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Review

Myc and mRNA capping ☆

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ABSTRACT

c-Myc is upregulated in response to growth factors and transmits the signal to proliferate by altering the gene expression landscape. When genetic alterations result in growth factor-independent c-Myc expression, it can become an oncogene. The majority of human tumour types exhibit a degree of c-Myc deregulation, resulting in unrestrained cell proliferation. c-Myc binds proximal to the promoter region of genes and recruits co-factors including histone acetyltransferases and RNA pol II kinases, which promote transcription. c-Myc also promotes formation of the cap structure at the 5′ end of mRNA. The cap is 7-methylguanosine linked to the first transcribed nucleotide of RNA pol II transcripts via a 5′ to 5′ triphosphate bridge. The cap is added to the first transcribed nucleotide by the capping enzymes, RNGTT and RNMT–RAM. During the early stages of transcription, the capping enzymes are recruited to RNA pol II phosphorylated on Serine-5 of the C-terminal domain. The mRNA cap protects transcripts from degradation during transcription and recruits factors which promote RNA processing including, splicing, export and translation initiation. The proportion of transcripts with a cap structure is increased by elevating c-Myc expression, resulting in increased rates of translation. c-Myc promotes capping by promoting RNA pol II phosphorylation and by upregulating the enzyme SAHH which neutralises the inhibitory bi-product of methylation reactions, SAH. c-Myc-induced capping is required for c-Myc-dependent gene expression and cell proliferation. Targeting capping may represent a new therapeutic opportunity to inhibit c-Myc function in tumours. This article is part of a Special Issue entitled: Myc proteins in cell biology and pathology.

1. Introduction

c-Myc is a potent cellular protein which is required for cell proliferation throughout development and in adult [1,2]. It is upregulated in response to growth factors and transmits the signal for proliferation by regulating gene expression. c-Myc is also a prevalent oncogene which is deregulated to some extent in most human tumour types, resulting in aberrant cell proliferation [1,2]. In recent years, many promising approaches to targeting c-Myc expression or function have been discovered, and many of these have exhibited promising results in mouse cancer models [3]. However, currently there are no therapeutic approaches in the clinic which specifically target c-Myc and therefore the need remains to understand the molecular mechanisms by which c-Myc functions. This review discusses the mechanisms by which c-Myc promotes mRNA cap formation, how this influences gene expression, and the opportunities for investigating capping as a therapeutic target to inhibit Myc function.

2. Discovery of c-Myc

In humans, the Myc family of proteins consists of c-Myc, N-Myc and L-Myc. c-Myc is believed to be expressed in all proliferating cells and is the Myc protein most commonly deregulated in tumours. Prior to the discovery of the mammalian Myc proteins, v-Myc was identified as one of the first-discovered viral oncogenes [4]. Subsequently, a cellular homologue of v-Myc, c-Myc, was identified as a nuclear protein. c-Myc (and all Myc proteins) were found to contain basic-helix–loop-helix leucine zipper motifs, which had been previously observed in sequence-specific DNA-binding proteins [5]. As a consequence of these observations, c-Myc was confirmed to be a transcription factor which regulates protein-encoding genes, resulting in regulation of mRNA expression in a gene-specific manner. When purified from cell extracts, c-Myc is isolated as a heterodimer with a basic-helix–loop-helix leucine zipper protein, Max. Max is required for c-Myc to bind to DNA and regulates transcription [6,7]. The N-terminus of c-Myc binds to co-factors, including histone acetyltransferases and RNA pol II kinases, which mediate transcriptional activation and repression [2,8]. The complex details of how c-Myc regulates transcription are tackled elsewhere in this special issue.

3. c-Myc is a transcriptional regulator

Our understanding of c-Myc as a transcription factor has evolved with and contributed to our understanding of mammalian transcriptional mechanisms. Initially c-Myc was identified as a transcription factor that increased and decreased expression of certain protein encoding genes. The advent of micro arrays allowed “whole genome” analysis for

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the first time. This revealed the surprising finding that, regardless of cell lineage, c-Myc regulates transcription of approximately 10% genome, and represses and activates genes by equivalent measure [1,8]. c-Myc was also observed to be a relatively weak transcriptional regulator, typically activating and repressing genes by 1.5–2-fold. In the 1990s, the first evidence came that c-Myc regulates transcription elongation [9,10]. Subsequently, most mammalian genes were found to have a pool of RNA pol II paused downstream of the promoter. Release of paused RNA pol II into elongation phase was recognised to be a rate-limiting step in transcription [11,12]. The mechanism of RNA polymerase II pausing and release is complex and major discoveries continue to be made concerning the mechanism and its regulation. Recognition that c-Myc has a pleiotropic effect on gene expression began with the discoveries that c-Myc globally upregulates chromatin acetylation and methylation associated with transcription, and that c-Myc globally increases RNA pol II C-terminal domain Serine-2 and Serine-5 phosphorylation, events associated with transcription initiation and elongation, respectively [13,14]. Subsequently, advanced RNA sequencing technologies have revealed that c-Myc globally amplifies transcription of the majority of RNA pol II genes [15,16]. In addition to increasing mRNA transcription, c-Myc has been found to influence mRNA translation by promoting RNA polymerase I and III-dependent transcription, thus upregulating rRNA (ribosomal RNA) and tRNA (transfer RNA) production [17–20].

