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Review

Myc and its interactors take shape ☆

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ABSTRACT

The Myc oncoprotein is a key contributor to the development of many human cancers. As such, understanding its molecular activities and biological functions has been a field of active research since its discovery more than three decades ago. Genome-wide studies have revealed Myc to be a global regulator of gene expression. The identification of its DNA-binding partner protein, Max, launched an area of extensive research into both the protein–protein interactions and protein structure of Myc. In this review, we highlight key insights with respect to Myc interactors and protein structure that contribute to the understanding of Myc's roles in transcriptional regulation and cancer. Structural analyses of Myc show many critical regions with transient structures that mediate protein interactions and biological functions. Interactors, such as Max, TRRAP, and PTEF-b, provide mechanistic insight into Myc's transcriptional activities, while others, such as ubiquitin ligases, regulate the Myc protein itself. It is appreciated that Myc possesses a large interactome, yet the functional relevance of many interactors remains unknown. Here, we discuss future research trends that embrace advances in genome-wide and proteome-wide approaches to systematically elucidate mechanisms of Myc action. This article is part of a Special Issue entitled: Myc proteins in cell biology and pathology.

1. Introduction

The *MYC* oncogene was one of the first human genes identified that can drive cellular transformation. Encoding the c-Myc (Myc) transcription factor, it was originally discovered as a cellular homolog to v-gag-myc, present in the avian myelocytomatosis virus (MC29) that causes avian leukemia [1–4]. Other transforming and more tissue-specific family members were subsequently identified in neuroblastoma and lung cancer as *MYCN* (N-Myc) and *MYCL* (L-Myc), respectively [5–7]. Myc functions as a central hub of the cell, integrating signals from numerous pathways to direct gene expression programs and regulate many biological functions, including cell growth, proliferation, apoptosis, differentiation, and transformation (Fig. 1) [8]. Myc levels in cells are normally highly controlled at multiple levels, including *MYC* gene expression and protein stability, but become deregulated in many human cancers. Recent cancer genome sequencing efforts affirm that *MYC* is one of the most frequently amplified genes across many cancer types [9,10]. Gene amplification, often used as a clinical marker of Myc aberration, is among the many mechanisms by which Myc is deregulated (reviewed in [11]). Chromosomal translocation of *MYC* is found frequently in hematopoietic malignancies. While generally considered an under-mutated gene, *MYC* is recurrently mutated in Burkitt's lymphoma [12]. Many additional mechanisms of Myc deregulation occur in cancer, including activation of upstream pathways, such as Wnt and Notch signaling, as well as increased mRNA and protein stabilization.

Myc's prominent role in tumorigenesis makes it an ideal candidate for therapeutic targeting. However, inhibiting Myc directly through pharmacological means has not been possible to date, largely because Myc does not possess enzymatic activity or binding sites that can be blocked by small molecules. Other strategies are required to develop an effective inhibitor of Myc activity. Blocking Myc's interaction with its essential partner protein, Myc-associated factor X (Max), using a dominant interfering protein (called Omomyc), provides an important proof-of-concept that inhibiting Myc and its protein–protein interactions is an effective therapeutic strategy with a tolerable tumor-normal index [13,14]. More recently, a small molecule inhibitor targeting the BRD4 bromodomain protein has been shown to downregulate Myc at the transcriptional level as part of its mechanism of action, with efficacy particularly in hematopoietic malignancies [15,16]. These collectively indicate that, while direct pharmacological inhibition of Myc has not been possible, a better understanding of Myc function and regulation

