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Review

siRNA-mediated transcriptional gene silencing: the potential mechanism and a possible role in the histone code

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Abstract. Epigenetics is the study of meiotically and mitotically heritable changes in gene expression which are not coded for in the DNA [1, 2]. Three distinct mechanisms appear intricately related in initiating and sustaining epigenetic modifications: RNA-associated silencing, DNA methylation and histone modification [1]. Recently, in human cells small-interfering RNAs (siRNAs) have been shown to mediate transcriptional gene silencing (TGS). The observation that siRNAs can function to suppress gene expression at the level of transcription has created a major paradigm shift in mammalian RNA interference. The putative mechanism(s) of siRNA-mediated TGS in both yeast and human cells will be discussed. Undoubtedly, the ramifications from this paradigm shift in which RNA has demonstrated a potent and specific capability to regulate the expression of the gene are immeasurable both therapeutically (i.e. directed control of gene expression) and biologically in understanding the evolution of the cell.

Key words: siRNA; RNAi; histone code; transcriptional gene silencing; DNMT3A.

Introduction

Evolution can be defined as 'the change in genetic composition of a population during successive generations, due to natural selection acting on the genetic variation among individuals, and resulting in the development of new species'. The consequences of evolution can be observed in the genomes of present-day organisms. An added layer of evolutionary complexity has emerged in our consensus of biological interpretation and is manifest during the cell cycle when not only is the genome replicated and passed on to the daughter cells but also the previous cells' gene expression profile, referred to as epigenetic inheritance [3, 4]. Epigenetics is the study of meiotically and mitotically heritable changes in gene expression which are not coded for in the DNA [1, 2]. Three distinct mechanisms appear to be intricately related and implicated in initiating and sustaining epigenetic modifications; RNA-associated silencing, DNA methylation and histone modifications [1]. It has now become clear in human cells that RNA-associated silencing can be transcriptional in nature, is operable through an RNA interference (RNAi)-based mechanism that is specifically mediated by small-interfering RNAs (siRNAs) and can silence target genes at the level of the chromatin. Undeniably these findings suggest that RNA plays a much broader and more profound role in the cell than previously envisioned. As such it is becoming apparent that RNA can actually regulate DNA and the expression profile of the genome.

RNA-associated silencing

Double-stranded RNA-induced post-transcriptional gene silencing (PTGS) is known as RNA interference (RNAi) in animals, as quelling in the filamentous fungus Neurospora crassa [5–7], and was first described in plants and termed co-suppression (reviewed [7]). RNAi is a process in which double-stranded RNA (dsRNA) induces homology-dependent degradation of messenger RNA (mRNA) [8–10]. The process of RNAi involves small interfering double-stranded RNAs (siRNAs), 21–22 bp in length, with 3' overhanging ends that can induce a homology-dependent degradation of the cognate mRNA [9]. It has been argued that the regulation of gene expression via an RNAi-mediated pathway is essentially a form of cellular based innate immunity used to protect against transposable elements or retrotransposons and RNA viruses [11–13].

The generation of siRNAs is the result of a multistep process that involves the action of Rnase III endonuclease known as Dicer [14–16] (fig. 1). The Dicer-processed ~21 bp siRNAs (reviewed in [17]) provide much of the specificity in the silencing process. Following the action of Dicer, the ~22 bp siRNAs are incorporated into the RNA-induced silencing complex (RISC), which identifies and silences by slicing the mRNAs complementary to the siRNA through interactions with Argonaute 2 [18–23] specifically in P-bodies located in the cytoplasm [24] (fig. 1). Precisely how siRNAs are able to localize with the RISC complex to the target mRNA still remains to be determined.

RNA-associated silencing

RNAi can also suppress gene expression through a transcription-mediated pathway: transcriptional gene silencing (TGS) [25]. TGS was first observed when doubly transformed tobacco plants exhibited a suppressed phenotype of a transgene. Careful analysis indicated that methylation of the targeted gene was involved in the suppression [26]. TGS mediated by dsRNAs was further substantiated in viriod-infected plants [27] and was shown to be due to RNA-dependent DNA methylation (RdDM). The RdDM requires a dsRNA to target DNA and is subsequently processed to yield short RNAs [27,

