2017

Activating the Chromatin by Noncoding RNAs

Laham-Karam Nihay

Mary Ann Liebert Inc

http://dx.doi.org/10.1089/ars.2017.7248

ARS FORUM REVIEW ARTICLE

Activating the chromatin by non-coding RNAs

Nihay Laham-Karam[1], Pia Laitinen[1]*, Tiia A. Turunen[1]*, Seppo Ylä-Herttuala[1,2,3]

[1] A.I. Virtanen Institute, University of Eastern Finland, Kuopio, Finland

[2] Heart Center, Kuopio University Hospital, Kuopio, Finland

[3] Gene Therapy Unit, Kuopio University Hospital, Kuopio, Finland

* These authors contributed equally to the work.

Running title: ncRNA activating the chromatin

Correspondence should be addressed to N.L-K. / S.Y-H.

Address: Yliopistonranta 1E, 70210 Kuopio, Finland

Phone: +358 40 355 3292

Email: nihay.laham-karam@uef.fi / seppo.ylaherttuala@uef.fi

Key words: non-coding RNA, chromatin, transcriptional activation, siRNA, miRNA, lncRNA, piRNA

Word count (excluding references and figure legends)- 8,095 (abstract 250)

Reference numbers- 168

Number of greyscale illustrations- 3

Number of colour illustrations- 3 (online 1 and hardcopy 2)

Abstract

Significance: The extent and breadth of transcription has recently been uncovered and this has revealed an extensive array of non-coding RNAs (ncRNAs). The biological role and significance of these ncRNAs have been realised and to date it appears that ncRNA may have many important regulatory functions. ncRNAs are multifaceted and they induce a complexity of different types of transcriptional and post-transcriptional regulation including gene activation.

Recent Advances: Association of ncRNAs with gene activation is an important finding. Not only enhancer RNA (eRNA) but other types of ncRNAs, including small RNA (sRNA), long non-coding RNA (lncRNA), micro RNA (miRNA) and PIWI-associated RNA (piRNA) have also been implicated in gene activation. Interestingly, they often coincide with histone modifications that favour an open chromatin. In addition, these ncRNAs can recruit key factors important for transcription including RNA polymerase II. They may directly bind the genomic DNA or act as scaffolds, alternatively they may loop the chromatin to enhance transcription.

Critical Issues: Although the role of small activating (sa)RNAs has been considerably studied, the roles of miRNAs and piRNAs in gene activation still need to be substantiated and issues of specificity require further studies.

Future Directions: The ncRNA field is coming out of its infancy and we are gaining a global picture of the importance of ncRNAs. However, detailed mechanisms of action of the different ncRNAs are still to be determined. This may reveal novel ways of transcriptional regulation which will facilitate our ability to utilise these regulatory pathways for research and therapeutic purposes.

Introduction

With the advent of high throughput genomic technologies, our knowledge of the transcriptional landscape has changed, such that we now recognise that the majority of the genome is transcribed (28,29). This has also altered our view of the RNA world and non-coding RNAs (ncRNAs). Different types of ncRNAs have been defined, mainly based on their size and some according to their function (see table 1). These include micro RNA (miRNA), enhancer RNA (eRNA), PIWI-interacting RNA (piRNA), small RNA (sRNA), long non-coding RNA (lncRNA) and promoter-associated RNA (paRNA). These ncRNAs (Figure 1) can arise from different parts of the genome, either from unidirectional or bidirectional transcription, as is the case for eRNA and paRNAs, and have variable stability and longevity [reviewed in (48,111,118,120)]. Interest in the ncRNAs has grown with increasing evidence of functionality and biological relevance. The ncRNAs are all found in the nucleus but not exclusively, for example miRNA and siRNA also have their effects in the cytoplasm and some lncRNA can also be found there. The nuclear localisation and level of expression of ncRNAs has supported a regulatory role for these RNAs. The type and means of ncRNA regulation are still being defined and although ncRNAs can often impact negatively on gene expression, as is often the case with miRNA, supporting evidence has also been accumulated that suggests a role for ncRNA in activation of gene expression. This has mainly been due to induced changes at the chromatin that allow for increased transcription. In this review, we will concentrate on ncRNAs that activate gene expression, including sRNA, lncRNA, eRNA, piRNA and miRNA. We will present evidence of their involvement in gene activation and discuss what is known to date about the mechanisms of their activity.

