lymph nodes. These diseases include leukemia, lymphoma, and multiple myeloma. Over the past few decades these cancers have been increasingly recognized as a genetic disease accumulating specific genetic mutations that aid in their diagnosis. Characterizing and classifying hematological cancers by taking into account the clinical behavior, morphology, immunophenotype, and cytogenetic data has led to better diagnosis and treatment.

Leukemia consists of several malignancies that originate in the bone marrow and are derived from clonal expansion of myeloid or immature lymphoid cells. Disease occurs when leukemic cells out compete normal bone marrow residents, resulting in a deficiency of blood platelets, white blood cells, and red blood cells. Lymphoma consists of several malignancies that originate from mature lymphoid lineages. Lymphomas commonly originate in lymph nodes and present as an enlarged node. Lymphoma is classified based on the predominant cell type and the degree of differentiation. Malignancies affecting the B cell lineage make up more than 90% of the human non-Hodgkin's lymphomas (NHLs) [43, 44]. Multiple myeloma is a tumor composed of plasma cells. Those are the cells that generate affinity matured antibodies in response to microbial infection. The genetics of multiple myeloma are fairly complex, and have not yet pointed to specific and recurrent genetic abnormalities in several different tumors.

MYC is the most commonly dysregulated genes in the cases of NHL [45–47]. The role of *MYC* in cellular transformation therefore has remained an area of intense study to better understand tumor biology as well as gain potential insights for the treatment of these life-ending diseases.

1.13
Mechanisms of *MYC* Dysregulation in Hematological Malignancies

Dysregulated *MYC* expression in hematological malignancies occurs by several different mechanisms. Cytogenetic and molecular investigations have provided evidence that chromosomal abnormalities such as translocation, gene amplification, and mutations in the *MYC* open reading frame or promoter/transcriptional regulatory regions can give rise to *MYC* overexpression. Dysregulated *MYC* has also been associated with viral infection and dysregulation of auxiliary proteins that stabilize *MYC*.

In normal cells *MYC* expression is tightly regulated at both the transcriptional and post-transcriptional levels. One feature common to Burkitt's lymphoma, a prototypic form of NHL, and a small portion of other leukemias is a chromosomal translocation that juxtaposes the *MYC* proto-oncogene with the regulatory elements of an immunoglobulin gene locus [43, 48–50]. As the heavy and light chain loci are transcriptionally activated during lymphocyte development and thereafter, these translocations lead to *MYC* overexpression. The continuous transcription of *MYC* by a powerful immunoglobulin promoter no longer allows for the carefully controlled, and transient, expression of *MYC* in response to physiological signals. Instead, there are high and consistent levels of *MYC* throughout.

Three types of *MYC* translocations have been identified in Burkitt's lymphoma cases. The most common translocation (t8;14) is *MYC* (chr. 8) to IgH (chr. 14), which is seen in 80% of Burkitt's lymphoma cases. About 15% of Burkitt's lymphoma cases have a t2;8 translocation, where the translocation occurs between *MYC* and kappa light chain gene, and the remaining 5% have a t8;22 translocation between *MYC* and lambda light chain gene [43, 49]. In cells that express immunoglobulin genes, these genomic rearrangements result in expression of *MYC* that would otherwise be tightly regulated [48, 51, 52]. A similar translocation of *MYC* into the alpha locus of the T cell receptor has also been reported for some T cell leukemias [53].

Amplification of the *MYC* locus is another genetic abnormality that is observed in some forms of leukemia or lymphomas. These may be observed in about 16% of diffuse large B-cell lymphomas (DLBCL), a common form of NHL that affects adults in North America and Europe [54]. Gene amplification is a cellular process whereby multiple copies of a particular gene accumulate, leading to overexpression of the gene product. Gene amplification can occur by the breakage-fusion-bridge cycle in a cell in which a sister chromatid that has incurred a DNA double strand break fuses to the other sister chromatid forming a bridge. At mitosis, the breakage of this giant inverted repeat leaves each daughter cell with a chromatid lacking one telomere. After replication, the broken sister chromatids fuse again, perpetuating the breakage-fusion-bridge cycle. Amplification occurs when the breakage of the fused sister chromatid is asymmetric and one daughter cell receives both allelic copies of a proto-oncogene [55–57]. Defects in NHEJ component have been observed in human cancers, including leukemia and multiple myeloma [58]. Amplification of the IgH/Myc fusion loci has been reported in some human B cell lymphomas and has been associated with poor prognosis.

