Transcriptional Regulation by Promoter Targeted RNAs

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Abstract: Small RNA molecules, including small interfering RNA (siRNA) and micro RNA (miRNA), have rapidly emerged as important regulators of gene expression. Recent articles have demonstrated RNA mediated complex induced transcriptional gene silencing (TGS) occurring in the nucleus. Originally the small RNA mediated TGS pathway has been reported in yeast and plants, currently a number of articles strongly suggest that this newly established gene silencing mechanism is present in mammals. RNA mediated TGS has been reported for various human promoters including inhibition of tumor susceptibility genes, X-chromosome inactivation and suppression of human chemokine receptor. Small RNAs can inhibit human viral infection through the TGS pathway. Prolonged HIV-1 transcriptional gene silencing by an RNA duplex targeting a sequence located within the HIV-1 promoter has been reported initially using a susceptible adherent cell line model and recently prolonged suppression of productive HIV-1 infection in a T-cell line model has been demonstrated by a retrovirally delivered short-hairpin RNA (shRNA) targeting the same region. RNA mediated gene silencing in HIV-1 infection can induce heterochromatin (closed) structure in the promoter regions, which is consistent to those changes seen in studies of various RNA directed TGS in various human promoter regions. More recent observations suggest transcriptional activation can be induced through RNA duplexes targeting the human promoter of E-cadherin, p21 and the progesterone receptor. Although the precise mechanisms of how RNA mediated transcriptional gene silencing or activation functions has yet to be elucidated, this review describes linkage of small RNA mediated gene regulation and induction of epigenetic regulation in the promoter region in mammals.

Keywords: Transcriptional gene silencing (TGS), siRNA/shRNA, RNAi, Heterochromatin formation, HIV-1.

Small RNA molecules, including small interfering RNA (siRNA) and micro RNA (miRNA), have been recently found to be important regulators of gene expression. RNA duplexes can induce transcriptional gene silencing (TGS) in the nucleus, which is distinct from post transcriptional gene silencing (PTGS), where small RNA induce slicing of specific mRNA containing homologous complementary sequences. TGS in human cells has been recently reported in a number of human promoter genes including those of suppression of tumor susceptibility genes [1, 2], X-chromosome inactivation [3] and inhibition of human chemokine receptor expression [2]. Our studies focus on RNA mediated TGS in HIV-1 or SIV infection. We have shown that prolonged HIV-1 transcriptional gene silencing can be induced by an RNA duplex targeting a sequence located within the NF-κB binding motif of the HIV-1 promoter, initially using a susceptible HeLa cell line. We recently demonstrated that very prolonged suppression of productive HIV-1 infection in a T-cell line (MOLT-4) could be induced by a retrovirally delivered short-hairpin RNA (shRNA) targeting the same region (κB-shRNA). These results complement other data showing silencing of reporter genes under the control of the HIV promoter region, the 5'LTR of the virus [4, 5]. RNA directed TGS is associated with induction of biochemical modification of critical residues in the histone tails within the target promoter region, which are in turn associated with repressive chromatin structures [6-8]. The process of induction of these changes in chromatin and the precise mechanisms underlying the induction of TGS by siRNAs are poorly understood. Complicating this field even further is the observation that siRNAs targeting promoter regions have recently been shown to be associated with transcriptional activation in studies of human promoter regions of the E-cadherin, p21 and the progesterone receptor [9-11]. In some genes this occurs in the context of the same RNA duplex inducing transcriptional silencing in certain culture conditions using the same cell line. The precise mechanism of how small RNAs can induce both silencing and activation of transcription is unknown. This new generation of RNA adapting innate human host machinery for prolongation of the regulating effect is described in this review.