Small RNA

sRNAs are short RNA sequences of 19-27 nucleotides (nt). The most well-known of these are the small interfering RNA (siRNA), which are processed from perfectly based double-stranded RNA (dsRNA) by DICER ribonuclease III (DICER) within the RNA interference (RNAi) system (Figure 2; (30)). In the cytoplasm, the siRNA associate with Argonautes (AGOs) and they silence gene expression by degrading perfectly complimentary target messenger RNAs (mRNA). The siRNAs can also be delivered from exogenous sources and this has been widely used to regulate gene expression. Although the siRNAs mainly function in the cytoplasm, sRNAs can also have nuclear targets which are often associated with gene.

Activity of saRNA *in vitro* and *in vivo*

sRNAs targeting promoters were first observed in plants (84) and subsequently in *Drosophila* (98) and mammalian cells (58). The first reports demonstrated that when expressed (84,98) or introduced into the cell (87), these promoter-targeted sRNA inhibited gene expression and this was termed transcriptional gene silencing (TGS) and distinguished it from the post-transcriptional gene silencing (PTGS) mediated by siRNA targeting the mRNA. However, already in 2004 Kuwabara et al (58) identified a small non-coding dsRNA that could activate neuron-specific genes at the transcriptional level and this was followed by Li et al (69) demonstrating activation of gene expression by delivery of sRNA targeting different gene promoters. These sRNA are now often referred to as small activating RNA (saRNA). This ability of saRNA to increase gene expression has now been reported for many different genes including those of the vascular endothelial growth factor (VEGF) family (59,69,131), receptors (39,44,79,122), transcription factors (39,109,137,144,145), cancer related genes (16,39,69,70,99,104,108,161) and other biologically important genes (148,157,158; summarised in Figure 3). Likewise, saRNA have been tested in cell lines from different

species and found to activate gene expression (39), suggesting conservation of this system in mammalian cells. Although, some saRNA can be active in various cell types, others demonstrate differential activity (69,80,131). The differential activity of these saRNA is likely to be a result of distinct pre-established methylation states of the gene in question, since the responsiveness of a cell line to a saRNA can be altered with chemically-induced demethylation of the cell (69,130).

The observed range of activation by saRNAs has on average varied between 2-15 fold depending on the gene promoter targeted. Recently even higher activation has been demonstrated for a type I promoter, indicating that there might be differences in the level of activity depending on the type of promoter that is targeted (141). Often the activation has been observed on both the mRNA and protein level but not always (59). This observation is in accordance with recent estimates that only approximately 40% of the protein variance is explained by mRNA expression and that the protein translation rate is the dominant factor in the protein variance (121), and is likely to be due to the interplay of other post-transcriptional and translational regulation. An important feature of promoter targeted saRNAs is that they are able to increase the expression of multiple isoforms of a gene as has been demonstrated for VEGF-A (69,130), thereby maintaining the physiological equilibrium of perhaps functionally distinct isoforms.

Functional output of saRNA

Importantly, saRNA-mediated increased gene expression has been correlated with functional outputs. Specifically, saRNA regulating cancer-related genes have reported decreased viability (55,69,105,145), inhibition of proliferation (16,156,158), invasion and/or migration of cancer cells (145,158). Alternatively, saRNA have been used to decrease resistance of cancer cells to treatment (70,157). In addition, following saRNA-mediated increase of Progesterone receptor (PR) (44), VEGF-A (34) or transcription factors (eg Krupple-like factor [KLF4] or Myc) (137,149), activation of downstream genes of the targeted gene product were also observed.

In vivo studies of saRNA have also been encouraging in the context of functional activation. Turunen et al (131) first demonstrated that activating small hairpin (sh)RNA targeting the mouse VEGF-A promoter were able to induce increased vascularity in a hindlimb ischemia mouse model. Likewise, the same saRNA was also effective in reducing the infarct size in a mouse heart infarction model (130). *In vivo*, functional activity has also been observed in rodent cancer models where saRNA were associated with either smaller tumour size and/or extended animal survival (50,110,165) in addition to decreased metastasis (66). Furthermore, saRNA-enhanced expression of inducible nitric oxide (iNOS) in the cavernous tissue improved intracavernous pressure in streptozotocin induced diabetic rats (148).

