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COMMENTARY

A new mode of transcriptional repression by c-*myc*: methylation

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The proto-oncogene c-*myc* is a central regulator of cellular proliferation and cell growth, which executes its multiple activities mostly through transcriptional regulation of its target genes. Although the role of c-*myc* in the activation of transcription is clearly established, the mechanisms of transcriptional repression by c-*myc* are not well understood. It has been proposed that c-*myc* represses gene transcription by at least two distinct mechanisms (Gartel and Shchors, 2003). One mechanism is limited to the binding of c-*myc* to the DNA-binding protein Miz-1, via its C-terminal domain, resulting in inhibition of Miz-1 transcriptional activity (Staller *et al*., 2001). The other mechanism is dependent on c-*myc* binding to the Sp1 transcription factor through its central region leading to inhibition of Sp1 transcriptional activity (Gartel *et al*., 2001). Interestingly, both Sp1 and Miz-1 bind to the proximal promoter of the p21 gene (Gartel *et al*., 2001; Seoane *et al*., 2002), which is a major target of Myc repression (Coller *et al*., 2000).

A new twist was added recently to this picture by Brenner *et al*. (2005), who suggested that c-*myc* may also repress transcription by recruitment of a DNA methyltransferase corepressor Dnmt3a. DNA methylation is the most important epigenetic modification in mammalian cells and it is associated with transcriptional repression. Three active DNA CpG methyltransferases, Dnmt1, Dnmt3a, and Dnmt3b, have been identified in mammals (Okano *et al*., 1998). Dnmt3a and Dnmt3b are required for *de novo* methylation (Okano *et al*., 1999) and they can act as corepressors to silence gene expression (Fuks, 2001). Using GST pull-down and co-immunoprecipitation experiments, Brenner *et al*. showed that Myc interacts with the corepressor Dnmt3a and associates with DNA methyltransferase activity. Since p21 is a well-characterized target of Myc repression (Gartel and Radhakrishnan, 2005), they decided to check if Dnmt3a could act together with Myc to silence p21 gene expression. They found that cotransfection of Myc along with Dnmt3a provided a synergistic repressive effect on p21 promoter-reporter expression. In addition, treatment of U2OS cells with Dnmt3a antisense oligonucleotide led to depletion of *Dnmt3a* mRNA and protein levels and to induction of p21 mRNA, indicating that Dnmt3a may repress endogenous p21 gene. Using chromatin immunoprecipitation experiments (ChIPs), Brenner *et al*. demonstrated that Dnmt3a could bind to the proximal p21 promoter only in the presence of Myc, suggesting that Myc is targeting Dnmt3a to the p21 promoter.

Since Myc does not bind directly to the p21 promoter but may be recruited through its association with Miz-1, they asked if Miz-1 is the factor that facilitates Myc and Dnmt3a binding to the p21 promoter. Using co-immunoprecipitation experiments, they found that Miz-1 can interact with Dnmt3a and can associate with DNA methyltransferase activity *in vivo*. In addition, they showed that Miz-1, Myc, and Dnmt3a could form a ternary complex that is involved in p21 transcriptional silencing. Furthermore, Brenner *et al*. determined that a Myc point mutant that is deficient in binding to Miz-1 but is able to bind to Dnmt3a loses its ability to repress p21 transcription, suggesting that corepression of p21 promoter by Dnmt3a and Myc is dependent on interaction between Myc and Miz-1.

To establish if DNA methylation is required for Myc-dependent repression of the p21 promoter, they tested how Myc modulates p21 transcription in the presence of the DNA methylation inhibitor 5-azacytidine. They found that the repressive effect of Myc on p21 promoter-reporter activity was substantially relieved in cells treated with 5-azacytidine, indicating that methylation may play a role in Myc-mediated repression. Conversely, bisulfite genomic sequencing revealed that the p21 proximal promoter is methylated in c-*myc*+/+ but not in c-*myc*−/− rat fibroblasts. In addition, the p21 promoter gained methylation when Myc expression was restored in c-*myc*−/− cells, suggesting that Myc expression is required for DNA methylation.

Ultimately, Brenner *et al*. proposed that Myc could repress the p21 promoter by switching Miz-1 from a transcriptional activator to a repressor by a dual mechanism: (i) by preventing recruitment of a coactivator to Miz-1 (Herold *et al*., 2002; Seoane *et al*., 2002) and (ii) by bringing the corepressor Dnmt3a to Miz-1 (Brenner *et al*., 2005). They also suggest that targeting of DNA methyltransferases to individual genes through their association with specific transcription factors may be a general mechanism by which DNA methylation is directed to selected loci.

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However, certain recent data indicate that mechanisms of Myc-mediated repression may be more complicated. For instance, if Miz-1 is a positive transcription factor for the p21 promoter in physiological conditions, we would expect p21 levels to go down with decreasing Miz-1. In contrast, p21 levels were upregulated after Miz-1 knockdown and it was suggested that p21 may not be a Miz-1 target (Ziegelbauer *et al*., 2004). Furthermore, it was found that c-*myc* impairs Ras-mediated induction of p21 by interfering with Sp1 transcriptional activity, but independently of Miz-1 (Vaque *et al*., 2005). In addition, it was shown that although Myc represses proximal GADD45a promoter, Miz-1 does not bind to this promoter and is not responsible for Myc-mediated repression (Barsyte-Lovejoy *et al*., 2004).

Recently, Herbst *et al*. identified a new region MbIII (aa 188-199) in the Myc protein, which is required for Myc transcriptional repression and inhibits its apoptotic activity (Herbst *et al*., 2005). The MbIII region does not overlap with the Miz-1 interacting region, but it overlaps with the Sp1 interacting region on the Myc protein (Gartel and Shchors, 2003). These data suggest that c-*myc* may repress gene transcription in a Miz-1-independent fashion and further experiments are required to determine if methylation is involved in Miz-1-independent mode of Myc repression. Since the oncogenic potential of c-*myc* and its ability to promote tumorigenesis may be directly linked to its repressor fuctions, it is very important to establish the precise mechanisms of c-*myc*-mediated transcriptional repression.

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