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POST TRANSCRIPTIONAL GENE SILENCING (PTGS) AND TRANSCRIPTIONAL GENE SILENCING (TGS)

RNA interference (RNAi) or small interfering RNA (siRNA) is a process by which double stranded RNA induces inhibition of gene expression. It was first described in *Caenorhabditis elegans* [12] and subsequently reported in *Trypanosoma brucei* [13] and Drosophila [14]. It has been used to suppress a range of mammalian genes (reviewed elsewhere in this series) and suppresses a range of pathogenic human viruses including poliovirus [15], hepatitis virus [16-19], human papillomavirus [20] and HIV-1 [21-24]. Double stranded RNA are ~22 nucleotides in length and

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can induce two different gene regulation pathways of gene silencing, Post Transcriptional Gene Silencing (PTGS) and Transcriptional Gene Silencing (TGS) (Fig. **1**). The PTGS pathway predominantly takes place in the cytoplasm, and is dependent upon the RNA-induced Silencing Complex (RISC), which is formed following the recruitment of RNA duplex-Argonaute (Ago) protein complex. This RNA-protein duplex is a central player in RISC, allowing the unwound anti-sense strand of the RNA duplex to bind to complementary mRNA. The anti-sense RNA strand guides RISC to the specific target mRNA, followed by cleavage of the target mRNA and subsequent degradation [25-29]. Notably it has also been reported that PTGS can be observed within nucleus in human cells [30, 31]. It is accepted that PTGS pathway can be induced by RNAi or siRNA in a wide range of genes in numerous cell types.

RNA duplexes can also utilize a second gene regulatory pathway which occurs in the nucleus through a mechanism distinct from PTGS. The TGS pathway can be induced by RNA duplexes homologous to certain sequences within the promoter regions of genes. This process was initially reported in plants, fission yeast and Drosophila, and more recently had been reported to occur in certain human cells [1,2,4,5,8,32-52]. Recent articles suggest that during siRNA induced TGS there is specific recruitment of Ago proteins to the promoter regions of the targeted gene [2, 43, 51, 53]. These data, therefore, suggest that Ago proteins may also play a central role in the formation of RNA-induced initiation of transcriptional gene silencing (RITS) complex which initiates the heterochromatin formation associated with siRNA induced repression of transcription [6, 7]. This process is related to changes to the histone code, involving alteration in the methylation status of histone tails (eg. dimethylation of lysine 9 of histone 3 (H3K9me2) and trimethylation of lysine 27 of histone (H3K27me3), and the recruitment of both histone deacetylase (HDAC) and the polycomb group protein, enhancer of zeste homolog 2 (EZH2) [2, 37, 42, 45, 46, 51]. Peptide nucleic acids (PNA) can also induce TGS and the associated chromatin changes. These have been designed to directly target the transcription start site and induce repressive changes in chromatin architecture of the promoter region, which extend through this site [1]. The mechanisms underlying heterochromatin formation are multiple, complex and can result from the actions of a number of different regulatory molecules.

The components of RITS in mammalian cells has not been well described. The components of RITS are better understood in lower organisms including fission yeast where siRNA induced transcriptional gene silencing appears to play an integral role in the regulation of centromeric heterochromatin. In these organisms it is thought that the process is initiated by RITS and the heterochromatin modifications are mediated by RNA polymerase complex, histone methyltransferase Clr4, adaptor protein Tas3 and heterochromatin protein 1 (HP1) Chip1 [54-60]. One of the difficulties in understanding the process of RNA induced TGS in mammalian cells is that some of the crucial components of RITS in fission yeast have no sequence homologues in the mammalian genome (Tas3 and RNA-direct RNA polymarase (RdRp)). One exception is that the Argonaute family of RNA binding proteins is highly conserved and is found in RITS complexes in each organism so far studied [61-63]. The clue to sorting out the other components of RITS in mammalian cells is that even in lower organisms there is little sequence