Targeted promoter sites and saRNA design

Some mystery still exists regarding the promoter areas that can be targeted by saRNA. Notably, not all saRNAs that have been designed have demonstrated activity in regulating gene promoters (39,59,69,161). The most successful targeting has occurred in the promoter area -1000bp to -100bp from the transcription start site (TSS), however more distal hotspots have also been reported (82). Often, known transcription factor binding sites, GC rich areas (CpG) and repeat elements are avoided in the design of the exogenous saRNA. These are intended to limit interference with known transcription mechanisms or to avoid off-

target effects that may result from targeting repeat elements. Algorithms to predict active sites have at best reported a 50% success (2). This is not surprising since shifting the saRNA sequence by as little as one base pair on the target site is enough to abolish its activity (44). A better promoter targeting efficiency will come as we gain better understandings of the mechanisms of saRNA activation.

In addition to the place of targeting, the actual sequence and chemical properties of the saRNA are also an important consideration. Some effort has gone into characterisation of the saRNA sequence requirements and kinetics (44,104,152). Although there are some similarties to siRNA design, this is by far not absolute. We and others (146), recommend the following features for saRNA design, a duplex of 19nt that has a GC content of 40 to 60% (too high GC content may hinder AGO processing of the RNA) and 5' base-pairing biased towards asymmetric thermodynamic stability. Sequence mismatches are often not tolerated in saRNA (44,149,161), particularly in the 5' end of the guide RNA where there is an absolute requirement for sequence complementarity to the targeted area (82,149). Furthermore, modifications of RNA duplexes to improve stability or resistance to endonucleases, have not been tolerated on the 5' guide strand of saRNA, however modifications including Locked nucleic acid (LNA) and 3'biotin as well as 2'F and 2'-O-methyl of the saRNA backbone were tolerated to varying degrees (79,82,104,149,152).

Since saRNAs can be generated as duplexes then either the sense or the antisense can be chosen as the guide strand to mediate activity, whereas the passenger strand is targeted for degradation. Examples of both usage of sense and antisense strands have been shown mainly through mutation and loss of function assays (149). The choice of the guide strand is thought to depend on its thermodynamic nature, favouring an asymmetric stability of the RNA (8). In particular, AGO2 loading is dependent on the comparative thermodynamic nature of the RNA 5' and 3' ends, such that saRNA with lower stability at the 5' end relative to the 3' are preferentially loaded. Recently, Portnoy et al (106) correlated the loading of either strand to their 5' thermodynamic stabilities. However, these same authors demonstrated that loading into AGO2 did not guarantee activity, indicating that other factors influence the choice of the active strand as well.

Mechanisms of saRNA activation

More information about the mechanisms of saRNA activation have appeared in the literature in recent years (summarised in Figure 4). saRNAs have demonstrated some dependence on RNAi factors, those required for RNA processing and loading. In particular, they have been associated with a requirement for AGO, especially AGO2 (18,69,104,122,131). AGO2 has not only been localised to the nucleus (3,18,31) but importantly AGO2 enrichment at saRNA target site has been demonstrated, indicating the possibility that saRNA guides the AGO2 to the promoter region (37,82,106). AGO2 has also been shown to associate with either the promoter DNA or the corresponding antisense transcripts (18,106,122). Interestingly however, AGO2 cleavage activity, that is its main function in RNAi, does not appear to be necessary for saRNA activation (18,78,79,122). AGO2 activity at the chromatin level is still unclear. Is ncRNA loaded in AGO2 recruiting histone modifiers or is it impacting on transcriptional units? In Droshophila, it has been shown that AGO2 associates with chromatin preferentially at transcriptionally active sites and that it interacts with core transcription machinery; in particular, in conjunction with DICR 2 it can impact on RNA polymerase II (RNA polII) positioning on promoters (13). Perhaps saRNA-guided localisation of AGO2 at the promoter can affect RNA polII dynamics, and thereby saRNA alters the transcriptome.

The evidence to date supports transcriptional activation by saRNA, and as such the chromatin needs to be in a state amenable for transcription, that is in an 'open' state. Chromosomal DNA is normally wound on histone comprising nucleosomes, different modifications of the histones determine the level of interaction between the DNA and the nucleosome and subsequently the level of openness of the chromatin (reviewed in (95,102)). For transcription to occur unwinding of the chromosomal DNA must take place and as such this is associated with specific histone modifications. These types of modifications of epigenetic marks at the targeted promoter site have been associated with saRNA activation. In particular, enrichment of histone modifications characteristic of active marks such as, H3K4me2/3 and H3K27ac or loss of repressive marks, such as H3K9me3 and H3K27me3 were correlated with saRNA activation (44,59,69,78,131,161). In addition, inhibition of histone methylation and deacetylation by specific inhibitors reduced the activation achieved by saRNA (44,58). However, this correlation of histone modifications with saRNA activation can be the consequence rather than the mechanistic cause. One putative mechanism that was quickly dismissed for saRNA is alterations in DNA methylation at the targeted promoter. These changes were not observed for activating sRNA (69), although changes in DNA methylation had been observed with silencing sRNA (43,83). To date, what has been lacking in the chain of evidence for saRNA mediating histone modifications is sufficient experimental demonstration of saRNA-mediated recruitment of histone modifying enzymes. Only recently, Matsui et al (78) demonstrated that saRNA activation of Cyclooxegenase-2 (Cox-2) promoter required expression of WD40 repeat-containing protein (WDR5), which is a protein scaffold to stimulate histone methyltransferase. Furthermore, Portnoy et al (106) demonstrated co-precipitation of CTR9 and polymerase associated factor (PAF)1 following saRNA activation, both of which participate in a larger complex that interacts with histone modification enzymes (168).