1.14
Mutation(s) in the *MYC* Gene in Hematological Cancers

MYC is rapidly metabolized in cells via the ubiquitin/26s proteasome pathway with a half-life of approximately 30 min [59–62]. When cells enter the cell cycle, *MYC* can accumulate resulting from stabilization of the *MYC* protein. The increase in *MYC* half-life is mediated by two Ras effector pathways, Raf/ERK and PI-3K/AKT that result in *MYC* phosphorylation at Ser-62 and Thr-58, respectively [63]. *MYC* levels then return to low basal levels as the cell progresses through the cell cycle. Mutations in *MYC* that increase the half-life of the protein result in an accumulation of *MYC* such that these cells continuously expand and do not differentiate [61]. The presence of point mutations in *MYC* proteins occurs in about 60% of all B cell lymphomas. These mutations occur in two main regions of the open reading frame – those encompassing amino acid 47–62 or amino acids 106–143. Mutations in the amino acids Thr-58 and Ser-62 alter *MYC* phosphorylation at these residues, resulting in a substantial decrease in *MYC* degradation [61, 64, 65].

Regulated expression of *MYC* also occurs at the level of mRNA stability. Mitogens that initiate a proliferative response such as lipopolysaccharide, concanavalin A, or platelet-derived growth factor cause an increase in *MYC* mRNA concentration and stability [66]. *MYC* mRNA has a relatively short half-life of approximately 15 min in cells [67]. The 5′ truncated *MYC* mRNA that results from the chromosomal translocation in B-cell lymphoma was found to be quite stable with a half-life of several hours [68]. Mutations leading to the removal of the 3′ untranslated region (UTR) destabilizing sequences observed in T cell leukemias also result in an accumulation of *MYC* mRNA [69, 70].

1.15
Role of *MYC* in Cell Cycle Regulation and Differentiation in Hematological Cancers

MYC is expressed in most proliferating cells and repression of *MYC* is required for terminal differentiation of many cell types, including hematopoietic cells [71, 72]. Studies investigating conditional *c-myc* knockout alleles demonstrate that loss of *c-myc* stops cellular proliferation and these cells exit the cell cycle [73, 74]. In murine myeloid leukemic cells, overexpression of c-Myc blocks terminal differentiation and its associated growth arrest [75].

Deregulated *MYC* can maintain cells in a constant state of proliferation; this increases the likelihood that mutations in tumor suppressor, anti-apoptotic, or pro-apoptotic genes accumulate. *MYC* mediates genomic instability through nucleotide substitutions, double-stranded breaks, gene amplification, and defects in the mitotic spindle checkpoint (reviewed in Reference [76]). Under normal circumstances, these mutations would elicit cell cycle arrest and either correction of the mutation or the cell would be lead down an apoptotic pathway. Overexpression of MYC in cells can lead to a loss of cells cycle arrest and inhibition of apoptosis, allowing cells to accumulate mutations until the cell becomes transformed (Figure 1.2).

