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Review Article

Epigenetic reprogramming of Myc target genes

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Abstract: Myc protein plays a fundamental role in regulation of cell cycle, proliferation, differentiation and apoptosis by modulating the expression of a large number of targets. Myc binding to its targets depends on the presence of the E-box binding sequence and by a chromatin context in which histone H3K4me3 lysine methylation favors Myc binding. Myc role in transcription is still an open question since Myc is able to either activate or repress target genes and the molecular mechanisms by which it exerts these functions span from chromatin remodeling to processive RNAPII elongation. Since the types and number of enzymes able to reversibly modify histones is recently growing, some of the acquisitions regarding Myc chromatin remodeling properties are being revaluated. Here, we summarize recent findings regarding the function of Myc in epigenetic reprogramming of its targets in transcription of differentiated as well as pluripotent cells.

Keywords: Myc, transcription, histones acetylation, histones methylation, epigenome

Introductioon

Myc is a helic-loop-helix zipper (bHLHZ) protein that forms heterodimer with the small bHLHZ protein Max and recognizes the consensus sequence CACGTG called E-box [1-3].

Myc as transcription factor has been found to have a role in activation and repression of gene expression. The transcriptional activity of Myc is crucial for its ability to induce tumorigenesis. E-box binding by Myc-Max has been historically associated with the capacity to recruit co-factors to target gene promoters. Many co-factors have been identified which mediate transcription by a variety of mechanisms from chromatin remodeling to initiation of transcription and transcription elongation. [4-6]. The advent of new technologies, as the high-throughput sequencing of ChIP DNA (ChIP-seq), allowed to determine that Myc targets are widespread in the genome and that DNA regions effectively bound by Myc are 10-15% of genomic loci in mammals [7]. Unexpected was the observation that a significant fraction of the loci occupied by Myc lacks obvious DNA element that resemble canonical E-boxes [7,8].

Since its discovery as transcription factor, different molecular mechanisms utilized to exert Myc functions as activator or repressor of gene transcription have been described but still controversial and mainly obscure is how Myc recognizes the targets that will be activated from those that will be repressed [3,4,9,10] The reason is mainly attributable to the fact that Myc behaves as a weak modulator of transcription at least in in vitro experiments. Consequently, even if a wide number of genes have been described as potential Myc targets, for the majority of them it is still not known if they are direct or indirect Myc targets in vivo as well as the involvement and consequence of Myc binding in modulating their expression [8,11]. Expression profiles of Myc bound targets show that the majority of bound loci are activated by Myc and a relative smaller group is repressed [7,8,12]. Moreover, it is becoming evident that the presence or absence of E-boxes is not per se informative for the effect that Myc recruitment will have on the targeted gene. Early studies have suggested that Myc mediated repression was distinct from Myc-mediated activation in that it does not typically require E-box element in the

repressed target; transcription repression was demonstrated to be the consequence of Myc indirect recruitment to its repressed targets via protein-protein interaction with the two zinc finger transcription factors Sp1 and Miz1 whose binding to their targets was necessary for activated transcription [13,14]. More recently E-boxes elements have been found in the core promoters of repressed genes and binding of Myc to some of its targets has been demonstrated to occur through heterodimerization with Max [7,15,16].

Moreover, global Myc binding sites mapping and expression profiles of the bound loci confirms that the presence of the E-box in the binding loci do not correlate with whether or how the associated genes are regulated [7,15,16].
Because it is becoming evident that Myc direct binding to the E-box cannot be predictive of Myc transcriptional effect, it is speculative to assess that Myc may recognize epigenetic modifications on its targets genes that are predictive of the transcriptional effects consequence of its binding.

Myc-induced histones modifications

Many efforts have been done to study specific histone post-translational modifications that correlate with genomic Myc binding. [10,17,18]. It has been found that Myc binding controls the genome-wide distribution of histone H3 and H4 modifications. Myc targets activation was first found to correlate with the induction of histone acetylation through Myc interaction and recruitment on the bound targets of protein complexes with histone acetylases activities [19-22]. More recent was the finding that Myc mediated repression could be the consequence of Myc mediated recruitment of histones deacetylases at its target genes [23-25]. Since the plethora of histone modifications reversible changes has exponentially grown in recent years, the role of Myc in chromatin remodeling is getting more and more complicated. More then 100 possible chemical modifications of histones have been described and the number of families of modifying and de-modifying enzymes is growing up, thus we expect that Myc recognition of Myc binding site and the functional effects of Myc binding may depend by a combination of more than a modification and that preexisting modification may help the occurrence of subsequent histones changes.