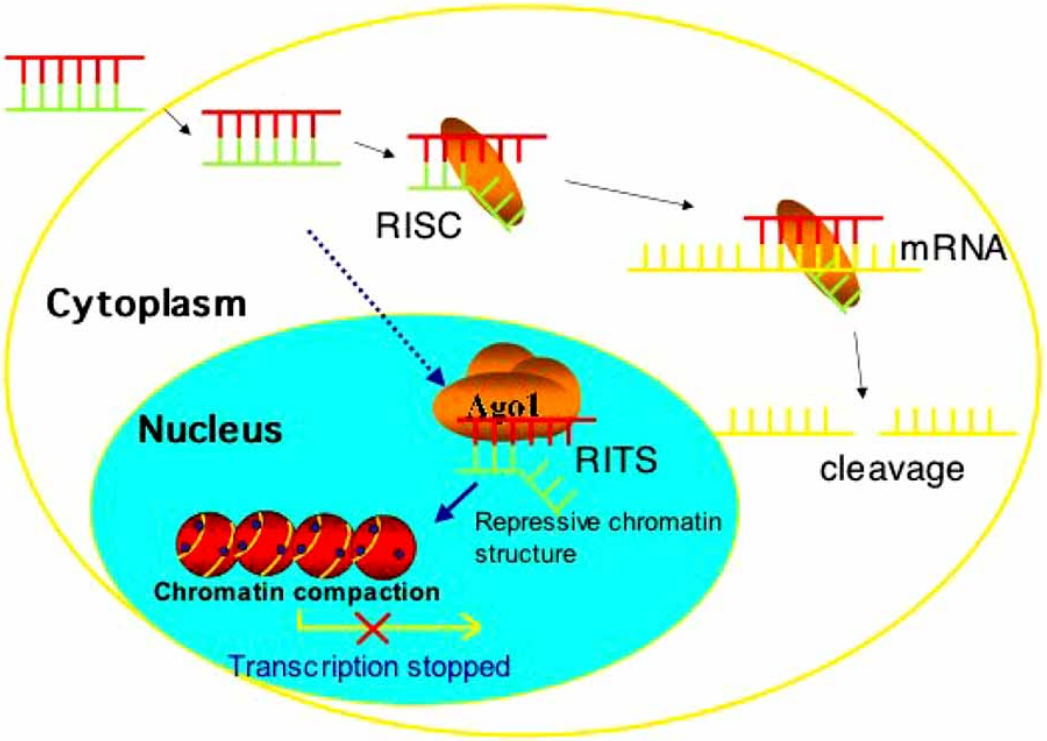

Fig. (1). siRNA can induce degradation of homologous mRNA though function of RISC and siRNA can also interact with promoter region to form repressive chromatin structure though RITS complex, inhibiting initiation of gene transcription.

homology between the other proteins involved in RITS but the proteins do have certain structural similarities [64]. It is likely that the process of TGS is initiated by an RNA duplex bound to an Ago protein providing both the specificity of the effect though complimentarity to the target sequence and the nidus to which the RITS complex is recruited to initiate the formation of heterochromatin. Although this process is best described as being associated with the induction of silencing, recent reports suggest that RNA duplexes targeted to promoter regions can also induce activation of gene transcription in certain human cells. Transcriptional activation has been demonstrated using promoter targeted double stranded (ds)RNAs to several mammalian genes including E-cadherin, p21 and the progesterone receptor [9-11]. The mole-cular mechanism of this transcriptional activation needs further studies. Small RNA induced transcriptional activation will be discussed later in this review.

MICRORNA MEDIATED GENE SILENCING

MicroRNA have also recently emerged as important regulators of gene expression. Initial reports showed that miRNAs are capable of suppressing specific genes at the translational level [9,65-68]. In addition to miRNAs' ability of translational repression of certain gene expression, they involve cognate mRNA cleavage [69, 70]. The miRNA pathway involves the production of mature single stranded miRNA from precursor RNA by RNA processing enzymes Drosha and Dicer [70-72]. MiRNAs have been shown to be involved in regulating a variety of physiological functions, such as cell differentiation, metabolism, and development. A large number of miRNAs have been identified in animals, plants, and viruses [73]. Recent articles show that miRNAs can also regulate tumor susceptibility genes [74-77], and expression profiling assays have discovered characteristic miRNA signatures in chronic lymphocytic leukemia, breast carcinoma and human solid cancers [78, 79]. MiroRNA-induced transcriptional gene silencing to form repressive chromatin structures by *de novo* DNA methylation or histone modification has been reported in both yeast and plants [80]. It has also been reported that miRNA could act as a cis-regulator to regulate transcription to modify gene expression in human cells. In this study heterochromatin formation has been shown through certain miRNA loaded into Ago1 complex to recruit EZH2 and induce H3K27me3 in a human promoter [48].

