Letter to the Editor

RNA activation: a diamond in the rough for genome engineers†

Running title: RNA activation

Dr. Luis María Vaschetto*[1,2]

Affiliations

[1] Instituto de Diversidad y Ecología Animal, Consejo Nacional de Investigaciones Científicas y Técnicas (IDEA, CONICET), Av. Vélez Sarsfield 299, X5000JJC Córdoba, Argentina.

[2] Cátedra de Diversidad Animal I, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, (FCEFyN, UNC), Av. Vélez Sarsfield 299, X5000JJC Córdoba, Argentina.

Corresponding author's phone and e-mails

Phone: 0054-3572- 445-6144

E-mails: luisvaschetto@hotmail.com (primary e-mail); luisvaschetto@conicet.gov.ar

†This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1002/jcb.26228]

Received 6 May 2017; Revised 18 June 2017; Accepted 20 June 2017
Journal of Cellular Biochemistry

DOI 10.1002/jcb.26228

Abstract

The ability to develop efficient and versatile technologies for manipulating gene expression is a fundamental issue both in biotechnology and therapeutics. The endogenous RNA interference (RNAi) pathway which mediates gene silencing was discovered at the end of the 20th century and it is nowadays considered as an essential strategy for knockdown of specific genes and for studying gene function. Remarkably, during the past decade, a RNA-induced mechanism of gene activation has also been reported. Likewise RNAi, the RNA activation (RNAa) process is also mediated by sequence-specific double-stranded RNA (dsRNA) molecules, and interesting resemblances between both RNA-based transcriptional mechanisms have been described. Small activating RNAs (saRNAs) and related molecules have been used for targeting of genes in species that are as different as nematodes and humans, and similar dsRNA-induced activation phenomena have also been observed in plants. The aim of this letter is to highlight recent molecular insights into yet unexplored RNAa mechanism and its potential for manipulating transcriptional activity.

Keywords: RNA activation; RNA interference; double-stranded RNA; small activating RNA; RNA-Induced Transcriptional Activation complex.

Main text

Over last decade, Li et al. (2006) observed that the introduction of double-stranded RNA (dsRNA) sequences targeting promoter regions of the E-cadherin, VEGF and p21 genes induced gene expression in human cultured cells. Independently, another research group used multiple dsRNAs to target the Transcriptional Start Site (TSS) of the Progesterone Receptor (PR) gene in breast cancer cell lines, obtaining similar results (Janowski et al. 2007). These examples, and many others, confirmed a targeted, promoter-specific mechanism of gene expression referred to as RNA activation (RNAa). In addition, synthetic dsRNAs targeting sequences beyond the 3' untranslated region of the PR gene were also found to induce RNAa-based activation (Yue et al. 2010). Although recently discovered, the RNAa mechanism has already shown to be an effective tool to study the expression of cancer-associated genes, being also potentially useful in the design of gain-of-function therapies involving tumor suppressors. For instance, the p21 cyclin-dependent kinase (CDK) inhibitor is a tumor suppressor gene downstream of p53 signaling pathway which modulates cell development by growth inhibition, senescence and differentiation. Accordingly, RNAa-induced p21 expression arrested cell cycle and lead to apoptosis in cancer cells (Yang et al. 2008). Moreover, the introduction of synthetic dsRNAs designed to target promoter regions of tumor suppressors genes such as KLF4 (Wang et al. 2010), WT1, NKX3-1, p53 (Huang et al. 2010) and HIC-1 (Pan et al. 2013) have been also shown to be able to induce targeted gene expression in mammalian cell lines.