In addition to modifying the chromatin to allow for transcription to take place, saRNAs may also activate gene expression by recruiting transcriptional units to the promoter sites. On the whole, active saRNAs have induced enrichment of RNAPII at the targeted promoters (18,37,79,103). It was also demonstrated that this RNA polII enrichment occurs in areas at or flanking the saRNA target site (37,82,106) as well as at the proximal promoter (106) including TSS (37). Furthermore changes in the different phosphorylated forms of RNA polII have been demonstrated with saRNA activation of a targeted promoter. Specifically, RNA polII with a Ser2 phosphorylation at the C-terminal domain (CTD) residues, which is indicative of an elongating RNA polII, was found enriched at the 5'-end and throughout the gene body of a saRNA-targeted promoter. Furthermore, this corresponded to a decline in the 'paused' form of RNA polII that is RNA polII Ser5 phosphorylation (106). It was also demonstrated that the sites of RNA polII recruitment, mediated by saRNA activation, were co-localised with nucleosome depletion near the TSS or at the proximal promoter and enhancer (141). This observation led the authors (141) to suggest that AGO2-loaded saRNA induces nucleosome depletion which would then allow for RNA polII binding in those regions and subsequently increase transcription.

In addition to RNA polII recruitment to saRNA-targeted promoters, recruitment of transcription factors have also been reported, in particular NFkappaB and CAMP responsive element binding protein (CREB)1 enrichment at the COX2 promoter following activation by saRNA(78). Likewise, activation by saRNA was also associated with specific heterogeneous nuclear ribonucleoproteins (hnRNPs) either by co-precipitation studies (122) or as identified by saRNA pull-down and identification of associated proteins with mass spectrometry (37). In the later study, it was observed that the hnRNP bound to the saRNA thereby localising to the target site. Loss of function analysis demonstrated significant albeit partial dependence of saRNA activity on the presence of hnRNP (37). In similar pull-down experiments, additional factors associated with a saRNA were identified, in particular saRNA activation of the p21 promoter was also

associated with transcriptional activators CTR9 and RNA helicase A (RHA) (106). Interestingly, these factors were enriched at the saRNA promoter target site and the corresponding TSS, furthermore knockdown of CTR9 and RHA significantly affected the saRNA activity. Since RHA is a nuclear RNA/DNA helicase II, which can directly bind to DNA, recruit RNA polII and bridge to transcription factors, and RNA polymerase-associated protein CTR9 homolog (CTR9) is a component of the PAF1 complex (PAF1C) which is involved in transcription initiation and elongation, then it was proposed that saRNA facilitates the assembly of RNA-induced transcriptional activation complex (106).

Clues to the mechanism of saRNA activation might come from better knowledge of the saRNA-target molecule. A number of studies have addressed the question as to whether the saRNA bind their target site on the genomic (g)DNA or activate by binding a corresponding promoter RNA (sense or antisense; Figure 5). saRNA mutation studies (see above) have demonstrated the requirement for target-specific sequences but these of course cannot delineate if sequence recognition is occurring via gDNA or RNA binding. Likewise, induction of mutations in the gDNA of targeted sites also reduced the activity of a saRNA (82). However, this too could not differentiate the saRNA binding since gDNA mutations would consequently affect antisense RNA sequences. Nevertheless, in several studies binding of saRNA to their gDNA target sites (p21, E-cadherin and PR) was exhibited by co-precipitation of targeted-gDNA with biotinylated-saRNA (37,82,106). Furthermore, the requirement of this binding for saRNA activation of the PR promoter was verified in a cell clone that carried a mutation in the targeted gDNA binding site that resulted in both reduced saRNA activity and reduced gDNA co-precipitation with the saRNA (82). These are in contrast to previous observations of saRNA association with the PR antisense promoter transcript and not the PR promoter DNA (122), albeit in different cell lines. Likewise, promoter sense RNA have been detected as saRNA targets (78).