4. mRNA 7-methylguanosine cap

A key process during transcription of protein-encoding RNA pol II transcripts, whether c-Myc-regulated or not, is the addition of the "cap" structure to the initiating nucleotide [21,22] (Fig. 1). The cap consists of 7-methylguanosine linked to the first transcribed nucleotide by a 5′ to 5′ triphosphate bridge (abbreviated to m7G). The cap structure is thought to be unique to the 5′ end of RNA pol II transcripts, selecting them for specific handling and processing required for their ultimate expression [23–25]. In mammals, the first and second transcribed nucleotides can also be O-2 methylated, forming part of the recognised cap structure [26].

Three enzymic activities catalyse m7G formation, a triphosphatase, guanylyltransferase and methyltransferase [26] (Fig. 2). RNA is synthesised with a 5′ triphosphate, denoted ppp(5′)Np (where N is the first transcribed nucleotide). A triphosphatase removes the terminal phosphate and a guanylyltransferase catalyses addition of an inverted guanosine cap to create the cap intermediate, G(5′)ppp(5′)G. A methyltransferase methylates the inverted guanosine cap on the N-7 position to create "Cap 0", denoted 7mG(5′)ppp(5′)Np. Methylation of the first and second transcribed nucleotides on the O-2 position of the ribose creates the structures known as Cap1 and Cap2, respectively.

Although the basic enzymic activities utilised to promote capping are similar in all eukaryotes, the genomic arrangement of the capping enzymes varies significantly in different species [26]. Since this review is concerned with the function of c-Myc in humans, we will focus on the discussion of the mammalian capping enzymes. In mammals, the triphosphatase and guanylyltransferase are contained on a single polypeptide, RNGTT (RNA guanylyltransferase and 5′ phosphatase) [27–29], and the methyltransferase is contained in a separate enzyme, RNMT (RNA guanine-7 methyltransferase) [29,30] (Fig. 2). RNMT is isolated from mammalian cells in a complex with an activating subunit, RAM (RNMT-activating mini-protein) [31,32]. The guanylyltransferase reaction is reversible whereas N-7 methylation is not, thus N-7 methylation "locks-in" the cap structure. CMTR1 and CMTR2 methylate the first and second transcribed nucleotides, although not all transcripts receive this modification [33,34]. Furthermore, the precise mechanisms of function of CMTR1 and CMTR2 are in the early days of characterisation and not mentioned further here.

The cap methylation reaction is not readily reversible, however the entire cap can be removed by a variety of "decapping enzymes" [35–37]. mRNA is unstable without the cap and therefore decapping either initiates degradation or is a later stage of the degradation process. The decapping enzyme complexes can potentially act on all transcripts but in vivo can exhibit specificity for certain transcripts. Furthermore, various auxiliary proteins can increase decapping enzyme activity and direct the decapping complexes towards specific transcripts, thus facilitating cellular regulation of the process.

5. Capping and transcription

Not only does formation of the cap occur during transcription, it is integral to the process. RNA is vulnerable to degradation during the early stages of transcription and addition of the cap structure protects RNA pol II transcripts from attack by exonucleases. Addition of the cap thus passively permits transcripts to be synthesised [38,39].

Capping occurs shortly after transcription initiation and is restricted to RNA pol II transcripts since the capping enzymes RNGTT and RNMT–RAM are only recruited to this polymerase [40,41]. The RNA pol II large subunit C-terminal domain (CTD) is a recruitment platform for enzymes and factors which regulate transcription and modify RNA, including the capping enzymes [12,42]. Recruitment of factors to the CTD is coordinated by a series of phosphorylation events and other post-translational modifications. RNGTT and RNMT–RAM are recruited at the initial phases of transcription when the RNA polymerase II CTD is phosphorylated on Serine-5 [43,44]. RNGTT is activated by interaction

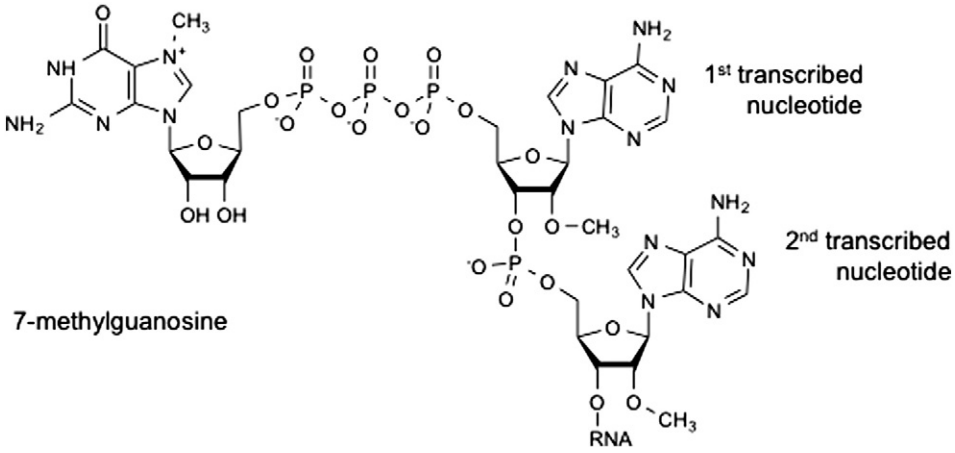

Fig. 1. Diagram of the mRNA cap. The mRNA cap is 7-methylguanosine linked via a 5′ to 5′ triphosphate bridge to the first transcribed nucleotide. The first and second transcribed nucleotides can also be methylated on the ribose 2-hydroxyl position creating Cap1 and Cap2, respectively.

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