The Figure 1 outlines molecular insights into RNAa and canonical RNA interference (RNAi) pathways. Mechanistically, the core of the RNAa transcriptional machinery includes the Argonaute 2 (Ago2) protein and dsRNAs referred to as small activating RNAs (saRNAs) or antigen RNAs (agRNAs) (Schwartz et al. 2008, Yang et

al. 2008). The saRNAs consist of 21 nucleotides duplexes that are processed by Ago2 and incorporated into the RNA-Induced Transcriptional Activation (RITA) complex also containing the CTR9 and RHA proteins (Portnoy et al. 2016). CTR9 is a subunit of the PAF1 complex (PAF1C) that regulates initiation and elongation of transcription by interacting with histone-modifying enzymes and RNA polymerase II (RNAP II), whereas RHA is an ATP-dependent DNA/RNA helicase that also recruits chromatin remodelers and factors implicated in formation of the transcriptional activation machinery. Thus, saRNA-Ago2 forms the binary core of the RITA effector complex which associates with heterogeneous nuclear proteins (hnRNPs), transcription factors and RNAP II in order to induce targeted gene expression (Schwartz et al. 2008, Hu et al. 2012, Portnoy et al. 2016). In addition, a recent work shows that other Argonaute family member, the Argonaute 1 (Ago1) protein, is also required to mediate RNAa-based activation of the Foxg1 gene, a key transcription factor during brain development (Fimiani et al. 2016).

It is interesting to note that RNAa shares important common features with the RNAi pathway such as the presence of Ago and RHA proteins also known to form part of the RNA-Induced Silencing Complex (RISC); however, the kinetics of both mechanisms are different, being it indicative of distinct signaling processes for each transcriptional pathway. Whereas small interference RNA (siRNA) oligonucleotides induce RNAi in few hours, the response to RNAa treatment is observed only after 2 days following saRNA transfection, with transcriptional effects more durable in the last (RNAa). The most significant resemblances between RNAa and RNAi might include the thermodynamic selectivity of the RNA strand used by Ago2 as guide, and the discovery that certain RNA interference micro-RNAs (miRNAs) targeting promoter regions can also induce activation (Place et al. 2008, Huang et al. 2012, Turner et al. 2014). Likewise RNAi, the RNAa process triggers epigenetic modifications associated in this case to

switch-on gene expression (see Fig. 1). Recently, the Li laboratory showed that RITA complex recruits chromatin modifying enzymes in order to induce both monoubiquitination of Histone 2B (H2Bub1) and methylation of Histone 3 at Lysine 4 (H3K4me), and subsequent transcriptional elongation by phosphorylation of RNAP II (Portnoy et al. 2016). In addition, a decrease in histone acetylation level may also be associated to RNAa (Janowski et al. 2007). Moreover, in petunia plants, it has been suggested that small dsRNAs derived from regulatory inverted repeat sequences might trigger RNA-directed DNA methylation (RdDM) and thus induce gene expression (Shibuya et al. 2009). Taken together, these findings indicate that RNA-based activation phenomena play a role in the establishment and modulation of specific epigenetic pathways.

The RNAa process is a regulatory mechanism encompassing a wide range of animal species and it may be induced by artificially designed saRNAs without need to transfect hazardous macromolecular complexes. Moreover, RNAa also can represent a versatile non-invasive platform for upregulating gene expression *in vitro*, as well as for overexpressing desired targets in specific tissues and/or developmental stages. In different mammal species, RNAa has been used to selectively activate transcription of essential genes in development (Huang et al 2010). However, it should be noted that several important characteristics on RNAa molecular functioning must still be elucidated, and further progresses in this area are required to shed light on these questions (Zhao et al. 2015). It is expected that advances in RNAa research come from genome engineering technologies and especially by programmable nucleases (ZFNs, TALENs and CRISPR/Cas9, etc.) methodological-based approaches for identifying and manipulating saRNAs targeting specific promoters. To conclude, RNAa constitutes a versatile but yet unexplored mechanism that exhibits an interesting potential to activate gene expression,

and therefore it should be taken into consideration in the design of new strategies to control transcription.

List of abbreviations

RNA interference: RNAi

RNA activation: RNAa

Double-stranded RNAs: dsRNAs

Small activating RNA: saRNAs

Progesterone Receptor: PR

Argonaute 2: Ago2

RNA-Induced Transcriptional Activation: RITA

RNA polymerase II: RNAP II

Heterogeneous nuclear proteins: hnRNPs

RNA-Induced Silencing Complex: RISC

Small interference RNA: siRNA

Micro-RNAs: miRNAs

Histone 2B monoubiquitination: H2Bub1

Histone 3 Lysine 4 methylation: H3K4me

RNA-directed DNA methylation: RdDM

Precursor miRNA: pre-miRNA

References

Fimiani C, Goina E, Su Q, Gao G, Mallamaci A. RNA activation of haploinsufficient Foxg1 gene in murine neocortex. Sci. Rep. 2016; 6:39311 DOI: 10.1038/srep39311.

