Short Conceptual Overview

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The TATA-box motif and its impact on transcriptional gene regulation by miRNAs

Abstract: Emerging evidence suggests that components of the small RNAs pathway interact with chromatin to regulate nuclear events, such as gene transcription. However, it has recently been reported that in some cases, gene transcription regulation by cellular miRNAs can occur via targeting the TATA-box motif without altering epigenetic modifications. This observation supports the notion that multiple mechanisms of miRNA-based transcriptional regulation exist, enhancing our understanding of the complexity of small RNA-mediated gene regulatory pathways. Here, we remark that miRNA-mediated transcriptional modulation, through the TATA-box motif, may be a synergistic approach for transcriptional control.

Keywords: AGO proteins; chromatin; miRNAs; TATA-box motif; transcriptional gene regulation.

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Introduction

MicroRNAs (miRNAs) are a type of endogenously expressed small regulatory non-protein-coding RNAs with a length of ~22 nt, and are typically derived from hairpin transcripts. MiRNAs interact with their targeted RNAs in a sequence-dependent manner, thereby functioning as post-transcriptional regulators of gene expression (1).

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The miRNAs are then incorporated into silencing-effector complexes, which they guide to complementary nucleic-acid targets (2). MiRNAs function as components of ribonucleoprotein (RNP) complexes, also known as RNA-induced silencing complexes (RISCs), and in that context they are referred to as either micro-ribonucleoproteins (miRNPs) or miRNA-induced silencing complexes (miRISCs), respectively (3). The most important and best characterized components of miRNPs are proteins of the Argonaute family (4, 5). Mammals possess four Argonaute (AGO) proteins, AGO1 to AGO4. AGO2 is the only AGO protein that functions in RNA-interference (RNAi) due to its RNaseH-like P-element induced wimpy testis (PIWI) domain, which cleaves mRNAs at the center of the miRNA-mRNA duplex (3). It has been estimated that more than 60% of human protein coding genes are predicted to contain miRNA binding sites within their 3′ UTRs (6, 7). The diversity and number of miRNAs suggest that a vast number of normal and pathological outcomes may be controlled, at least in part, through miRNA-mediated repression (8). Post-transcriptional regulation by miRNAs typically relies on miRNA interaction with sequences within 5′ and 3′-UTRs of messenger RNAs. However, small non-coding RNAs-binding proteins AGO1/2 have been found in the nucleus (9–12) and are reported to participate in transcriptional regulation, associated with the RNA polimerase II (RNAP II) core transcription machinery (12–15). In this regard, there is growing evidence that some miRNAs display a remarkable capability to act as sensors and recruiters of RISC components, repressor protein complexes, and chromatin modifiers to promote miRNA-induced transcriptional gene silencing (TGS) (2).

Small RNA-directed transcriptional gene regulation

Several pieces of evidence support an important role for small RNAs in the modulation of chromatin structure and TGS in plants, fungi, and animal cells (16). For example,

studies in *Schizosaccharomyces pombe* and other multicellular organisms suggest that effector complexes, directed by small RNAs, target nascent chromatin-bound non-coding RNAs and recruit chromatin-modifying complexes (16). Through various mechanisms, including DNA methylation and histone modification, the end effect of such modulation is often TGS.

Recent high-throughput genomic approaches allowed the mapping of AGO footprints on either mRNA or DNA sequences *in vivo* under different physiological conditions, thus highlighting the multi-functional roles of these proteins, particularly in the nucleus (17). Interestingly, AGO proteins are known to play important roles in the establishment of heterochromatin as well as the silencing of repetitive sequences (18). There are several examples in mammals, in which small RNAs function in the nucleus to modulate gene expression, including silencing and activation at the epigenetic level, and in an AGO-dependent manner (18–24). In this regard, miR-223 binds to complementary binding sites within the promoter of its target gene *Nfia*, repressing transcription by influencing epigenetic events (20). MiR-373 can readily induce the expression of E-cadherin and cold-shock domain-containing protein C2 (CSDC2) by targeting sites within their promoters (21). Another miRNA, miR-423-5p, induces transcriptional silencing by targeting a conserved region in the promoter of progesterone receptor (PR) gene (22). MiR-744 and miR-1186 may induce *Ccnb1* expression, thus modulating mouse cell proliferation through binding sites in the gene promoter (19). A similar report suggests the capacity of miR-589 to upregulate both *COX-2* and *PLA2G4A* gene expression (23). In all these observations, there is a common feature that involves RISC components, repressor protein complexes, chromatin modifiers, and the miRNAs bound to target genes to promote transcriptional regulation. However, an outstanding question remains: Could miRNAs promote gene silencing without chromatin modifiers?