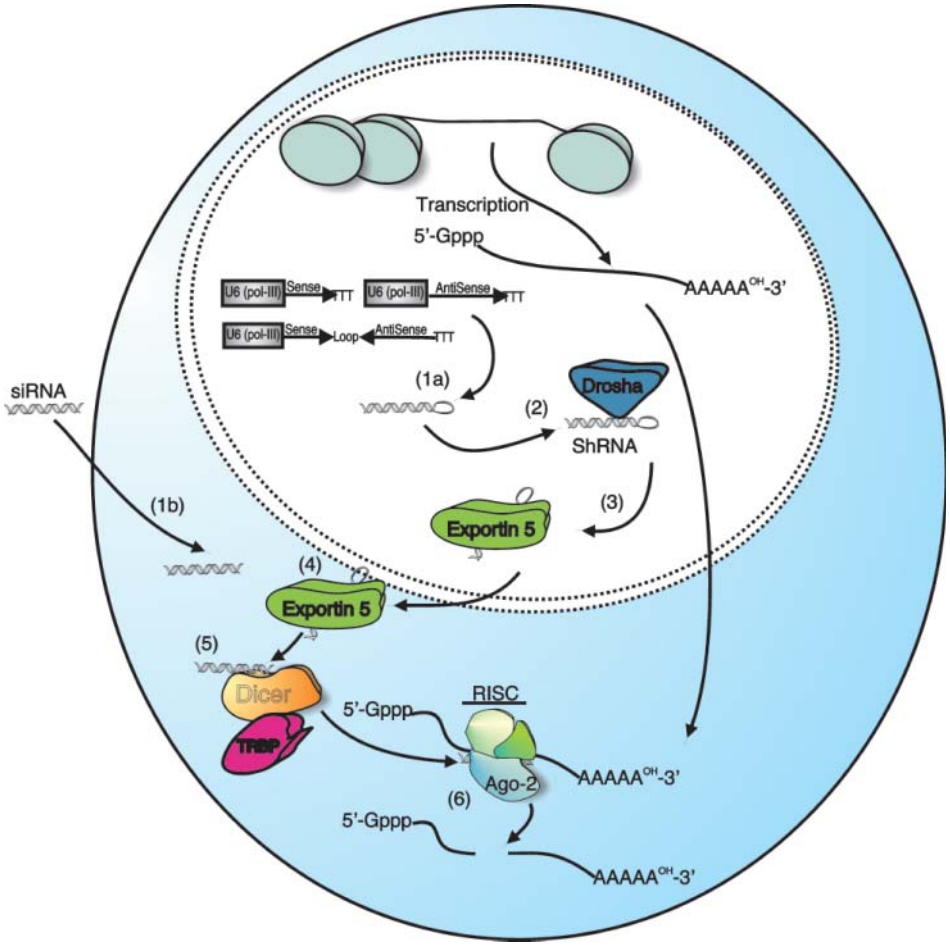

Figure 1. *PTGS pathway in mammalian cells.* RNAi is operative at the post-transcriptional level when either the synthetic siRNAs are directly transfected into the target cell (**1b**) or stably transduced with a lentiviral vector that expresses siRNAs either from 2 independent promoters (U6 Pol-III) or a single promoter driving the expression of a hairpin shRNA targeting a particular gene of interest (**1a**). The expressed siRNAs are probably bound and processed (dicer substrage, 3' end) in the nucleus by Drosha (**2**) and then exported out of the nuclues by Exportin 5 (**3**) (Lee, Ahn et al. 2003; Lund, Guttinger et al. 2004), and handed off to Dicer (**4**) which then cleaves the loop from the hairpin (**5**) producing the siNA that is then loaded into RISC ultimately leading to slicing of the target mRNA (**6**) essentially driving PTGS.