Debate has been ongoing as to the possible role of the promoter RNA transcripts in particular the antisense in the activation mechanisms of saRNA. Although the initial studies proposed an involvement of promoter antisense RNA in the activation by saRNA (18,79,122) more recent studies have negated a role for the promoter antisense (82). Initial proposals for a role for the promoter antisense were either based on association of the promoter antisense with the saRNA (122) or; association of AGO with the antisense transcript following saRNA treatment (18,79,122). Furthermore, a knockdown study of the PR promoter antisense RNA resulted in reversal of saRNA-activation of the PR promoter (122). In these studies no reduction in antisense RNA following saRNA treatment or degradation of the antisense RNA by AGO was detected (18,79,122), therefore putative mechanisms did not rely on changes in antisense RNA as had been suggested (86). The role of the antisense overlapping the promoter was subsequently proposed as a shuttle, specifically it was proposed that saRNA shifted the interacting proteins from the promoter to the antisense thereby remodelling the protein interactions at the promoter. This too was suggested for saRNA activation mediated via the promoter sense RNA (78). However, not all promoters have a corresponding antisense transcript, for example Hu et al (37) were unable to detect non-coding transcripts for the p21 promoter and yet they observed saRNA activation of that promoter, likewise Voutila et al (137) were unable to detect antisense transcripts for the KLF promoter. Consistently, lack of changes in the antisense have been noted with saRNA activation for different gene promoters (18,79,122,152,161). More recent studies interrogating the role of the antisense in saRNA activation, demonstrated that siRNA knockdown of the overlapping antisense did not attenuate the saRNA activity on the PR promoter (82). It is possible that multiple mechanisms can be activated by the saRNA depending on the molecular targets that are available, as such the debate on the role of the promoter non-coding transcripts on saRNA activation will only be resolved once the entire mechanisms are elucidated (164).

Specificity

One of the issues of saRNA activity is the specificity of its action. There are two aspects to the non-specific effects that have likewise been a concern in the siRNA field [reviewed in (42)]: 1) Activation of the innate immunity by dsRNA and; 2) Activation of miRNA-like mechanisms that mediate post-transcriptional silencing due to binding of sRNA to sequences of partial homology. Activation of either of these can result in off-target effects (88,154) and potentially complicate interpretation of saRNA activation (105).

In the siRNA field, it was known early on that double-stranded RNA molecules can induce type I-Interferons (IFNs) and thereby induce the expression of IFN-responsive genes [reviewed in (113)]. Subsequently, this IFN induction may then be responsible for the observations of gene activation by saRNA, and not specific effects of the saRNA on the gene promoter target site. To address this issue, assessment of the IFN response activation has been carried out and to date no significant changes in expression of IFN-regulated genes have been reported with saRNA treatment (69,79,137). In addition, type I-IFN-treatment did not affect E-cadherin or p21 expression (69) nor did induction of IFN by poly-inosinic-polycytidylic acid (poly I:C) affect Low density lipoprotein receptor (LDLR) expression (79), although these same genes were induced by a corresponding saRNA. Chemical modifications of the saRNA also can improve their specificity and reduce their IFN activation (46).

Since the saRNAs are only 19-24 nt then there is a potential for partial homology binding to sequences other than the targeted site. In doing so saRNA may activate miRNA-like mechanisms targeting other RNA transcripts. This is more difficult to track and requires more global assessment of gene expression such as micro-array analysis, as has been performed for shRNA regulating VEGF-A (40), or deep sequencing to determine altered genes following saRNA treatment. Of course, saRNA activation of any particular gene promoter may not be influenced by off-target effects occurring in the cell (105), likewise not all promoter-targeted sRNA induce off-target effects (127), however this is an important check in saRNA applications.