Considerable progress has been made in elucidating RNA-directed chromatin-based alterations, particularly those that are brought about by non-coding RNAs (25). Interestingly, RNA-mediated transcriptional regulation of some genes (e.g., progesterone receptor), occurs in the absence of histone modification or DNA methylation, which are two common TGS mechanisms (26). However, previous studies have yet to identify any feature common to all miRNA-binding sites. Paradoxically, small RNAs (including miRNAs) can also induce transcriptional gene activation (TGA). Several common features suggest that the TGA and epigenetic-TGS are functionally related as both processes are mediated by AGO proteins, and are dependent upon the presence of promoter proximal non-coding transcripts [reviewed in Ref (27)]. However, the mechanism of miRNA-mediated TGA has yet to be fully understood. Given that RNA can also form duplexes with DNA, it is reasonable to think that miRNAs require direct hybridization with genomic DNA to specifically find regulatory regions. Often, a search for consensus sequences for miRNA-binding sites in promoter regions is conducted in order to explain potential instances of miRNA mediated-TGS (24). There is no common mechanism to control transcriptional processes; thus, it is difficult to predict whether non-coding RNAs targeting a specific sequence in the promoter will have a repressive or activating regulatory effect, and it is likely that the presence of specific transcription factors and other DNA- or RNA-binding proteins in the relevant tissue or cell line are important determinants. Further explorations are needed to determine the more specific roles of miRNAs-regulating transcription.

Promoter regulation by miRNAs beyond methylation and acetylation: thinking inside the TATA box

In eukaryotic systems, the initiation of transcription is a complex process requiring RNA polymerase II (RNA Pol II) and numerous auxiliary transcription factors. The TATA-box motif is present in up to 35% of RNA Pol II-transcribed human genes, representing the most conserved and widespread core promoter (28). The TATA-box motif is located within a narrow distance range, which is immediately upstream the transcription start site (TSS), wherein the pre-initiation complexes (PICs) are assembled (29) (Figure 1). During transcription initiation, TBP first binds to the TATA box of a gene and nucleates the assembly of PICs (28, 30). Recently, Fan et al. designed and evaluated the potential of TATA-box-targeting small interfering RNAs (siRNAs) in the regulation of gene expression. They also identified four characteristics that contribute to the high efficiency of TATA-box-targeting activating siRNAs on *IL-2* promoter activation: UA at the 3′ end of the antisense strand (23 nucleotides in length), which targets the center of TATA-box (~34 bp upstream the TSS), as well as 2′-OMe modified bases at the 3′ terminus of the antisense strand (31). In other recent studies, the same group revealed that a novel HIV-1-encoded miRNA, miR-H3, could target the TATA-box motif within HIV-1 5′ long terminal repeat (LTR) and enhance viral replication (32). Furthermore, chemically-synthesized siRNAs, which target the same

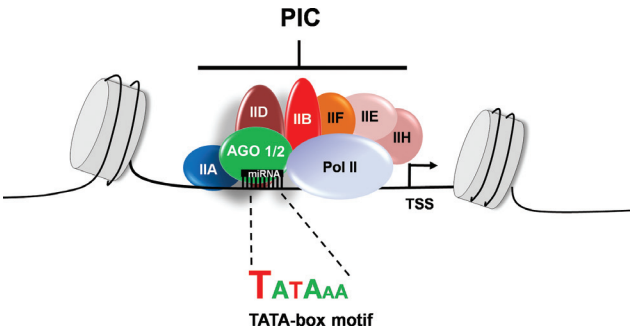

Figure 1: TATA-box motif as a potential target of regulation by miRNAs.
TATA-box motif is one of the most prevalent core promoters, located immediately upstream the transcription start site (TSS), wherein the pre-initiation complexes (PICs) are assembled. The PICs comprise several general transcription factors, including RNA polymerase II core unit, TFIID (or TATA-box–binding protein, TBP), TFIIA, TFIIB, TFIIE, and TFIIF (29, 30). In some cases, transcriptional regulation by miRNAs can occur by targeting the TATA-box motif without altering epigenetic modifications.

site, activate HIV-1 viral production from the CD4+ T cells isolated from HIV-1-infected patients receiving suppressive antiretroviral therapy. More recently, Zhang et al. found an association between some miRNAs (including let-7i, mir-145 and mir-16) and Pol II employing an RNA chromatin immunoprecipitation and quantitative real-time RT-PCR approaches, in human peripheral blood mononuclear cells. Interestingly, the authors found that a fraction of 1%–40% of miRNAs localized in the nucleus can be associated to the general transcription factors and that these miRNAs interact with the DNA sequences (e.g., TATA-box motif) close to the transcription start site (TSS) (Figure 1).