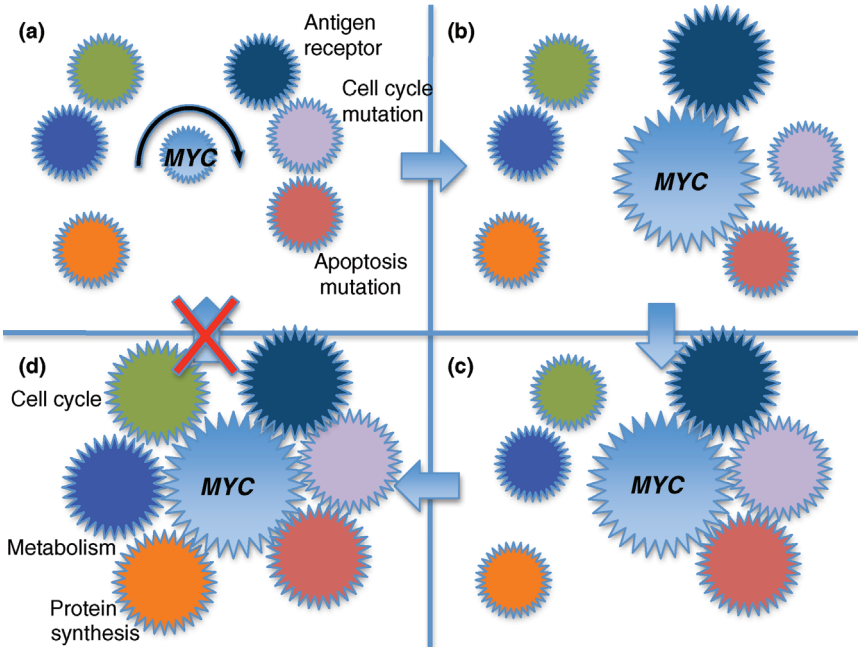

Figure 1.2 How cancer develops: *MYC*'s role in the development of lymphoid cancers is a multistep process: *MYC* levels are tightly regulated in a normal cell (a); *MYC* overexpression in a cell can synergize with normal antigen receptor signaling to destabilize normal cell function (b); additional mutations in cell-cycle and/or apoptotic machinery are required for full transformation (c); a tumor cell fails to return to homeostatic levels of *MYC* but is dependent on antigen receptor signaling and super-physiologic levels of *MYC* (most likely to drive the internal efforts to meet the increased physiological demands placed on the cell to maintain continuous growth and proliferation) (d).

1.16
Role of BCR Signaling in Conjunction with MYC Overexpression in Lymphoid Malignancies

The notion of a role for chronic inflammation in lymphomagenesis has been with us for many years. Multiple observations suggest that antigenic stimulus can play a role in lymphomagenesis. First, infection with *Helicobacter pylori* is an apparent cause of human lymphomas in mucosal associated lymphoid tissue (MALT) and gut associated lymphoid tissue (GALT) [77]. Treatment with antibiotics to eradicate infection elicits remission of these tumors, as if they might have been sustained by antigenic stimulus from the microbe [78, 79]. Along these lines, a more recent report has shown that cells obtained from MALT lymphoma tumors express a unique, and restricted, antibody repertoire with frequent reactivity to rheumatoid factor [80]. The restriction in the BCR repertoire strongly suggests stringent antigenic selection. Second, mice with graft versus host disease consequent to bone marrow

transplantation frequently develop B-cell lymphomas that contain integrations of ecotropic murine leukemia proviruses; these tumors were host-derived, and required histoincompatibility and T-cell help [81]. Third, the gene expression profiles of diffuse large B-cell lymphomas resemble those of B-cells that have mounted a response to antigen [82]. Moreover, the tumor cells obtained from either BL or diffuse large B-cell lymphoma (DLBCL) tumors display high-affinity antigen receptors on their surface, as if they had been subjected to the selective pressure of an antigen [82–87]. These findings prompt the hypothesis that an antigenic stimulus may cooperate with other tumorigenic influences in the genesis of lymphoma [88].

In normal B-cells, the BCR binds antigen and subsequently triggers growth and proliferation of B-cells and production of antigen-specific immunoglobulin. The role of BCR stimulation in conjunction with Myc overexpression in the formation of lymphoid malignancies has been investigated using Eμ–*MYC* transgenic mice [31]. Transgenic mice were generated that express *MYC* under a lymphoid specific promoter, B cell receptor to hen egg lysozyme (BCR^{HEL}), and the cognate antigen, soluble HEL (sHEL). The Eμ-*MYC*/BCR^{HEL}/sHEL mice formed fatal lymphomas as early as five weeks of age. Evidence of tumor in the Eμ-*MYC*/BCR^{HEL} and the Eμ-*MYC*/sHEL did not occur until 18 and 22 weeks, respectively [31]. These data provide evidence that BCR stimulation, in conjunction with *MYC* overexpression, can lead to lymphomagenesis (Figure 1.2).