Long non-coding RNAs in chromatin regulation

To date, knowledge of the specific functional roles of lncRNA is still lacking. This is partly due to the breadth of this class of lncRNAs. lncRNAs have been defined based mainly on their size and non-protein coding function, and it is not likely that all of these transcript share similar functions or mechanisms, and some of them may be only by-products of transcription. Some attempts have been made to re-categorize these transcripts; Mukherjee et al. for example defined seven different RNA classes based on the metabolism (e.g. synthesis, processing and degradation) of the transcripts (90). As expected, in these RNA classes mRNAs and lncRNAs differed in some properties, such as the rate of transcription and degradation. However, two of the new classes contained both mRNAs and lncRNAs, suggesting maybe similar processing pathways. Some studies have shown that lncRNAs can also code for peptides with biological function, such as the 46 amino acids peptide that is structurally similar to sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) inhibitors phospholamban and sarcopilin (4). This peptide was shown to directly interact with SERCA, decreasing Ca^{2+} reuptake (4). The same research group also found another muscle-specific mircropeptide that is 34 amino acids in size which enhanced SERCA activity (91). Many lncRNAs have computationally annotated open reading frames (ORF) but not so much translational activity as mRNAs (12). Computational prediction

identified lncRNA ORFs as less functional with poorer start codon and short ORF, thus resulting in less activation and small peptides (92). The studies so far that have described individual peptides encoded by lncRNAs, have described only short micropeptide formation. It also seems that lncRNA-ORFs are relatively rare considering how much of the genome is transcribed into ncRNAs. New technologies will provide platforms to generate more information about lncRNAs and their function. For example screening using Clustered regularly interspaced short palindromic repeats (CRISPR) was recently used to identify the lncRNAs that have specific function in cell growth in human cells (75). This study, like many before, showed also that the lncRNA functions are highly cell-type specific.

Transcriptional gene regulation on the chromatin level has been indicated for some lncRNAs. Many lncRNAs have been shown to induce gene silencing, often by recruiting the polycomb repressor complex (PRC2) to their loci (51). A very well-known example is the X-inactive specific transcript (XIST) that in female cells participates in the inactivation of the X chromosome gene expression (10,24). However, on the other X chromosome the XIST expression needs to be restricted in order to maintain active gene expression on one of the chromosomes. lncRNA TSIX was identified to be expressed antisense to XIST and therefore repressing it on the active chromosome (64). Recently the mechanisms have been characterized in more detail and it has been shown that TSIX recruits chromatin-modifying enzymes that induce H3K36 trimethylation on the XIST promoter (93). The process of X chromosome inactivation seems to be very complex and the mechanism is not yet resolved. Another lncRNA, X active coating transcript (XCAT) was found expressed specifically in humans from the active X chromosome and later studies have shown that it antagonizes XIST accumulation in early stage of X chromosome inactivation (134,135). Likewise regulation of Homeobox A cluster (HOXA) genes have been linked to histone modifications, in particular lncRNA Gm15055 in mouse embryonic stem cells was shown to recruit PRC2 to HOXA gene cluster and thus maintain stable, repressive H3K27me3 levels on their promoter (74). This study interestingly showed by 3C method that the Gm15055 directly interacts with multiple sites on HOXA gene cluster. A very well-known example of lncRNAs in chromatin regulation is the HOTAIR RNA transcript, which was first characterized in 2007 (112). HOTAIR is expressed from the HOXC locus and mediates the repression of HOXD locus by interacting with PRC2, thus mediating the methylation of H3K27 at the locus. However, a recent study showed that the gene silencing induced by HOTAIR can be independent of PRC2 binding, where only tethering of HOTAIR to chromatin was able to lead to gene silencing events, after which PRC2 can be recruited to the loci (107).

Even though the role of lncRNAs in transcriptional gene silencing has been more widely studied, an increasing amount of evidence also supports a functional role for lncRNA in gene activation. There are many mechanisms by which this transcriptional activation may occur (summarized in Figure 6). The molecular mechanisms have not yet been characterized in detail but an increasing number of publications on the topic suggests a high correlation between specific lncRNA expression levels and gene regulatory events (54,56,132). Many studies have been based on global characterization of expression in response to different cellular status, associating specific lncRNA expression changes with certain disease (such as cancer or, cardiovascular disease; 125,129,133). These studies offer insights into the possible use of lncRNA expression as a biomarker for such disease states. However, they often tell little about the actual function of such RNA transcripts. Further characterization has been done in some cases by overexpression or knockdown experiments to provide more details into the mechanism of action, but deeper understanding of the action of different lncRNAs has not yet been reached.