Evidences that Myc binding occurs in regions of chromatin enriched in specific histone modifications are emerging. It has been found that histone methylation at Myc target loci is critical for recognition of Myc targets; promoters occupied by Myc show a strong correlation with the histone H3 lysine trimethylation (H3K4me3 and H3K79) prior to the induction of Myc expression and upon its binding changes in the methylation patterns of histones have been observed. [10,16,21].

Significantly, in this context interaction of Myc with H3K4 methylation-demethylation enzyme complexes has been described; Myc binds and inhibits the activity of thritorax proteins that act as H3K4 de-methylases. In Drosophila Myc has been found to inhibit the activity of the thritorax proteins Lid, Rbp2 and Plu1 [26-28]; expression of dMyc abrogates Lids enzymatic activity blocking demethylation of H3K4 maintaining a chromatin mark that has positive correlation with active genes. In this case Lid behaves as a dMyc co-activator for the expression of the growth regulator Nop60B gene. Since H3K4 trimethylation is a pre-requisite for Myc binding, inhibition of Lid demethylase activity by Myc may results in maintenance of local H3K4 trimethylation to favour Myc additional binding [26].

Myc binding and recruitment of the LSD1, a flavin dependent demethylase, has also been described [29]; in this case H3K4me2 demethylation occurs at genomic regions surrounding Myc binding sites; also in this case demethylation determines activation of Myc target genes but through a different molecular mechanism. LSD1 recruitment through Myc interaction favors local and temporary H3K4me3 demethylation at Myc genomic targets and consequence of this reaction is the production of hydrogen peroxide that locally is responsible of modification of guanines in 8-oxodG.

In this case demethylation of H3K4me2 is a very early event that seems to be instrumental to produce nicks on promoter regions of Myc targets repaired in locus by the Ape1 and OGG1enzymes involved in repair of DNA oxidized breaks. The mechanism that has been hypothesized is that Myc mediated recruitment of the LSD1 demethylase along with the repairing enzymes is the first step necessary to unwind locally chromatin to initiate Myc mediated tran-

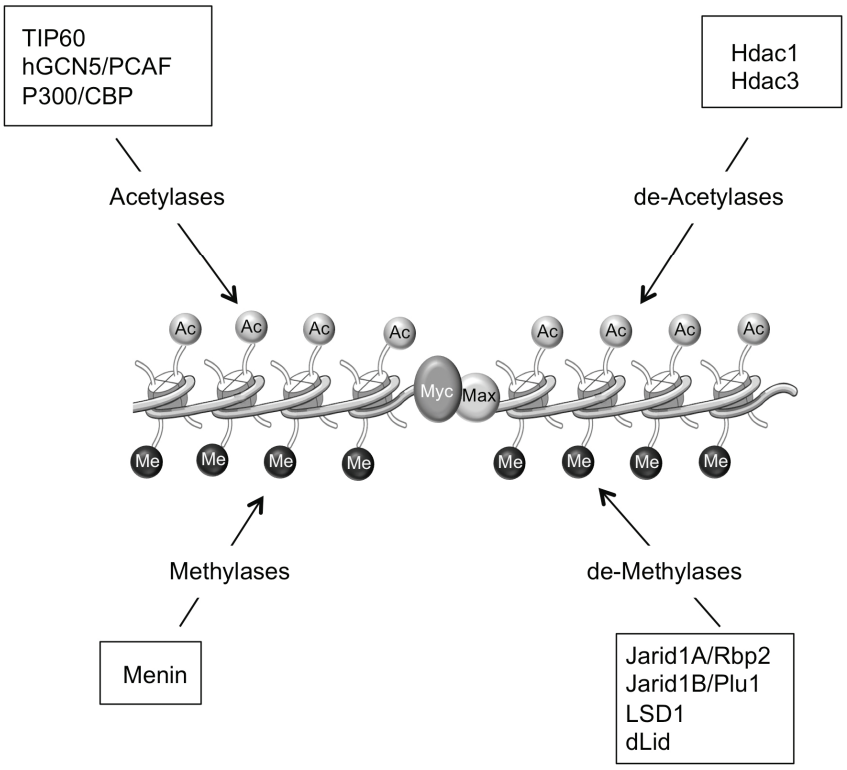

Figure 1. Myc recruitment of histones modifying enzymes. A canonical gene is shown with histones modified by acetylation and methylation in nucleosomes. The Myc-Max complex on chromatin is able to recruit several histone-modifying complexes; indicated In the squares are the Myc protein partners of these complexes responsible of the modifying enzymatic activity.

scription [4,29].