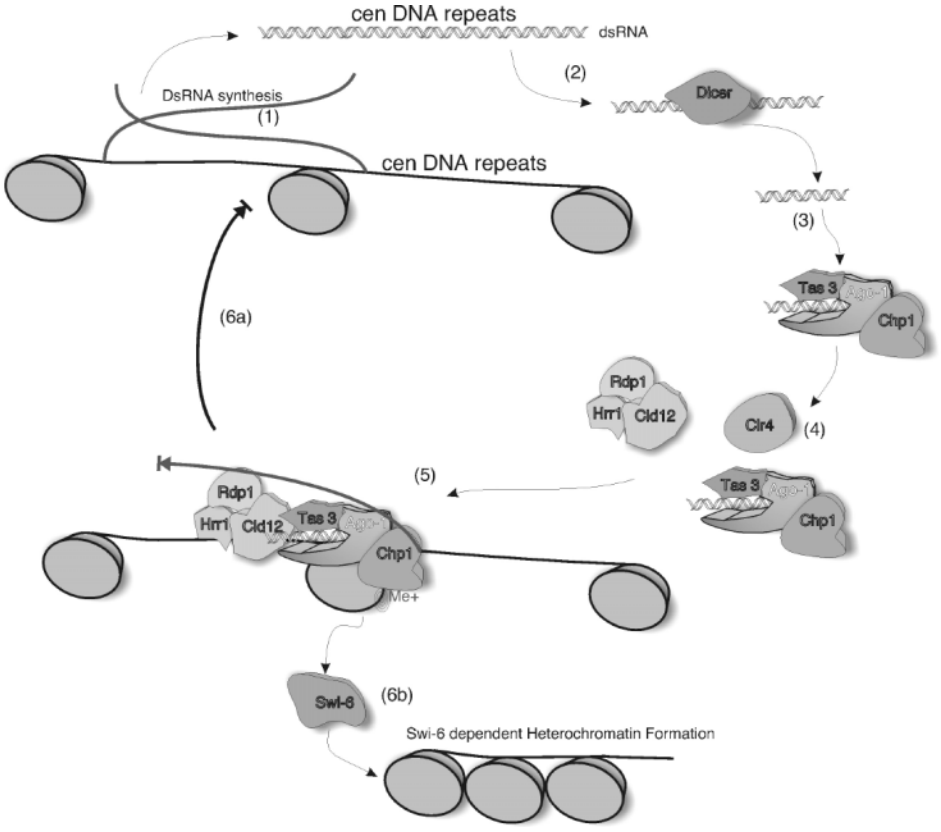

Figure 2. *dsRNA-mediated TGS in S. pombe.* dsRNAs are generated from bidirectional transcription of the centromeric DNA repeats [*cen* DNA repeats, (1)], which are then processed by Dicer (2) to siRNAs (3) and loaded into the RITS complex (4). The RITS complex then interacts with the RNA-dependent RNA polymerase complex [RDRC, (5)], which leads to H3K9 methylation (5) and silencing of the *cen* DNA repeats (6a) and/or swi-6-dependent heterochromatin formation (6b). This figure and proposed model was adapted from [33].

28]. These short dRNAs happened to include sequences that were identical to genomic promoter regions and in turn proved capable of inducing methylation of the homologous promoter and subsequent transcriptional gene silencing. Transcriptional gene silencing in plants has been shown to be carried out by a generally larger size class of siRNAs, 24 26 nt in length [29, 30]. These data suggest that longer siRNAs are involved in RdDM, how applicable the size classes are in other organisms has not yet been determined.

To date the greatest amount of investigation on RNAi, specifically TGS in eucaryotic systems, has been done on *Schizosaccharomyces Pombe.* RNAi-mediated TGS in *S. pombe* has been implicated in regulating heterochromatic silencing through histone 3 lysine 9 methylation (H3K9) [31]. In *S. pombe* mutants in *dcr1* (Dicer homolog) and *ago1* (Argonaute homolog) were shown to be reduced in centromeric repeat H3K9 methylation, which is necessary for centromere function [31]. These data denote a link between siRNA-specific targeting of histone modifications to specific genomic sequences which subsequently recruit or interact with Swi6, resulting in regulation of the heterochromatic state [31]. Additional investigation demonstrated that the *dcr1* processed dsRNAs, which correspond to centromeric repeats in *S. pombe*, interact with *ago1*, Chip1 (chromodomain protein) and Tas1 (previously uncharacterized) to form the RITS complex [32]. The RITS complex, specifically *ago-1* and Chp1, then associate with chromatin binding factors Swi6 and Clr4 (Suv39H6 homolog) to silence targeted genomic regions [32]. The presence of siRNAs in the RITS complex was shown to require Rdp1, *Hrr1* (*helicase required* for *RNA*-mediated heterochromatin assembly *1*) and Cid12 (a 38-kDa protein involved in mRNA polyadenylation) [33]. Consequently, in *S. pombe* siRNAs generated from centromeric regions can trigger silencing of the centromeric region as depicted (fig. 2) based on models proposed [33].

Recently, siRNA-mediated TGS in *S. pombe* has also been shown to require RNA polymerase II (RNA Pol-II), suggesting that transcription of the homologous target is required to intiate TGS [34, 35]. The observation that RNA Pol-II is involved in siRNA-mediated TGS suggests two potential models. The first model would be that there is an RNA Pol-II-expressed transcript homolgous to the targeted gene which somehow remains associated with the gene, possibly affiliated with the nucleosome, and as such allows for the RNAi machinary to direct chromatin modifications of the targeted genomic region, ultimately leading to TGS. This siRNA/RNA model could be envisioned to act as a local 'address' to allow chromatin and RNAi modification complexes, guided by siRNAs, access to the targeted gene (fig. 3A). Supporting this siRNA/RNA model is the observation that

in *S. pombe* T7-mediated transcribed genomic regions were not susceptible to siRNA-mediated TGS while RNA Pol-II regions were [35], and the observation that heterochromatin formation in mouse cells involves HP1 proteins and treatment with RNase causes a dispersion of heterochromatin-associated protein (HP1) from pericentromeric foci [36].

