Emerging Roles for Maf1 beyond the Regulation of RNA Polymerase III Activity

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Abstract

Maf1 was first identified in yeast, and studies in metazoans have primarily focused on examining its role in the repression of transcription that is dependent on RNA polymerase III. Recent work has revealed a novel and conserved function for Maf1 in the maintenance of intracellular lipid pools in *Caenorhabditis elegans*, mice, and cancer cell lines. Although additional Maf1 targets are likely, they have not been identified, and these recent findings begin to define specific activities for Maf1 in multicellular organisms beyond the regulation of RNA polymerase III transcription and suggest that Maf1 plays a more diverse role in organismal physiology. We will discuss these newly defined physiological roles of Maf1 that point to its placement as an important new player in lipid metabolism with implications in human metabolic diseases such as obesity and cancer, which display prominent defects in lipid homeostasis.

Introduction

One mechanism utilized by organisms in response to cytotoxic stress, such as DNA damage and starvation, is regulation of the transcriptional activities of RNA polymerase (pol) I, II, and III [1,2]. RNA pol I is involved in ribosomal RNA (rRNA) transcription and regulation of its activity is complex but is in part controlled at transcription initiation and elongation and is also sensitive to ribosomal DNA copy number variation [3,4]. RNA pol II produces messenger RNAs and many noncoding RNAs. One level of regulation is through changes in the phosphorylation status of its C-terminal repeat domain, a tandem repeat of the heptapeptide sequence YSPTSPS [5], which alters its binding affinity to a variety of nuclear factors [6,7]. RNA pol III generates rRNA, transfer RNAs, 5S rRNA, and the remaining complement of small noncoding RNAs [8]. RNA pol III transcripts comprise essential components of the cellular protein synthesis machinery, which must be synthesized in high copy to fulfill the cell's biosynthetic demand during growth. At the same time, RNA pol III activity must be amenable to negative regulation in order to impede growth during unfavorable conditions. This level of regulatory precision is mediated by the activities of multiple factors that influence cell cycle progression and cellular transformation when deregulated [9]; they include c-Myc [10] and tumor suppressors pRB (retinoblastoma protein) [11] and p53 [12].

Not all species have clear orthologs of these well-studied regulatory factors of RNA pol III [13] mentioned above. *Saccharomyces cerevisiae*, for example, utilizes other well-conserved transcriptional regulators, like Maf1, to regulate RNA pol III and coordinate cellular growth and responses to stress [13]. Maf1 is a phosphoprotein that has been characterized in *S. cerevisiae* [14], *Drosophila melanogaster* [15], *Caenorhabditis elegans* [16], and mammals [17] and shown to integrate stress signaling and cellular growth and proliferation pathways by regulating transcription that is dependent on RNA pol III. The yeast, worm,

Table 1. Summary of experimental findings of altering Maf1 across species

Manipulation	Organism	Tissue/ type	Phenotype	Reference
Overexpression	*C. elegans*	All *mafr-1* expressing tissues	RNA pol II and III Lipid homeostasis Reproduction	[16]
	Human cell	Glioblastoma	RNA pol I, II, and III Anchorage-independent growth	[23] and [38] [38]
		Liver	Triglyceride levels	[23]
RNAi	Human cell	Glioblastoma	RNA pol I, II, and III Cellular morphology	[38]
	C. elegans	Ubiquitous	RNA pol II and III Lipid homeostasis Reproduction	[16]
		Intestine		
	Drosophila	Fat body	Organismal growth	[15] and [42]
Knockout	Mouse	Ubiquitous	Food intake Body weight Reproduction	[41]

mouse, and human research communities use different nomenclature for genes and proteins. For clarity, we use "Maf1" when referring to Maf1 in general terms. Specific references to *C. elegans* RNA and proteins are written as *mafr-1* and MAFR-1, respectively.

Maf1 may have evolved as an early mediator of cellular growth while other factors surfaced later to enhance regulation in more complex eukaryotes that require more sophisticated layers of control. The presence of Maf1 across species is suggestive of its necessity to modulate essential cellular functions, which facilitate its persistence to withstand evolutionary pressures. The study on Maf1 across multiple species has not only uncovered insightful information regarding Maf1 function (Table 1) but also raised new questions. This piece will summarize existing and newly identified roles that Maf1 plays in cellular homeostasis—across organisms of diverse complexity—and will give insights into the physiological impacts that Maf1 activity has in regard to nutrient signaling and cellular growth pathways.

Cellular growth is an energy-intensive process, and under favorable conditions, nontransformed cells preferentially utilize carbohydrate metabolism for ATP generation [18]. When dietary sources of sugar become scarce, cells switch to lipolysis of cellular lipid stores and generate energy through mitochondrial and peroxisomal beta-oxidation [19]. In times of nutrient excess, surplus carbohydrates can be utilized to synthesize cellular lipids through lipogenesis [5], which can lead to obesity, diabetes, and other metabolic syndromes when left unchecked [20]. Cellular growth requires lipogenesis pathways, not only as a maintenance component of energy homeostasis but also as a vital system for the synthesis of cellular membranes and signaling molecules such as steroid hormones and prostaglandins [21]. Whereas cancer cells have upregulated lipid synthesis to drive deregulated cell division, mutation of lipid biosynthesis genes has

been shown to severely impair growth and development in multiple species [22].

Findings by Palian *et al.* [23] and Khanna *et al.* [16] have highlighted a noncanonical role for Maf1 in the regulation of intracellular lipid stores. These two papers demonstrated that, in addition to regulation of cellular biosynthetic capacity that is dependent on RNA pol III, Maf1 also regulates select RNA pol II genes, such as *Acc1* and *Fasn*, which encode enzymes for the first two steps in *de novo* lipogenesis (discussed below). While some of the molecular mechanisms that regulate Maf1 function have been identified, it is clear that other posttranslational modifications of Maf1 that influence cellular functions remain to be uncovered. However, the extent of diversity in modifications to specific residues of the Maf1 protein, which are that are essential for Maf1 functions, remains to be elucidated.

Maf1 Structure, Function, and Regulation

A large body of work has characterized the role of yeast Maf1 as a repressor of RNA pol III transcription in a TFIIIB (*t*ranscription *f*actor for polymerase *III B*)-dependent manner [24]. Subsequent research has demonstrated biochemical evidence of human Maf1 interaction with RPAC2 (alpha-like subunit of RNA pol III), RPC1 (the largest subunit of RNA pol III), and Brf1 (subunit of TFIIIB) [17]. It is believed that this direct interaction with the RNA pol III machinery facilitates the ability for Maf1 to regulate transcriptional output. Maf1 proteins in all species share three regions of high similarity, named A, B, and C box. The domains defined in these regions are unique to Maf1 and do not contain sequence motifs of known function. With the use of mutant alleles of human Maf1, it was shown that the A box is required for Maf1 to interact with the large RNA pol III subunits and that the B box is required for interaction with Brf1 [17].