TGS IN HIV-1 INFECTION

We have previously demonstrated that RNA duplexes targeting the promoter region of the integrated forms of the retroviruses, HIV-1 and SIV, can induce sustained suppression of viral replication through TGS [4, 5, 49, 51]. The most effective of these duplexes, κB-siRNA, has a sequence homologous to a section of the tandem repeat of NF-κB motifs within the U3 promoter region of the HIV 5'LTR (Fig. **2**). Initially we designed four siRNAs targeting different regions of the HIV-1 promoter up stream of the transcription start site to assess their relative efficacy. The kinetics of viral suppression varied among the siRNAs tested. Reverse transcriptase levels of culture supernatant, which indicate newly released HIV-1 from the infected cells, was reduced over 1000-fold in the cultures transfected with κB-siRNA, when compared to mock transfected cultures, for over 30 days after a single transfection of the siRNA, while an siRNA targeting the Sp1 binding motif did not induce any significant viral suppression. We have recently shown that prolonged (>1yr) HIV-1 gene suppression can be achieved in a T-cell line stably expressing a short hairpin RNA (shRNA) targeting the same sequence within the NF-κB binding motif [5].

We observed the strong RNA duplex mediated TGS targeting NF-κB region of U3 HIV-1 promoter was induced by both siRNA and shRNA in independent experimental settings. Both suppressed viral mRNA expression and newly formed virus in the culture supernatants, but cell associated proviral HIV-1 DNA was detected in these culture at all time points suggesting that viral suppression occurs at a post integration stage of the viral life cycle. This is consistent with viral transcription being inhibited by suppressive chromatin formation in the HIV promoter region, induced by the small RNAs. This is associated with elevated levels of H3K9me2, H3K27me3, decreased level of histone 3 acetylation (H3Ac) and recruitment of HDAC1 [4,5,49,51] The induced chromatin modifications through small RNAs across the HIV-1 promoter region are consistent with those previously characterized in studies of *in vitro* models of latent HIV-1 infection [81-87]. This indicates that siRNA mediated TGS in HIV-1 infection leads to a state that resembles the chromatin architecture in the HIV-1 promoter region of latent HIV-1 infection.

Nuclear run-on assays provide a direct assessment of transcription and can distinguish transcriptional from post-transcriptional effects. Run-on assays of the nuclei isolated from infected cultures indicate that transcription of HIV-1 was fully suppressed in both siRNA and shRNA mediated TGS [4, 5]. This was confirmed by silencing of a luciferase reporter construct driven by the 5'LTR of HIV-1 by the same κB-shRNA construct [5]. During the process of reverse transcription, HIV-1 creates two identical LTR regions: the 5'LTR which acts as a promoter, and the 3'LTR which plays a role in the termination of transcription of mRNA, providing the machinery for polyadenylation [88]. Therefore siRNAs with homology for the U3 region of the LTR have the potential to act as inducers of PTGS mediated degradation of all HIV mRNA species, both spliced and unspliced. In order to evaluate the contribution of these 3'LTR sites to the observed reduction in viral mRNA and viral replication, our siRNAs were tested using a HeLa cell clone stably transfected with the HIV-3'LTR expressed under the immediate early CMV promoter. In this system, no significant reduction in LTR mRNA was observed with either κB-siRNA and κB-shRNA [5,51], suggesting that both show limited capacity to induce PTGS. These data strongly support the contention that κB-siRNA and the κB-shRNA construct inhibit productive HIV-1 infection primarily through TGS.