A study by Ørom et al identified already in 2010 that lncRNAs played a role in regulating the neighboring genes (96). This study found that knocking-down many of these lncRNAs resulted in decreased expression of

the neighboring protein-coding gene. These lncRNAs were serving as “enhancers” for the protein-coding genes, even though the chromatin signature was different than with typical enhancer. These lncRNAs were also not transcribed bidirectionally as eRNAs, and they were also more often polyadenylated. A later study by the group showed that these activating enhancer-like ncRNAs act by bringing the Mediator complex to the vicinity of their neighboring genes via chromatin looping (60).

lncRNAs have been shown to transcriptionally silence genes by recruiting repressive enzymes and factors to their loci. Likewise, recruitment of histone modifiers has been observed with lncRNAs in transcriptional gene activation. Hoxb genes for example have been shown to be activated by HoxBlinc lncRNA transcript that binds to Hoxb genes, this followed by recruitment of the enzyme complex Setd1a/MLL1 which maintains H3K4me3 histone modification levels (26). Moreover, deletion of HoxBlinc by CRISPR genome editing impaired Hoxb gene expression, but also chromatin looping. lncRNA transcripts may therefore serve an important role in the three dimensional organization of chromatin. An interesting finding in HOXA gene regulation was lncRNA HOTAIRM1, which has been identified as a biomarker in many cancer types [e.g. (27,140)]. This lncRNA was found to have different variants that can either repress or activate HOXA gene expression in different cell types (150). These transcripts all originate from the same HOTAIRM1 gene and influence H3K4me3 and H3K27me3 histone marks. The basal level of HOTAIRM1 expression and histone methylation marks determined the effects that the lncRNA had on the activation: in cells with higher expression of HOTAIRM1, the H3K27me3 levels were lower and the HOTAIRM1 function was mediated by only increased H3K4me2 levels. In another cell type, where the basal level of HOTAIRM1 was lower, the promoters were marked with high levels of both H3K4me3 and H3K27me3 and the induction of HOTAIRM1 expression led to significantly lower levels of H3K27me3 on HOXA genes, as well as to increased H3K4me3 methylation at HOXA1 and HOXA2 gene promoters. This study also showed direct chromatin interaction of HOTAIRM1 with the HOXA genes. The lncRNA variants recruited UTX/MLL or PRC2 complexes to activate or silence, respectively, HOXA gene expression. Another example is the HOTTIP transcript that is expressed from the HOXA locus. HOTTIP activates HOXA genes by binding directly to WDR5 and through chromosomal looping, can bring the WDR5/MLL complex to target gene loci (147). This leads to increased levels of H3K4me3 and activates the transcription of these HOXA genes.

Interesting feature of lncRNA action has been their ability to guide genes to active compartment of the nucleus. lncRNAs were demonstrated to participate in the nuclear organization, as was seen for example with lncRNA Nuclear Paraspeckle assembly transcript 1 (NEAT1) that structurally enables paraspeckle formation (20). A study by Yang et al. showed that specific lncRNAs contribute to the translocation of E2F1-regulated growth control genes to active nuclear compartment upon serum stimulation (162). This was dependent on histone demethylase KDM4C. Two different lncRNAs were important for this regulation, taurine up-regulated 1 (TUG1) and NEAT2. The promoters that were found to interact with TUG1 were associated with a corepressor complex and histone modifications that are considered marks of repressed gene expression. On the other hand, NEAT2 was found to interact with promoters that had histone modifications that imply active gene transcription and was shown to bring these genes to transcriptionally active nuclear compartment. These studies imply that lncRNAs play an important role in forming transcriptionally repressive or active nuclear chromatin compartments. Acting as scaffolds, lncRNAs recruit many different types of chromatin modifying enzymes to their associated genes thereby directly affecting gene regulation.

In addition to their role as scaffolds, lncRNAs can also activate genes by sequestering away repressive proteins and miRNAs. In T-cells derived from immune thrombocytopenic purpura patients, lncRNA Maternally expressed 3 (MEG3) expression was increased and was shown to bind miR-125a-3p (67). This

group had previously shown that miR-125a-3p was associated with pathogenesis of the disease by regulating C-X-C motif chemokine ligand 13 (CXCL13), and so now suggested that MEG3 is regulating the levels of this miRNA. Interestingly, DNA hypomethylation of MEG3 promoter and a corresponding increase in expression of MEG3 and another miRNA cluster have also been detected in atherosclerotic lesions (1), suggesting that MEG3 may play a role in different diseases. Another example of lncRNA activation potential is that of muscle-specific lncRNA, lnc-MD1, which has been shown to directly interact with miRNAs miR-133 and miR-135. This 'sponging' interaction acts to sequester the miRNAs and thereby regulate muscle-specific gene expression of transcription factors Mastermind-like protein (MAML)1 and Myocyte enhancer factor 2C (MEF2C) (14). In Duchenne muscular dystrophy cells the level of lnc-MD1 was decreased and the expression levels of MAML1 and MEF2C were altered. The studies of these competing endogenous RNAs imply that lncRNAs participate in a complex network of regulatory events to achieve necessary gene expression for the cells, and can direct tissue specific gene expression. Many of these functions in sponging other factors have been so far shown in the cytoplasm, but similar ncRNA function may be found within nucleus. lncRNA HOXA11-AS was shown to bind PRC2, Lysine-specific histone demethylase 1 (LSD1) and DNA methyltransferase (DNMT)1 in the nucleus and to regulate protease, serine 8 (PRSS8) and KLF2, while it also functioned as a competing RNA in the cytoplasm by interaction with miR-1297 (126).