An alternative model, the siRNA/DNA model, could be envisioned to operate by RNA Pol-II essentially unwinding the targeted DNA and allowing the siRNA and RNAi machinary access to the targeted promoter (fig. 3A). Supporting this model is the observation that RNA Pol-II has been shown to associate with complete unfolding of 1.85 out of 3 nucleosomes upstream of the transcription start site for the PH05 promoter [37], and the siRNA EF52 target site, shown to initiate TGS in human cells, is ~1 nucleosome upstream of the TATAA transcriptional start site [38]. Moreover, RNA Pol-II is associated with nearly 60 subunits and a mass in excess of 3 million Da, including the cresent-shapped Mediator, which essentially envelopes the RNA Pol-II core subunits [37]. Overall, these reports suggest, at least in human cells, that a good hypothesis for the role of RNA Pol-II in siRNA-mediated TGS is to essentially open up the targeted promoter to allow the promoter-directed siRNAs access to their respective target.

siRNA-mediated TGS in human cells

While dsRNAs can induce sequence-specific methylation of DNA in plants and histone methylation in yeast, regulating gene expression at the transcriptional level, it was not known until recently how applicable this phenomenon was in mammalian cells. In human cells, gene silencing induced by RNAi was initially thought to be restricted to action on cytoplasmic mRNA or RNA at the nuclear pore [23], similar to most reports in *Caenorhabditis elegans* and *Trypanosoma brucei* [5, 8, 39]. Recent reports have, however, documented that siRNAs targeted to three different genes, specifically the promoter regions, can induce transcriptional silencing via DNA methylation in human cells [38, 40, 41]. The role that DNA methylation plays in the observed silencing is questionable, as siRNA-mediated TGS has been shown to occur in the absence of increased DNA methylation at the targeted promoter [40, 42], while others find that siRNA or antigene RNA mediated TGS correlates with increased histone methylation [6, 43].

A priori one would assume that the siRNAs must access the nucleus as well as the targeted promoter for gene silencing to occur, and indeed that proved to be the case with synthetic siRNAs [38]. There may, however, exist a mechanism in human cells that actively transports the promoter-targeted siRNAs to the nucleus, as synthetic and tRNA vector-based siRNAs, which are predominantly localized in the cytoplasm, were efficacious at targeting the oncogene erbB2 promoter and inducing CpG-specific methylation [41], and short hairpin RNAs (shRNAs) directed to the RASSF1A promoter were capable of inducing DNA methylation [40]. Such a mechanism may involve an Argonaute-related complex [44], as Argonautes have been shown to localize in the nucleus and are involved in siRNA-mediated TGS in *S. pombe* [32, 33, 35]. However, the mechanism by which the promoter-directed siRNAs are guided to and gain access to genomic DNA in human cells remains unknown. It is possible that a small fraction of an uncharacterized siRNA-protein complex might be transported to the nucleus, similar to what has been observed in plants [45, 46], or alternatively, siRNAs might gain access to genomic DNA during cell division, when the nuclear membrane disappears. Moreover, it is currently unknown in human cells whether siRNA-mediated TGS is operable through an RNA/RNA intermediate [i.e. siRNA recognizes a long transcript which overlaps with the targeted promoter (R. Allshire, personal communication [35] and fig. 3B] or whether the siRNAs interact directly with the targeted promoter in an RNA/DNA-dependent fashion (fig. 3B). What is known about the mechanism of siRNA-mediated TGS in human cells is that it involves DNA methyltransferase 3b (DNMT3b) [41]. In addition, recent unpublished data suggest that in human cells DNMT3a is also involved and co-localizes with one strand of the targeted promoter siRNA, a silent-state histone methylation mark at the targeted genomic region, and that the mechanism of action requires RNA Pol-II [K.V. Morris unpublished data] (fig. 3B).

DNA methylation (the DNA methyltransferases)

The DNA methyltransferase family of proteins [DNMT1, 3a and 3b (fig. 4)] function to establish and maintain genomic methylation patterns which are established during embryogenesis. Specifically, the DNA methyltransferases add a methyl group to the 5 -carbon of a cytosine located proximal to a guanine (5 -CpG-3). Deletion of any of the three major DNMTs proves detrimental to murine development [47]. DNMT1 is thought to function in maintaining the set methylation patterns (i.e. maintenance methyltransferase), while DNMT3a and -3b are thought to function primarily as de novo DNA methyltransferases (reviewed in [48]). DNMT3a and 3b map to chromosomes 2p23 and 20q11.2, respectively, with DNMT3a expression being relatively ubiquitous and DNMT3b expression being relatively limited to low-level expression in most tissues [49]. Both DNMT3a and -3b share 98 and 94% amino acid and sequence homology, respectively, with the COOH terminal catalytic do-