Two different single stranded RNAs targeting the HIV-1 promoter region, which are about 100-bases upstream (LTR-247antisense) and 7-bases downstream (LTR-362antisense) of our targeted NF-κB region have also been shown to mediate transcriptional silencing [42, 46, 50]. These RNAs

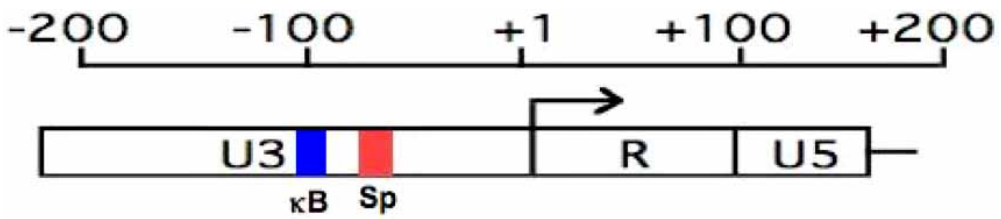

Fig. (2). The location of the sequence targeted by two siRNAs are illustrated. HIV-1 5' LTR region consists of U3, R, U5 region. HIV-1 promoter is located in U3. The positioning of the effective siRNA, which shows strong transcriptional repression of HIV-1 gene is painted in blue referring to kB. The siRNA, which fails to induce TGS, is in red referring to Sp. Transcription start site of HIV-1 is indicated by +1.

are delivered by expression plasmid or HIV-2 based lentiviral vector and small RNAs are expressed under the U6 promoter [42,50] Both the 362antisense and 247antisense vectors appear to induce some level of transcriptional silencing, however, only 362antisense was effective in sustaining virus production for periods up to one month *in vitro* [50]. The TGS is accompanied by elevated H3K27me3 and recruitment of HDAC1 and HDAC3a in the region of the 5'LTR. Further, LTR-362antisense induced TGS has been observed to be associated with the following epigenetic changes: Ago1 is loaded onto the antisense strand for the initiation of TGS, then DNA methyltransferase3a (DNMT3a) has been shown to co-immunoprecipitate with the small RNAs at the targeted promoters as well as to co-immunoprecipitate with Enhancer of zeste 2 (EZH2) and HDAC1 [50, 89, 90]. Ezh2 is the histone methyltransferase involved in generating the H3K27me3 epigenetic mark, which has been observed in the silencing event. These induced modifications specifically at the HIV-1 promoter through LTR-362antisense are consistent with our data regarding the epigenetic changes associated with κB-siRNA and κBsh-RNA mediated TGS [4, 5, 51]. Recent reports have shown that even the antisense strand of siRNA duplexes, acting alone, interacting with a RNA polymerase II transcribed promoter associated RNA species, can direct sequence-specific transcriptional gene silencing in human cells [42,46,91]. These data suggest that the anti-sense strand of the RNA duplex could play an important role for induction of the silencing components through the RITS complex. It is notable that we have previously demonstrated the effects of κB-siRNA and κB-shRNA are virus specific, having no effect on: cell growth characteristics, expression of CD4 or chemokine co-receptors for HIV, or on other genes regulated by NF-κB [4, 5]. However, off-target effects must be accounted for as they have been described with siRNAs or antisense RNA designed to induce TGS. The interpretation of data presented above with respect to the gene silencing effect through the LTR-247antisense is complicated by the presence of off-target effects. The antisense RNA are targeting a non-coding RNA, C10orf76, which appear to exhibit a cell wide non-specific effect [46]. These effects are difficult to predict and slight off setting of target sequences can make substantial changes to the extent of off-target effects seen [92].

REGIONAL HETEROCHROMATIN FORMATION IN THE HIV-1 PROMOTER

Recent reports regarding siRNA induced TGS in yeast have revealed that heterochromatin specific chromatin modifications, such as H3K9me2, spread beyond the nucleation site provided by the siRNA induced RITS complex, resulting in regional heterochromatin formation [8,58,93]. Using Chromatin Immunoprecipitation (ChIP) analyses for H3K9me2 and HDAC1 at sites substantially upstream and downstream of the targeted area in the HIV-1 promoter we were able to demonstrate a similar phenomenon with enrichment of HDAC1 and H3K9me2 associated with HIV-1 DNA 300-base pairs upstream of the target site and enrichment of H3K9me2 in the *gag* coding region of the viral genome 800-base pairs downstream of the site targeted by κB-siRNA [51]. These data suggest siRNA induced TGS of HIV-1 is associated with extended regional heterochromatin formation which extends from the nidus formed by the target site. The mechanism of this spread of heterochromatin markers is unclear at this stage but a possible model is presented in Fig. (**3**).