Myc also has been found to directly interact with enzymes with H3K4 methyltransferase actity as the Menin protein, a subunit of MLL1, 2 Mixed Lineage Leukemia complexes that are transcriptional regulators of gene expression. Given the pre-existence of H3K4me$_3$ and its independence upon Myc, the recruitment of MLL with methyltransferase activity might be relevant to restore the H3K4me3 chromatin environment on promoters at which Myc binding up or down regulates H3K4me3 levels [10,30]. Myc protein partners with enzymatic activities responsible of modification of histones tails by acetylation and methylation are summarized in **Figure 1**.

It has also to consider that it is difficult to distinguish Myc effects on chromatin that occur through Myc mediated recruitment of histones modification complexes from Myc mediated modulation of targets directly involved in chromatin remodeling. As examples of Myc indirect role on chromatin remodeling is the Myc mediated transcriptional activation of the gene encoding the histone acetyltransferase hGCN5, the insulator protein CTCF or the nucleosomal remodeling complex regulator MTA1 [31-33]. In modulating the activity of these proteins Myc exert broad effect on the overall chromatin structure of the eukaryotic cell [8,22,23].

Myc mediated global epigenetic reprogramming

Functional genomic studies have been conducted in a global unbiased manner in the human genome examining Myc chromatin activity widespread in the genome including intergenic regions in which no canonical E-box is distinguibile. From these studies strong evidences emerge that Myc is required to maintain the euchromatic state in a widespread manner including intergenic sites [10,17,18,22,34]. Even if it are still obscure the mechanisms underlying these Myc functions, it has been suggested that the epigenetic state at intergenic sites may be a Myc uncharacterized enhancer function to regulate genes at distance. In this view the recent demonstration that Myc recruitment on chromatin facilitates a histones methylation driven oxidation of DNA [4,29] and the consequent unwinding of DNA open the possibility that this mechanism could operate at intergenic sites to favor transcription of adjacent Myc target

genes.

While Myc is most famous for its role in tumorigenesis, the pivotal role of Myc in reprogramming fibroblasts in cells whose chromatin state resemble induced pluripotent stem cells (iPS) and murine embryonic stem cells (mESC) has been one of the most exciting discoveries of the last decade [35-38]. Determination and maintenance of pluripotency is related to an epigenetic state characterized by an open chromatin conformation. Myc function in maintaining self-renewal and in inhibiting differentiation of iPS and ESC cell is still mainly obscure but several reports suggest that Myc contribution is due to its capacity to globally maintain the histone acetlation and methylation euchromatin state of those cells [39,40]. How does Myc elicit changes in histone H3 methylation patterns of promoters in these pluripotent cells needs of more extensive characterization of functional genomic analysis of Myc and histone modifications during cells reprogramming. The comprehension of the molecular mechanisms involved in this process will indoubtly take advantage of the great deal of previous works that has been accumulated in dissecting Myc functions as epigenetic transcription regulator.

Although Myc binding is clearly coupled to changes in transcription efficiencies of its targets, there are significant number of binding sites that are not directly associated with changes in gene expression [10]. These sites can be considered to represent targets that are regulated contingently depending on a specific tissue specific or developmental context and dedicated experimental conditions are necessary to discover the role of Myc in their regulation. It is intriguing that Myc targets can be divided in subset of genes that are binding sites for common transcription regulators and combinatorial binding of different transcription factors can impart transcriptional synergy or interference between their activities. Based on these considerations these targets can be instrumental to elucidate the cooperativity effects of Myc binding with adjacent modulators of transcription in order to determine which targets are regulated by Myc alone and which are dependent on Myc in conjunction with other transcription factors.

Myc recruits distinct complexes to specific subsets of target genes and probably different complexes depending on the specific cellular context; since is not possible to make an easy classification of Myc targets based on cis regulatory regions predictive of transcriptional effect of Myc recruitment, it remains an open question how Myc recognizes targets to be activated from target to be repressed. It is likely that Myc recognizes a histonic code that is predictive of the transcriptional activated or repressed fate of its targets prior to choose dedicated partners to recruit on chromatin. Systematic classification of Myc targets on the basis of histone modifications predictive of an open or closed chromatin environment and the study of the changes occurring after Myc recruitment may help in the future to get light on the question of what determines activation versus repression.