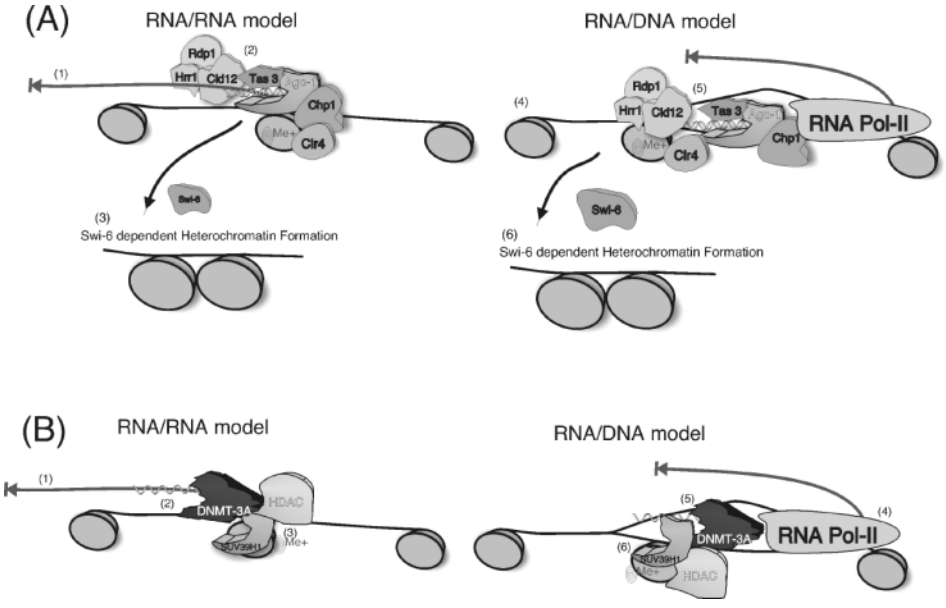

Figure 3. *Proposed mechanisms for TGS in S. pombe and humans.* Two models for siRNA-mediated TGS are proposed here, either an RNA/RNA or an RNA/DNA-mediated mode of silencing. In S. *pombe* (A) the siRNAs may interact with a long non-coding transcript which spans the targeted chromatin (1), subsequently allowing the RITS/RdRP complex to localize to the targeted region, (2) resulting in gene silencing (3) [proposed by R. Allshire, personal communication]. Alternatively, siRNA-mediated silencing may function through an RNA/DNA intermediate. The siRNAs may gain access to the targeted DNA by the effects of RNA Pol-II opening up the targeted region (4) for the siRNA/RITS/RdRP complex to gain access (5), leading to gene silencing (6). In human cells (B) only one strand from the initial promoter-targeted siRNA [K.V. Morris, unpublished data] may be targeted to a low level expressed non-coding transcript (1), subsequently allowing a complex containing the siRNA, DNMT3A, HDAC and Suv39H1 to localize to the targeted chromatin (2), leading ultimately to histone methylation and initial silencing of the targeted promoter (3). Alternatively, the single strand of the siRNA may gain access to the targeted promoter by the effects of RNA Pol-II on the targeted promoter (4), resulting in the recruitment of the siRNA/DNMT3A/HDAC/Suv39H1-containing complex (5), leading to histone methylation and silencing of the targeted chromatin (6).

main remaining relatively conserved and the NH_3 terminus containing the majority of variability [49]. The role of this variable NH_3 region remains to be determined but might somehow be involved with a yet-to-be determined RNA-mediated transcriptional silencing complex. Such a complex might be envisioned to contain an argonaute, similar to findings in S. pombe [35].

DNA methylation predominates on CpG dinucleotides in the mammalian genome, specifically in regions containing many repetitive elements, and is thought to function to silence these repetitive elements, essentially serving as a host defense system to reduce transposition [50]. DNA methylation can directly prevent binding of some transcription factors to their respective DNA binding sites and thus subsequently inhibit transcriptional activity of the methylated genomic region [51]. While DNA methylation can impair transcriptional activation it is apparent that the state of the chromatin also plays a role in transcriptional activity [52]. The question is, Which comes first in silencing? DNA methylation or