Hu J, Chen Z, Xia D, Wu J, Xu H, Ye ZQ. Promoter-associated small double-stranded RNA interacts with heterogeneous nuclear ribonucleoprotein A2/B1 to induce transcriptional activation. Biochem. J. 2012; 447:407-416.

Huang V, Qin Y, Wang J, Wang X, Place RF, Lin G, Lue TF, Li LC. RNAa is conserved in mammalian cells. PloS one 2010; 5(1):e8848.

Huang V, Place RF, Portnoy V, Wang J, Qi Z, Jia Z, Yu A, Shuman M, Yu J, Li LC. Upregulation of Cyclin B1 by miRNA and its implications in cancer. Nucleic Acids Res. 2012; 40(4):1695-1707.

Janowski BA, Younger ST, Hardy DB, Ram R, Huffman KE, Corey DR. Activating gene expression in mammalian cells with promoter-targeted duplex RNAs. Nat. Chem. Biol. 2007; 3(3):166-173.

Li LC, Okino ST, Zhao H, Pookot D, Place RF, Urakami, S, Enokida H, Dahiya, R. Small dsRNAs induce transcriptional activation in human cells. Proc. Natl. Acad. Sci. USA 2006; 103:17337-17342.

Pan S, Wang Z, Chen Y, Chen X, Ji J, Zhang J, Li J, Cai Q, Liu B, Zhu Z, Yu Y. Inactivation of tumor suppressor gene HIC1 in gastric cancer is reversed via small activating RNAs. Gene 2013; 527(1):102-108.

Place RF, Li LC, Pookot D, Noonan EJ, Dahiya R. MicroRNA-373 induces expression of genes with complementary promoter sequences. Proc. Natl. Acad. Sci. USA 2008; 105(5):1608-1613.

Portnoy V, Lin SHS, Li KH, Burlingame A, Hu ZH, Li H, Li LC. saRNA-guided Ago2 targets the RITA complex to promoters to stimulate transcription. Cell Res. 2016; 26:320–335.

Schwartz JC, Younger ST, Nguyen NB, Hardy DB, Monia BP, Corey DR, Janowski BA Antisense transcripts are targets for activating small RNAs. Nat. Struct. Mol. Biol. 2008; 15(8), 842-848.

Shibuya K, Fukushima S, Takatsuji H. RNA-directed DNA methylation induces transcriptional activation in plants. Proc. Natl. Acad. Sci. USA 2009; 106:1660-1665.

Turner M, Jiao A, Slack FJ. Autoregulation of lin-4 microRNA transcription by RNA activation (RNAa) in C. elegans. Cell Cycle 2014; 13(5):772-781.

Wang J, Place RF, Huang V, Wang X, Noonan EJ, Magyar CE, Huang J, Li LC. Prognostic value and function of KLF4 in prostate cancer: RNAa and vector-mediated overexpression identify KLF4 as an inhibitor of tumor cell growth and migration. Cancer Res. 2010; 70(24):10182-10191.

Yang K, Zheng XY, Qin J, Wang YB, Bai Y, Mao QQ, Wan Q, Wu ZM, Xie LP. Up-regulation of p21 WAF1/Cip1 by saRNA induces G1-phase arrest and apoptosis in T24 human bladder cancer cells. Cancer Lett. 2008; 265(2):206-214.

Yue X, Schwartz JC, Chu Y, Younger ST, Gagnon KT, Elbashir S, Janowski BA, Corey DR. Transcriptional regulation by small RNAs at sequences downstream from 3′ gene termini. Nat. Chem. Biol. 2010; 6(8):621-629.