PROMOTER-TARGETED SIRNAS INDUCE VIRAL SILENCING IN SIV-INFECTED CELLS

We have extended our observations regarding siRNA mediated TGS to simian immunodeficiency virus (SIV) infection. We designed five siRNAs, targeting separate sequences upstream of the transcription start site in 5'LTR of SIVmac251. We used CEMx174, a human lymphoid cell line, as the substrate for SIV infection [94]. One of these double stranded siRNA, targeting a site located about 200 base pairs upstream of the transcription start site, demonstrated a strong TGS mediated silencing effect, showing over 10^4-fold reduction of virus production at day-12 post transfection [49]. ChIP analyses revealed enrichment of heterochromatin markers H3K9me2, H3K27me3 in the SIV LTR in the silenced cultures. This process was inhibited by the HDAC inhibitor, trichostatin-A (TSA) further supporting a role for epigenetic changes in RNA mediated TGS. Essentially these observations recapitulate the observations made in the HIV-1 model and provide the basis for possible *in vivo* testing of these approaches for silencing of retroviruses [4, 5, 51].

SMALL RNAS INDUCE TRANSCRIPTIONAL ACTIVATION

As we mentioned before, understanding the role of siRNAs in regulating gene expression at the transcriptional level has become more complex. Initial observations suggested that these siRNAs repress transcription, but more recently small RNAs, especially those targeting the transcriptional start site of certain genes have been shown to

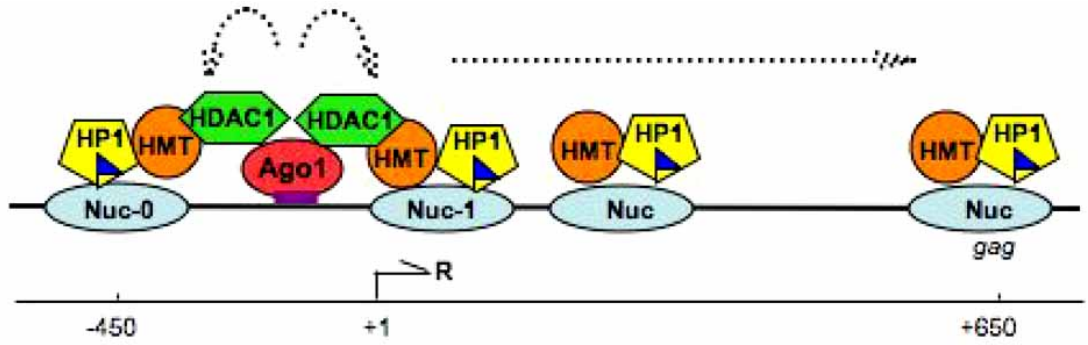

Fig. (3). Model of heterochromatin formation induced by siRNA targeting HIV-1 promoter region. siRNA acts as a nucleation centre for recruitment of the RNA-induced transcriptional silencing (RITS) complex and closed chromatin formation extends both up stream and down stream to include adjacent promoter and mRNA coding regions. siRNA (purple line) is loaded into Ago1. The RITS machinery, including histone deacetylase (HDAC1) and histone methyltransferase (HMT) induces H3K9me2 (blue flag) and recruits heterochromatin protein 1 (HP1) to the area surrounding the siRNA target site. Complexes including HP1 and HMT spread beyond the initial site of recruitment, creating broad domains of heterochromatin structure as indicated by H3K9me2 status of both the upstream promoter and downstream gag regions. Schematic figures show the relative positions of nucleosomes within the 5'LTR of HIV-1. The nucleosomes (Nuc-0, -1, -2) are precisely positioned in the HIV-1 LTR after integration of viral DNA into host genome (diagram modified from [81]). The number is relative to transcription start site. Fig. **3** was modified from published article [51].