Additionally, through co-immunoprecipitation assays, the authors further investigated the interaction between AGO1 and AGO2 with Pol II and TBP in HEK-293T cells. Taken together, these results suggest that cellular miRNAs and AGO proteins are associated with the RNA Pol II core transcription machinery in human cells (33) (Figure 1). Essentially, this is a mechanism independent of acetylation and/or methylation at histones, which is capable to enhance promoter activities via binding to the TATA-box motifs of eukaryotic genes, such as those encoding IL-2, insulin, calcitonin, or c-myc (33).

Previously, we proposed a miRNA-DNA·DNA triple-helical structure formation mediated by a miRNA:AGO complex, as a plausible non-canonical gene regulation strategy targeting important nuclear events, such as transcription (24). In this sense, miRNA-directed TGS may occur by means of a parsimonious mechanism through triplex formation structures on promoter sequences, which are capable of inducing gene silencing, without the chromatin modifiers (24) that potentially could also occur through the TATA-box motif.

Expert opinion

Previously, David Corey targeted promoters using double-stranded RNAs to induce TGS and interestingly, the effect observed was neither a result of increased promoter DNA methylation, nor an effect of the inhibition of DNA methyltransferase activity (34). The discovery of chromatin modification-independent promoter regulation by miRNAs – particularly through the TATA-box motif – raises some exciting and challenging new questions about the mechanistic differences between silencing and activation. For example, is a perfect and contiguous base pairing of miRNA nucleotides (e.g., 2–8) necessary for miRNA-DNA association? 2) How do GU pairs or mismatches and bulges in the seed region affect repression or activation? 3) How many bulges or mismatches can be tolerated in the miRNA-DNA interaction for AGO-binding to the promoter region? 4) What would be the minimum degree of complementarity that should exist between one miRNA and the TATA-box motif to stabilize the interaction? 5) Are AGO proteins needed to generate a direct interaction on the TATA-box motif?

The majority of miRNA-targeting prediction programs for mammalian genomes are based on the assumption that, within transcripts, miRNAs target recognition requires conserved Waston-Crick pairing to the 5′ region of the miRNA centered on nucleotides 2–7 of the miRNA (the seed region) (35). However, many experimental results show that some ‘non-seed’ miRNA target sites are highly biologically functional (36–38). On the one hand, these non-seed sites contain single mismatches, GU wobbles, insertions, or deletions in the seed-match regions (35). On the other hand, there is evidence that perfect complementarity between miRNAs and their targets is not necessary for silencing, and that some miRNA nucleotides are more important than others (39, 40). Accessibility of binding sites might have an important effect on miRNA-mediated repression (41). Moreover, some experimentally characterized sites diverge significantly from these rules and can, for example, even require a bulged nucleotide in the seed region pairing (37, 42). Given that the rules of target recognition are not completely understood, each prediction tool may utilize a different combination of criteria for miRNA target site prediction on either UTRs or promoter

regions. In addition, combinations of multiple binding sites within a sequence can require a specific configuration (e.g., separation by a stretch of nucleotides of specific sequence and length) for efficient repression or activation (42). However, these findings have made it more difficult to explain how miRNAs regulate gene expression at the transcriptional level.

The biological outcome of the miRNA-TATA-box motif interaction may be altered by several factors that contribute to the binding strength, site accessibility, sequences flanking TATA-box motifs and RNA secondary structure, which in turn, may influence the repressive or activating effect of the promoter. While the regulation of genes possessing TATA-box can be independent on the participation of remodeling chromatin proteins, it is possible that genes lacking this consensus sequence could be regulated through miRNA-DNA·DNA triplex structure formation, stabilized by AGO proteins (24) or by different epigenetic events.

Outlook

The underlying mechanisms linking RNA and chromatin remain unclear. At present, we are beginning to appreciate the unanticipated roles of the small RNA machinery in regulating gene transcription. Undoubtedly, more systematic analyses for transcriptional control by noncoding RNAs and, particularly by miRNAs, are required to increase our knowledge. Such knowledge will allow us to decipher the regulatory information encoded by the genome, which in turn, will enable us to envision miRNA-based transcriptional artificial regulation across several metazoan species.

List of abbreviations

AGO	Argonaute
miRISCs	miRNA-induced silencing complexes
miRNA	microRNA
miRNPs	micro-Ribonucleoproteins
PIC	Pre-initiation complex
PIWI domain	RNaseH-like P-element induced wimpy testis
RISC	RNA-induced silencing complex
RNAi	RNA interference; RNAP II, RNA polymerase II
siRNA	Small interfering RNA
TBPs	TATA box-binding proteins
TGS	Transcriptional gene silencing
TSS	Transcription start site
UTR	Untranslated region

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