Zhao XY, Voutila J, Habib NA, Reebye V. RNA Activation. In Innovative Medicine. Springer Japan 2015; pp. 241-249.

Figure caption:

Figure 1. Schematic model for RNAa and RNAi-based pathways. In the microRNA (miRNA) pathway, a pri-miRNA is cleaved by the RNAse III endonuclease Drosha to generate a stem-loop precursor miRNA (pre-miRNA). Both pre-miRNA and long siRNA precursors are recognized by Dicer, another RNAse III enzyme, which cleaves them into mature microRNA (miRNA) and small interfering RNA (siRNA), respectively. The siRNA/miRNA duplex is further processed and the guide-strand is assembled into the Argonaute-containing RNA-Induced Silencing Complex (RISC) that promote RNAi-mediated responses such as, for instance, targeted RNA degradation and spreading of repressive epigenetic marks (e.g., DNA methylation, H3K9me, etc.). Similarly, in the RNAa mechanism, a small activating RNA (saRNA) is bound by Argonaute 2 (Ago2) protein and the guide-strand is loaded into the RNA-Induced Transcriptional Activation (RITA) complex. RITA functions to assemble the transcriptional machinery on target promoters and to recruit chromatin remodelers that trigger activation-associated epigenetic modifications (e.g., H2Bub1, H3K4me, etc.) or loss of repressive marks. It is thought that RNAa and RNAi may alter the chromatin structure in order to maintain the active and repressive states, respectively.

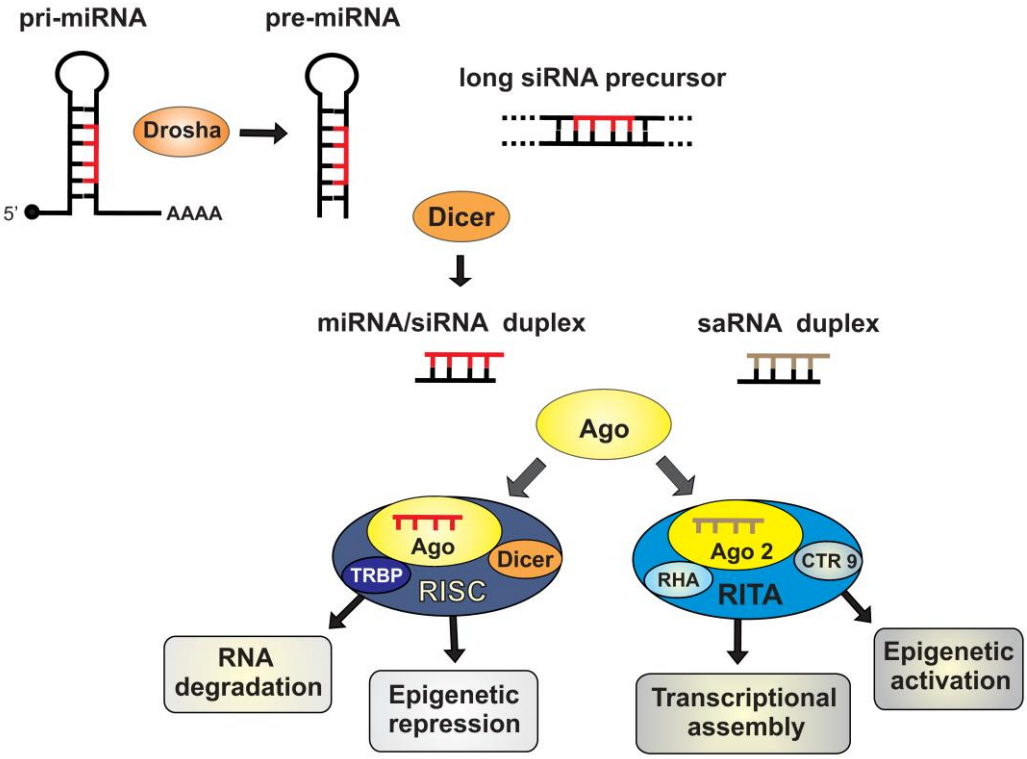

Figure 1