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References

[1] Adhikary S and Eilers M. Transcriptional regulation and transformation by Myc proteins. Nat Rev Mol Cell Biol 2005; 6: 635-645.

[2] Wasylishen AR and Penn LZ. Myc: The beauty and the beast. Genes and Cancer 2010; 1:532-541.

[3] Meyer N and Penn LZ. Reflecting on 25 years with MYC. Nat Rev Cancer 2008; 8: 976-990.

[4] Amente S, Lania L, Avvedimento EV and Majello B. DNA oxidation drives Myc mediated transcription. Cell Cycle 2010; 9: 3002-3004.

[5] Eilers M and Eisenman RN. Myc's broad reach. Genes Dev 2008; 22: 2755-66.

[6] Cowling VH and Cole MD. Mechanism of transcriptional activation by the Myc oncoproteins. Semin 2006 Cancer Biol ; 4: 242-252.

[7] Zeller KI, Zhao X, Lee CW, Chiu KP, Yao F, Yustein JT, Ooi HS, Orlov YL, Shahab A, Yong HC, Fu Y, Weng Z, Kuznetsov VA, Sung WK, Ruan Y, Dang CV and Wei CL. Global mapping of c-Myc binding sites and target gene networks in human B cells. Proc Natl Acad Sci U S A 2006; 103: 17834-17839.

[8] McMahon SB. Emerging concepts in the analysis of transcriptional targets of the Myc oncoprotein: are the targets targetable? Genes and Cancer 2010;1: 560-567.