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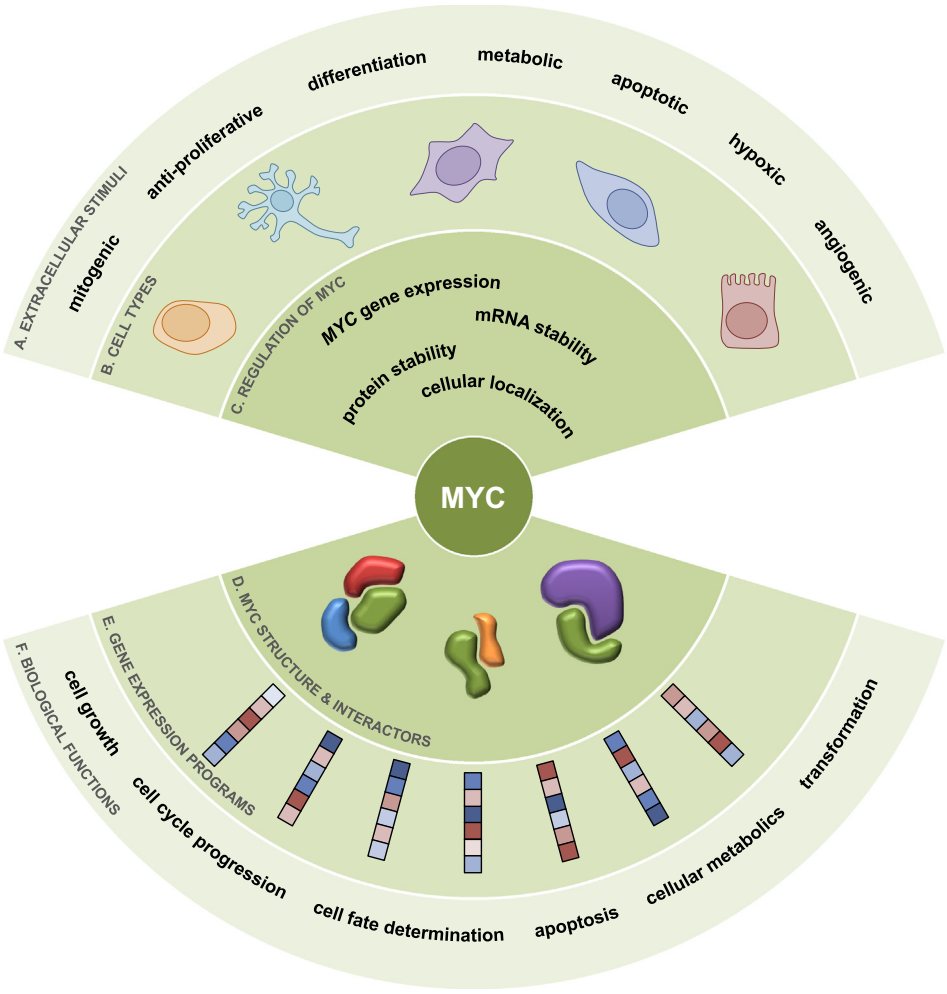

Fig. 1. Myc is a central hub in the cell. Multiple extracellular stimuli activate different signal transduction pathways that converge on Myc (A). The variety of stimuli and pathways is highly dependent on the specific cell type (B). These signaling pathways regulate Myc at various levels, including *MYC* gene expression, mRNA and protein stability, and cellular localization (C). Myc integrates these input signals through interactions with different proteins (Myc as green shapes and other proteins in other colors); the intrinsically disordered nature of its structure mediates transient conformations to allow for varied protein interactions (D). Different Myc interaction complexes occur on chromatin to mediate specific gene expression programs (represented by schematic heat maps) (E), which manifest as a multitude of biological outputs (F). Aberrations at any levels can contribute to Myc deregulation and oncogenesis.

through its protein–protein interactions and upstream signaling will lead to the promising alternative strategies of inhibiting oncogenic Myc.

In this review, we focus on the regulation and function of Myc as a transcriptional regulator as well as potent oncoprotein, and highlight a number of Myc interactors that contribute to these activities. We also discuss the structural features of Myc that will be important to consider when developing Myc inhibitors designed to block Myc–protein interactions.

2. Myc regulates transcription through protein–protein interactions

Early efforts to study Myc as a transcriptional regulator have focused on identifying direct target genes primarily to better understand the mechanism of how Myc regulates numerous biological activities (reviewed in [17]). For example, Myc's ability to regulate cell cycle progression is orchestrated in part by transcriptionally activating cyclins D1 (*CCND1*) and D2 (*CCND2*), and repressing the cyclin-dependent kinase (CDK) inhibitor $p21^{Cip1}$ (*CDKN1A*) and growth arrest gene *GADD45*. With the advent of genome-wide technologies, the Myc transcriptional network has been considerably expanded. Myc is estimated to bind 10 to 15% of the genome [18–22], and controls transcription mediated by all three RNA polymerases. In addition to the large number of RNA polymerase II (RNA Pol II)-transcribed protein-coding genes and non-coding RNAs, Myc also regulates ribosomal and transfer RNAs (rRNA and tRNA) that are transcribed by RNA polymerases I and III (RNA Pol I and III) [23–26]. These findings establish a central role for Myc in regulating global gene expression, protein biosynthesis, and cell growth.

Characterizing interacting proteins with respect to Myc target gene expression and/or promoter binding are complementary approaches to better understand Myc as a regulator of gene transcription. Traditionally Myc interactors were identified one protein at a time and then evaluated for their potential role in regulating Myc function on a single or a small number of target gene(s). Only a few Myc interactors have been evaluated on a genome-wide scale. Incorporating this global approach to known and novel interactors is a key area for investigation that promises to unveil the relationship of Myc-interacting proteins to Myc target gene regulation. To this end, established Myc interactors involved in the control of gene transcription will be discussed.

2.1. Max as an essential Myc interactor

Max is the primary partner for Myc [27]. Through their respective basic helix–loop–helix leucine zipper (bHLH-LZ) regions, Myc and Max heterodimerize to bind DNA at enhancer (E)-boxes with a canonical CACGTG sequence not only within gene promoter regions, but also enhancer regions and regions with no obvious connection to specific genes [18,28–30]. The level of Myc protein expression has been shown to modulate Myc binding to additional regions with non-canonical

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