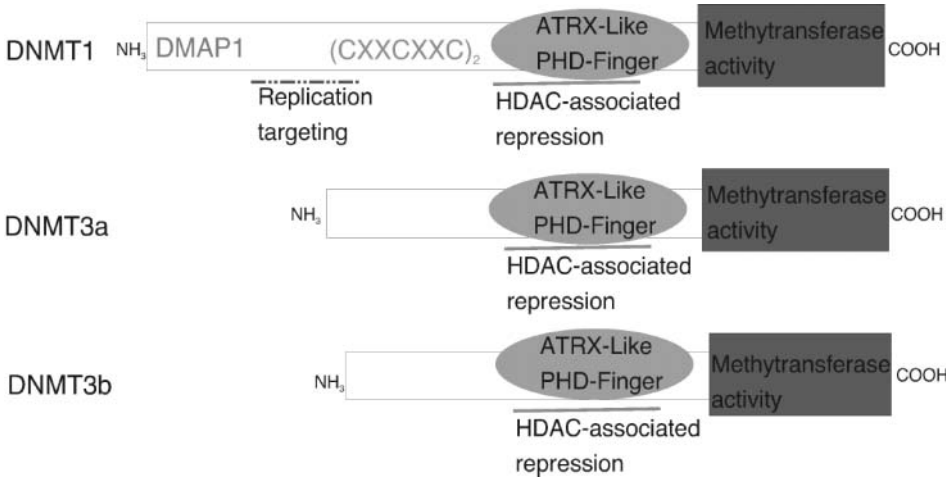

Figure 4. *Comparative analysis of DNA methyltransferase 1, 3a and 3b.* The DNA methyltransferase active site is shown in bold in the COOH terminus of each DNA methyltransferase (DNMT). DMAP1 refers to the binding region for the transcriptional co-repressor protein, which is 5' of the PCNA binding site shown as replication targeting. The $CXXCXXC_2$ cysteine-rich zinc-binding motif of DNMT1 is also shown, as well as regions involved in HDAC-associated repression shown as (HDAC-associated repression).

histone modifications affecting the chromatin structure [53]. Alternatively, there may be an amalgamation of both DNA methylation and histone modifications occurring simultaneously. Indeed, there is evidence that both DNA methylation and chromatin-modifying complexes work in tandem, as DNMT3a and -3b have both been shown to repress gene expression in concert with histone deacetylase (HDAC) activity through interactions with the ATRX domain (fig. 4) [54]. Interestingly, the modulation by sumoylation of DNMT3a, via the action of SUMO-1, has been shown to regulate the interaction of DNMT3a with HDAC1/2 [55]. Of particular note is the fact that DNMT3a copurifies with HDAC1, and the purified fractionations contain histone methyltransferase activity that is specific for H3K9 [56], whereas DNMT3b copurified with DNMT1 and contained fractions lacking histone methyltransferase activity [56]. These data suggest that DNMT3a might not only function as a de novo DNA methyltransferase but also participate in complexes which are involved in histone modifications, specifically histone deacetylation and methylation.

Importantly, the observed siRNA-mediated TGS in human cells not only induced silencing of the targeted gene but also H3K9 methylation [41, 42]. The methylation of H3 can occur on lysines (K) 4, 9, 27, and 36 and as such generate an opportune environment for gene silencing [57]. Interestingly, the addition of 5-azacytadine (5-AzaC) and trichostatin A (TSA) completely reversed the observed siRNA-mediated TGS in human cells [38, 43]. Indicating that histone and DNA modifications both play pivotal roles in the observed siRNA-mediated TGS, similar to work carried out with reactivation of aberrant CpG-methlylated genes in cancer [58]. Taken together, the mechanism of siRNA-induced TGS seems to be far more complicated and might actually involve a chromatin-remodeling complex that also contains DNMT3a, at least at some stage of the silencing. Regardless, these data along with Dicer knockouts [59, 60] strongly suggest that RNAi is involved with heterochromatic silencing in mammals, similar to observations in plants and *S. pombe* [61].

Histone modification

Chromatin is composed of nucleosomes which consist of ~146 bp of DNA wrapped around an octamer of histones (i. e. two copies of each histone, H2A, H2B, H3 and H4) [62]. Histones are basic proteins containing a globular domain and an N-terminal tail that protrudes from the nucleosome. It is these protruding tails which can serve as targets for covalent post-translational modifications such as acteylation, methylation, phosphorylation, sumoylation and ubiquitination (reviewed [70]). The acetylation of histone tails by histone acetyltransferase (HAT) results in the relaxation of the chromatin, a disruption of histone-DNA interactions and gene activation. Conversely, the deacetylation of histones by HDACs results in condensation of the chromatin and transcriptional repression (reviewed in [64]). The HDACs are divided into three classes: class 1 HDACs (HDACs 1, 2, 3 and 8), class 2 HDACs (HDACs 4, 5, 6, 7, 9 and 10), and class 3 HDACs, which form an NAD-dependent enzyme class similar to yeast SIR2 (reviewed in [63]). HDACs have been shown to be involved in transcription, cell cycle progression, DNA replication, gene silencing and cellular differentiation (reviewed in [63]). Of particular note are the class 1 HDACs, as they contain functional catalytic domains which have been implicated in complexes directly linked to gene-specific regulation.