activate transcription [9-11]. This has been most extensively described in relation to the gene coding for the progesterone receptor. Small RNAs targeting the transcription start site of this receptor were initially reported to suppress transcription, however, under certain circumstances the same RNA duplex can activate progesterone receptor expression [1, 10, 11, 43]. It appears that duplex RNAs complementary to promoter regions can repress or activate gene expression in different cellular contexts. The RNA duplexes targeting promoter region of progesterone receptor inhibit transcription in T47D breast cancer cells, which express high levels of progesterone receptor [1, 43]. The same RNA duplexes activate progesterone receptor expression in MCF7 breast cancer cells, which endogenously express low levels of progesterone receptor [10]. Furthermore when using the T47D cells they demonstrated that identical RNA duplexes could induce either transcriptional activation or suppression of progesterone receptor. Under growth conditions that lead to high progesterone receptor expression in T47D cells, the RNA duplexes repress gene expression. However, under growth conditions that lead to low progesterone receptor expression in the T47D cells, the same RNA duplexes can activate gene expression [11]. Thus, different outcomes can be achieved in the same cell line depending on basal expression levels. The authors of these papers contend that antisense transcripts of the promoter region are the targets for the RNA duplexes. They hypothesize that the key mediators controlling transcription of progesterone receptor may be noncoding transcripts that overlap miRNAs across the promoter region [11,95]. However, there are no clear biochemical pathways to describe how miRNAs control noncoding transcripts and how the siRNAs regulate activation or inhibition in the transcription of progesterone receptor based on basal antisense transcripts level. Their data suggest that controlling basal expression level of the antisense transcripts is the initial regulator of progesterone receptor transcription, because basal levels of expression affect whether activation or silencing is observed. MiRNAs have been described with near-perfect complementarity to gene promoters in either the sense or antisense orientations [11]. Other research has revealed that both sense and antisense transcripts are commonly found spanning these areas of promoter regions [96-98]. The role of antisense transcripts, notably 'aberrant' transcripts, in siRNA regulation of transcription have been extensively studied in lower eukaryotes, such as yeast, which can be suppressed by histone deacetylase complexes to recruit HP1 [99, 100]. Current models for RNAi-mediated heterochromatin assembly propose that siRNAs guide the RITS complex to nascent centromeric transcripts, where these noncoding transcripts serve as platforms for the recruitment of siRNA-mediated RITS complex [58-60, 101-103]. The RITS complex containing Ago1 then recruits the RNA polymerase complex (RDRC) and Dicer, which mediates the siRNA amplification, resulting in the recruitment of histone methyltransferase Clr4 to form the methylation of H3K9 and which creates a binding site for HP1 proteins Swi6 and Chp1 (important components of heterochromatin formation) [54-59]. Human cells lack several components of RDRC, therefore the same mechanism cannot be directly applied to siRNA mediated TGS in human cells.

Other observations suggest that an imbalance in bidirectional transcription levels may determine whether a promoter targeted siRNA results in activation or silencing of transcription [104, 105]. Studies of the promoter region of human gene E-cadherin, p21, [9, 104] have shown that siRNA mediated gene activation of p21 is not the result of direct promoter targeting, but rather functions *via* post-transcriptional suppression of a p21 antisense RNA of arising from the promoter region of sense strand of transcript. PTGS is widely accepted to take place in cytoplasm, however, some reports show that PTGS can be induced in nucleus [30, 31]. They hypothesize a model which suggests that at steady state, endogenous expression of p21 is associated with comparable levels of both sense and antisense transcripts across the promoter region. When a reduction in p21 antisense transcription in the promoter region occurs through an siRNA induced PTGS pathway, in