1.17
Deregulation of Auxiliary Proteins in Addition to MYC in Hematological Cancers

The transformation of normal hematopoietic cells is largely caused by genetic mutations resulting in activated oncogenes and inactivated tumor-suppressors. These mutations give rise to various pathologic features in the neoplasm, including proliferation, immortalization, blocked differentiation, genomic instability, and resistance to apoptosis. The requirement of multiple genetic mutations has been demonstrated for several proto-oncogenes, including *MYC*, Bcl2, Bcl6, and many more. Cells harboring a single mutation that leads to altered expression of these proto-oncogenes do not give rise to cancer [89]. For example, Eμ–*MYC* transgenic mouse in which *MYC* is overexpressed in B-cell progenitors under control of the immunoglobulin heavy chain enhancer develop clonal pre-B and B-cell lymphomas only after acquiring a secondary mutation [89]. Crossing Eμ–*MYC* transgenic mice with either Eμ-Bcl2 or p53 $+/-$ mice led to accelerated lymphoma development [90–92]. The aggressiveness of hematological malignancies seems to correlate with the accumulation of additional mutations affecting pro-survival, anti-apoptotic, and apoptotic factors. For example, the transformation of an indolent malignancy (follicular lymphoma) to an aggressive malignancy (diffuse large cell lymphoma) has been correlated with secondary mutations involving *MYC*, p53, Bcl2, or p16/INK4a [93, 94].

Importantly, while alteration to *MYC* expression and stability can increase the total amount of *MYC* protein present in the cell at a particular point in time, they do not

seem to affect the function of *MYC*, as is the case in activating point mutations for some oncogenes (i.e., Ras). Ras is a proto-oncogene that encodes a GTP-binding protein that plays a role in cell growth and survival [95]. Activated Ras initiates a number of signal transduction pathways that include Raf/MAPK (ERK) and PI3 kinase/AKT that are involved in cell proliferation and survival, respectively [96–98]. Mouse models evaluating Ras, *MYC*, or Ras and *MYC* overexpression show that overexpression of *MYC* alone resulted in tumor formation only after a long latency period of 15–20 weeks while overexpression of Ras led to tumor formation in mice beginning at 4 weeks of age. Mice overexpressing both Ras and *MYC* formed tumors in mice beginning at 3 weeks of age [99].

MYC overexpression accompanied by inactivating mutations for tumor suppressor genes (i.e., p53 and ARF) also lead to a more aggressive malignancy. p53 is a DNA-binding protein that can induce cell-cycle arrest or apoptosis in response to DNA damage and expression of mitogenic oncogenes, such as *MYC* [100, 101]. ARF is upstream of p53 and activates p53 by interfering with its negative regulator, Mdm2 [102–104]. Eμ-*MYC* mouse models evaluating the onset of lymphoma show that the onset of lymphoma mice harboring an additional mutation in ARF or p53 is greatly accelerated relative to Eμ-*MYC* alone [92, 105]. Analyses of many human Burkitt's lymphomas where *MYC* is overexpressed also showed that Arf and p53 mutations occur spontaneously during tumor development [106, 107].

1.18 Conclusion

MYC proteins have important roles in the regulation of a large number of distinct cellular programs that are key for the normal physiological function of a cell. In fact, studies in genetically modified mice suggest that *MYC* is critical in the ability of a cell to respond to extracellular signals and integrate several such signals at any one point in time. The effects of *MYC* on gene expression are still not entirely clear, although this is probably the means by which *MYC* is able to participate and regulate such a distinct number of functions in the cell (Figure 1.1). The mRNA encoding for *MYC* and the protein itself have very short longevity. This is a probably due to its powerful and pleiotropic functions, and the need to tightly control such a factor. The dysregulation of *MYC* expression or turnover has dramatic consequences, as observed in many types of cancer. When *MYC* is overexpressed, the normal functions are extended to confer a competitive advantage to those cells when they meet adverse conditions. Such cells hyperproliferate in a manner that is independent of exogenous growth and survival factors. Those mutant cells can also divide and survive in conditions that would normally counter growth, such as hypoxia. Ultimately, the presence of an excess of *MYC* is likely to foster the development of additional genetic defects through the accumulation of other mutations and large-scale chromosomal abnormalities (Figure 1.2). The different levels of complexity encountered in the studies of *MYC* are quite unique and can tremendously benefit from the influx of

additional types of ideas from computational biologists and statisticians. The absence of a consensus on the function of *MYC* makes this a ripe field for computational modeling and hypothesis generating approaches to biology.

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