The histone modifications resulting from HAT or HDAC activity have been proposed to assist in the formation of various combinations of epigenetic marks on the histone tails, ultimately resulting in a 'histone code'. These 'marked' histone tails are then capable of directing the recruitment of various specialized chromatin-remodeling factors [65, 66]. One important histone modification is the dimethylation of H3K9, which has been described to occur in fission yeast by means of Clr4 [67]. Interestingly, H3K9 and H3K27 methylation have been mapped to regions encompassing promoters of silenced genes on the mammalian inactive X chromosome [68]. In mammalian cells H3K9 methylation is directed by Suv39H1 [69]. H3K9 methylation directly recruits Swi6/HP1, and this recruitment coincides with spreading in H3K9 methylation in *cis* [70]. The chromodomain of HP1 binds to methylated H3K9 [71]. In human cells HP1 associates with HDACs through interactions with methylated lysines in the histone tail and this interaction mediates transcriptional repression by the recruitment of histone methyltransferases [72]. The histone methyl-lysine residues recognized by HP1 also serve as substrates for deacetylation by HDACs, subsequently allowing for regulation of HP1 and its association with a variety of transcriptional repressors [73–75]. Consequently, the removal of the acetyl group from H3K9 by HDAC allows for H3K9 methylation to occur, which permits interactions with HP1. HP1 can then associate with many various transcriptional regulators, ultimately resulting in an epigenetic mark and subsequent regulation of local gene expression.

Proposed model for siRNA-mediated TGS: writing the histone code

A model for the putative mechanism of siRNA-mediated TGS in human cells is emerging and is based both on published works as well as recent unpublished experimental evidence. The model is proposed to operate temporally as

(i) the siRNA is bound to a complex that already contains or recruits DNMT3a (K.V. Morris unpublished data and [76]), DNMT3b may also be involved in this complex [44]. This resultant complex can then (ii) be directed by the siRNA to the targeted promoter leading to promoter site recognition. Next, (iii) the DNMT3a and siRNA containing complex may contain or recruit HDAC-1 and Suv39H1 [56, 77, 78] which could (iv) lead to the removal of the acetate and subsequent methylation of H3K9 [41, 42] and/or H3K27 [K.V. Morris, unpublished data]. The result of H3K9 and/or H3K27 methylation is the suppression of the particular targeted gene expression (fig. 5). Finally, if the gene silencing is re-enforced and positively selected for by the cell, then DNA methylation and permanent silencing of the siRNA-targeted gene may ensue. Interestingly, HDAC-1, DNMT3a, siRNAs and the NuRD chromatin-remodeling complex [56, 76, 79] can all be linked, indicating one potential pathway to siRNA-mediated TGS in human cells.

Once the siRNA has localized the putative complex to the targeted promoter, other chromatin-remodeling and/or gene regulatory complexes might then work in concert to further remodel the targeted genomic region (fig. 5). Currently, there are six well-defined gene regulatory HDACs that appear to be involved in the suppression of gene expression (reviewed [80]). Of these six complexes, the nucleosome-remodeling HDAC (Mi2/NuRD), which contains retinoblastoma (Rb), HDAC1&2, the histone methyltransferase (Suv39H1) and possibly HP1, appears immediately intriguing with regard to possible mechanism(s) underlying siRNA-mediated TGS in mammalian cells.

In the MI2/NuRD complex, H3K9 methylation has been shown to be involved in X inactivation in the mouse [81] and the recruitment of HP1 [31, 70]. Methylation of H3K9 creates a binding site for HP1 [73]; notably, H3K9 was found to be methylated in promoter-specific siRNA-treated cultures [41, 42]. HP1 is an adaptor protein that recognizes methylated lysines within the histone tail and mediates transcriptional repression by recruiting histone methyltransferases [72]. Importantly, HP1 contains a hinge region that has been found to exhibit RNA binding activity, and HP1 binding to chromodomains seems to require both an intact chromodomain as well as an RNA binding component [36]. The localization of H3K9 methylation and HP1 to pericentromeric heterochromatin in