the nucleus there is a reduction in the low-level H3K27me3, suppressive marks, within chromatin associated with the p21 resulting in an increase in transcription [104]. This hypothesis is consistent with H3K27me3 is enriched, when there is a reduction of sense transcript of p21 promoter induced through siRNA induced PTGS pathway.. Regardless such a molecular pathway, where an imbalance in bidirectional transcription leads to dominant antisense RNA expression, resulting in directed transcriptional gene silencing of the sense promoter, might explain observations of epigenetic silencing of tumor suppressor genes, such as p21, in several human cell cancers [106]. However, there is still a need for clear biochemical analysis to describe how the imbalance in bidirectional transcription is induced. The authors also suggest that miRNAs might interact with antisense or sense transcripts of the promoter region, and that overlapping bidirectional transcripts might be controlled by natural miRNAs [11,95,104,105,107,108]. There are still many questions remaining to be answered especially with understanding the role of how small RNAs and proteins interact to control chromosomal DNA status. Finding out the precise mechanism linked with small RNA mediated TGS will facilitate therapeutic application.

THERAPEUTIC APPLICATIONS OF TGS

The challenges for the therapeutic use of TGS are similar to those facing other gene therapies. Effective therapy requires specifically targeted intracellular delivery of siRNA molecules or their precursors. In addition the delivery system has to ensure that sufficient quantities of the constructs get to the nucleus. These challenges are probably surmountable as a number of siRNAs that mediate PTGS are well progressed in the development pathway and have reached early stage clinical trials [109-112]. It is likely that similar approaches will be adapted to siRNA mediated TGS delivery including non-viral and viral vector systems. The other challenges are ensuring specificity and monitoring for off-target effects.

The stability of RNA duplexes can be increased by chemical modification of the two nucleotides at the 3'-overhangs of RNA duplex. These modifications include substituting in 2'-O-Methylpurines or 2'-fluoropyrimidines at both end of RNA-duplex. This approach has been used in most of the clinical applications of RNA duplexes mediating PTGS. Expression vectors for RNA duplexes usually use polymerase III (Pol III) promoters for shRNA expression or polymerase II (Pol II) promoters for expression of long hairpin RNAs. Seed-sequence-inducing off-target effects need to be avoided and non-specific interferon response from host cells has to be taken into consideration. In order to deliver RNA duplexes to the specific target cell, systemic delivery with selective targeting of siRNAs to specific cell-surface receptors is considered for the reduction of potential Seed-sequence-inducing off-target effects in non-target cells. Non-viral based delivery solutions include fusing siRNAs to antibody fragments and aptamers. Alternatively siRNAs can be packaged into nanoparticles coated with receptor-targeting ligands. Viral delivery systems for RNAi have been developed, but there are associated to safety issues. However, the advantage of using Lentiviral vectors is that they transduce both dividing and non-dividing cells, allowing stable shRNA expression from a construct integrated into the host genome. Vectors derived from adenoviruses (or AAVs) are used for more transient expression of shRNAs in therapeutic strategies against cancers and other diseases in which long-term RNAi is not desired. These non-integrating vectors largely remain episomal, and transduce both dividing and non-dividing cells. However, repeated administration of adenoviruses or AAVs can trigger strong immune responses.

Despite limitations in our understanding of how precisely small RNA species mediate TGS, they may play an important role in future therapeutic options to treat various diseases such as chronic infection and cancers. This new generation of drugs will provide siRNA-TGS-based therapies in near future based on the new concept of adapting human host machinery to control transcription through epigenetic modification to achieve prolonged gene silencing.

ABBREVIATIONS

ChIP assay	=	Chromatin Immunoprecipitation assay
EZH2	=	Enhancer of zeste homolog 2
shRNA	=	Short hairpin RNA
H3Ac	=	Histone 3 acetylation
H3K9me2	=	Dimethylation of lysine 9 of histone 3
H3K27me3	=	Trimethylation of lysine 27 of histone 3
HDAC	=	Histone deacetylase
HP1	=	Heterochromatin protein 1
HIV	=	Human immunodeficiency virus
PNA	=	Peptide nucleic acids
SIV	=	Simian immunodeficiency virus
RDRC	=	RNA polymerase complex
TSA	=	Trichostatin-A

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