[9] Dang CV, O'Donnell KA, Zeller KI, Nguyen T, Osthus RC and Li F. The c-Myc target gene network. Semin Cancer Biol 2006; 16: 253-264.
[10] Martinato F, Cesaroni M, Amati B and Guccione E. Analysis of Myc-induced histone modifications on target chromatin. PLoS One 2008; 3: e3650.
[11] Zeller KI, Jegga AG, Aronow BJ, O'Donnell KA and Dang CV. An integrated database of genes responsive to the Myc oncogenic transcription factor: identification of direct genomic targets. Genome Biol 2003; 4: R69.
[12] Patel JH, Loboda AP, Showe MK, Showe LC and McMahon SB. Analysis of genomic targets reveals complex functions of MYC. Nat Rev Cancer 2004; 4: 562-568.
[13] Gartel AL and Shchors K. Mechanisms of c-myc-mediated transcriptional repression of growth arrest genes. Exp Cell Res 2003; 283: 17-21.
[14] Wu S, Cetinkaya C, Munoz-Alonso MJ, von der Lehr N, Bahram F, Beuger V, Eilers M, Leon J and Larsson LG. Myc represses differentiation-induced p21-CIP1 expression via Miz1-dependent interaction with the p21core promoter.Oncogene 2003; 22: 351-360.
[15] Mao DY, Watson JD, Yan PS, Barsyte-Lovejoy D, Khosravi F, Wong WW, Farnham PJ, Huang TH and Penn LZ. Analysis of Myc bound loci identified by CpG island arrays shows that Max is essential for Myc-dependent repression. Curr Biol 2003; 13: 882-886.
[16] Herkert B and Eilers M. Transcriptional repression the dark side of Myc. Genes and Cancer 2010;1:580-585.
[17] Guccione E, Martinato F, Finocchiaro G, Luzi L, Tizzoni L, Dall' Olio V, Zardo G, Nervi C, Bernard L and Amati B. Myc-binding-site recognition in the human genome is determined by chromatin context. Nat Cell Biol 2006; 8: 764-770.
[18] Varlakhanova NV and Knoepfler PS. Acting locally and globally: Myc's ever-expanding roles on chromatin. Cancer Res 2009; 69: 7487-7490.
[19] McMahon SB, Wood MA and Cole MD. The essential cofactor TRRAP recruits the histone acetyltransferase hGCN5 to c-Myc. Mol Cell Biol 2000; 20: 556-562.
[20] Vervoorts J, Luscher-Firzlaff JM, Rottmann S, Lilischkis R, Walsemann G, Dohmann K, Austen M and Luscher B. Stimulation of c-MYC transcriptional activity and acetylation by recruitment of the cofactor CBP. EMBO Rep 2003; 4: 484-490.
[21] Frank SR, Parisi T, Taubert S, Fernandez P, Fuchs M, Chan HM, Livingston DM and Amati B. MYC recruits the TIP60 histone acetyltransferase complex to chromatin. EMBO Rep 2003; 4: 575-580.
[22] Knoepfler PS, Zhang XY, Cheng PF, Gafken PR, McMahon SB and Eisenman RN. Myc influences global chromatin structure. EMBO J 2006; 25: 2723-2734.
[23] Kurland JF and Tansey WP. Myc-mediated transcriptional repression by recruitment of histone deacetylase. Cancer Res 2008; 68: 3624-3629.
[24] Iraci N, Diolaiti D, Papa A, Porro A, Valli E, Gherardi S, Herald S, Ellers M, Bernardoni R, Valle GD and Perini G. A SP1/MIZ1/MYCN Repression Complex Recruits HDAC1 at the TRKA and p75NTR Promoters and Affects Neuroblastoma Malignancy by Inhibiting the Cell Response to NGF. Cancer Res 2011; 71:404-412.
[25] Kleine-Kohlbrecher D, Adhikary S and Eilers M. Mechanisms of transcriptional repression by Myc. Curr Top Microbiol Immunol 2006; 302: 51-62.
[26] Secombe J, Li L, Carlos L and Eisenman RN. The Trithorax group protein Lid is a trimethyl histone H3K4 demethylase required for dMyc-induced cell growth. Genes Dev 2007; 21: 537-551.
[27] Secombe J and Eisenman RN. The function and regulation of the Jarid1 family of histone H3 lysine 4 demethylasess: the Myc connection. Cell Cycle 2007; 6:1324-1328.
[28] Ge Z, Li W, Wang N Liu C Zhu Q, Bjorkholm M, Gruber A and Xu D. Chromatin remodeling:recruitment o histone demethylase RBP2 by Mad1 for transcriptional repression of a Myc target gene, telomerase reverse transcriptase. Faseb J 2010; 2:579-586.
[29] Amente S, Bertoni A, Morano A, Lania L, Avvedimento EV and Majello B. LSD1-mediated demethylation of histone H3 lysine 4 triggers Myc-induced transcription. Oncogene 2010; 29: 3691-3702.
[30] Bres V, Yoshida T, Pickle L and Jones KA. SKIP interacts with c-Myc and Menin to promote HIV-1 Tat transactivation. Mol Cell 2009; 36: 75-87
[31] Zhang XY, De Salle LM, Patel JH, Capobianco AJ, Yu D, Thomas-Tikhonenko A, and McMahon SB. Metastasis-associated protein 1(MTA1) is an essential downstream effector of the c-Myc oncoprotein. Proc Natl Acad Sci USA 2005; 102: 13968-13973.
[32] Flinn EM, Wallberg AE, Hermann S, Grant PA, Workman JL and Wright AP. Recruitment of Gcn5-containing complexes during c-Myc dependent gene activation. Structure and function aspects.J Biol Chem 2002; 277:23399-233406.
[33] Chau CM, Zhang XY, McMahon SB, and Lieberman PM. Regulation of Epstein Barr virus latency type by the chromatin boundary factor CTCF. J Virol 2006; 80: 5723-5732.
[34] Cotterman R, Jin VX, Krig SR, Lemen JM, Wey A, Farnham PJ and Knoepfler PS. N-Myc regulates a widespread euchromatic program in the human genome partially independent of its role as a classical transcription factor. Cancer Res 2008; 68: 9654-9662.
[35] Park IH, Zhao R, West JA, Yabuuchi A, Huo H,

Ince TA, Lerou PH, Lensch MW and Daley GQ. Reprogramming of human somatic cells to pluripotency with defined factors. Nature 2008; 451: 141-146.

[36] Takahashi K and Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126: 663-676.

[37] Kim J, Woo AJ, Chu J, Snow JW, Fujiwara Y, Kim CG, Cantor AB and Orkin SH. A Myc network accounts for similarities between embryonic stem and cancer cell transcription programs. Cell 2010; 143: 313-324.

[38] Chen X, Xu H, Yuan P, Fang F, Huss M, Vega VB, Wong E, Orlov YL, Zhang W, Jiang J, Loh YH, Yeo HC, Yeo ZX, Narang V, Govindarajan KR, Leong B, Shahab A, Ruan Y, Bourque G, Sung WK, Clarke ND, Wei CL and Ng HH. Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. Cell 2008; 133: 1106-1117.

[39] Lin CH, Lin C, Tanaka H, Fero ML and Eisenman RN. Gene regulation and epigenetic remodeling in murine embryonic stem cells by c-Myc. PLoS One 2009; 4: e7839.

[40] Knoepfler PS. Why myc? An unexpected ingredient in the stem cell cocktail. Cell Stem Cell 2008; 2: 18-21.