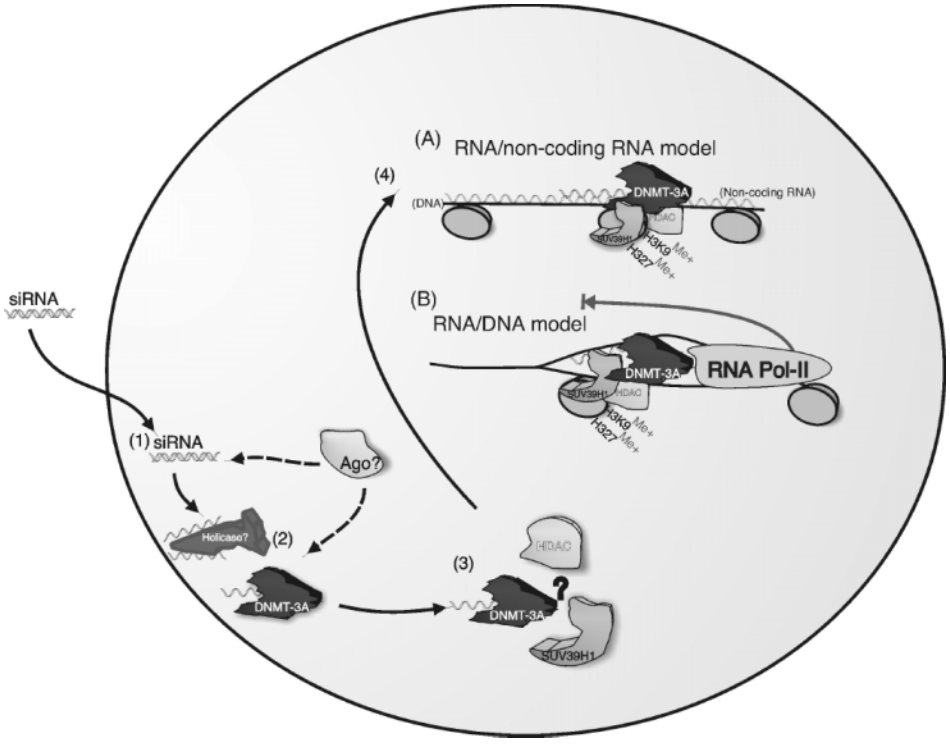

Figure 5. *Model for siRNA-mediated TGS in human cells.* SiRNAs are introduced by nuclear specific MPG-based transfection [37] into the target cells (1). Once inside the nucleus the siRNA is presumably unwound, and only one strand is bound by a complex that also contains DNMT3a [K.V. Morris, unpublished data (2)] [DNMT3b may also associate with this complex [41, 76] and (K.V. Morris, unpublished observation)]. Next, the single strand from the original duplex siRNA/DNMT3a-containing complex may interact directly or already contain HDAC1 and/or Suv39H1 [77, 78] (3). The siRNA probably then directs the DNMT3a or 3b-containing complex with or without the HDACs and/or Suv39H1 to the targeted promoter region, possibly via an interaction with a non-coding transcript that is associated with the targeted chromatin (RNA/RNA model) or with the targeted DNA when access is gained as the result of RNA Pol-II (RNA/DNA model) (4) where HDAC can deacetylate the respective histones (H3K9 and/or H3K27). The deacetylation of H3K9 and H3K27 would then permit Suv39H1 to methylate H3K9 and possibly H3K27, resulting in initial silencing of transcription (5). If the silencing is reinforced, the gene may become methylated and permanently silenced. It should also be noted that Argonaute 2 (Ago-2) may also be involved in the observed promoter-specific silencing through the proposed pathway or via an alternative yet-to-be-described pathway.

the mouse is abolished by RNase treatment, suggesting an RNA component is involved in chromatin remodeling as well as gene silencing that has been overlooked or not yet suitably characterized. Indeed, in *S. pombe* RNA Pol-II function is required to initiate siRNA-mediated TGS [35]. In addition, treatment of cells with HDAC inhibitors such as TSA results in the disruption of heterochromatin and loss of HP1 binding [82]. Interestingly, HP1 forms a complex with Rb and Suv39H1. The histone methyltransferase Suv39H1 not only binds HP1 but also DNMT1 and DNMT3a [77, 78]. Finally, in *S. pombe* there is a link between siRNA and chromodomain-containing proteins which are homologs for SUV39H1 and HP1 [31]. This is extremely intriguing, as the Mi2/NuRD complex could be envisioned to associate with siRNA through interactions with HP1, DNMTs or an unknown factor and selectively target or enhance the tethering of the complex to chromatic regions containing the promoter targeted by the siRNAs, thus regulating gene expression.

In the end, it might very well be that the siRNA interacts with one of or an amalgamation of the five other known complexes not mentioned here or that the promoter-directed siRNAs are bound to an undefined complex containing one or more of the argonaute proteins. Undeniably in plants, argonaute 4 is actively involved in DNA and histone methylation [30] and argonaute 2 has been shown to be the functional slicer in RNAi in mammals [20, 83] and is involved with RNA Pol-II in TGS of *S. pombe* [35].

Conclusion

Clearly, the role of an RNAi mechanism in protecting the cell from virus infection appears conserved throughout the various kingdoms. The recent discovery in human cells that siRNAs can modulate gene expression in a transcriptional fashion [37, 40–43, 84], when further substantiated with other promoters being targeted, will undoubtedly lead to a paradigm shift in the way we view the cell. In light of these seminal papers, it is now possible to literally connect siRNA with two pathways involved in direct control over the human genome, the DNMT and HDAC pathways. Importantly, the observation that siRNAs can essentially regulate DNA and that this regulation occurs through epigenetic modifications is pivotal in our understanding of gene regulation and has a direct impact on the potential theoretically to treat diseases involving the deregulation of certain genes. Moreover, the observation that siRNAs direct histone modifications, such as H3K9 methylation in the regulation of DNA, suggests that siRNAs might in fact function as mediators involved in writing the histone code and subsequently regulating gene expression. Overall, the implications of siRNA-mediated control of DNA suggest that RNA plays a pivotal and underappreciated role in regulating the cell that, once fully understood, could conceivably be used to treat human diseases resulting from gene dysregulation.

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