lncRNAs have also been shown to induce signaling pathways through direct interaction, whereby they activate not only a few specific targets in the genome but induce more widespread downstream effects. lncRNA NRB2 was shown to activate kinase signaling by direct interaction with AMP-activated protein kinase alpha (AMPKα) subunit following energy stress (76). The system worked in a feed-back loop where AMPK pathway increased NRB2 expression. Both lncRNAs CCHE1 and Metastasis associated lung adenocarcinoma transcript 1 (MALAT1) were shown to participate in the activation of ERK/MAPK kinase pathway in hepatocellular carcinoma (101) and in neuronal cell differentiation (15), respectively. In stroke, neuronal death is increased by activation of p53 that was induced by lncRNA MEG3 that directly binds p53 (160). Together these examples show that lncRNAs can initiate cascades of cellular function by direct interaction and activation of kinases or transcription factors.

lncRNAs act also in derepression of genes, activating poised genes. For example in *S. cerevisiae*, metabolic GAL genes were activated by GAL lncRNAs from the same cluster (22). The lncRNAs promote faster recruitment of RNA PolII and their expression decreases the binding of co-repressor protein Cyc8, thus inducing the activation of GAL genes when environmental conditions are suitable. Studying the mechanism further, Cloutier et al found that this de-repression occurs through R-loop formation between the lncRNA and GAL gene cluster DNA (21). This mechanism of action of lncRNAs enables fast induction of gene expression for certain critical genes. ncRNAs are likely to play a role in rapid responses to environmental stimuli since RNA transcription may occur more instantly than increasing production of proteins, such as transcription factors that would then further regulate other genes.

The regulation of gene expression is a complex network of multiple factors. The role of lncRNAs in this process is emerging and the functions of lncRNAs seem versatile. The expression of many individual lncRNAs has been associated with different disease states, but also with developmental processes (5,120,139). Fully understanding the action of lncRNAs will be important for comprehending the cellular processes concerning both basal cell functions, as well as therapeutic benefits in modifying the expression of these transcripts.

Enhancer RNA

Although eRNAs may resemble lncRNAs in size, ranging from 0.1-9Kb, they are a distinct class of ncRNAs, distinguished by their associated histone profile (25). The eRNAs are often, but not exclusively, derived from bidirectional transcription following RNA polII recruitment and binding to enhancer regions within the genome (Figure 1; reviewed in (62)). These enhancer regions are characterised by enrichment of H3K4me1 and low H3K4me3, they are bound by lineage-determining transcription factors, as well as enriched occupancy of RNA polII and other transcriptional co-regulators. Importantly, enhancers regulate transcription at gene promoters. The majority of eRNA transcripts are 5'capped, unspliced, not polyadenylated, have short half-lives, but are also dynamically regulated following signalling and correlate with increased expression of neighbouring genes (Table 1; (53)). Although there has been some debate as to whether eRNAs have a functional role in regulating transcription or are coincident to enhancer transcription, growing evidence suggests that they do indeed function in transcriptional activation (reviewed in (62,115)). This has included knockdown studies of eRNA, either by siRNA-mediated RNAi (61,81,96), or antisense oligonucleotide-activation of RNase H degradation (71), which led to a specific decrease in the corresponding mRNA expression. Equally, gain-of-function studies of eRNA using assays tethering eRNA to the promoter or enhancer sites, also demonstrated transcriptional activation (71,81).

The mechanisms by which eRNAs mediate their function are still to be defined. Enhancer regions loop to their corresponding promoter, this looping is mediated in part by Mediator and cohesins (47,60,143). Enhancer looping may facilitate eRNA tethering to the promoter. Alternatively eRNA may participate in mediating the DNA looping. It has been shown that eRNAs are enriched preferentially at enhancers that are actively looping to promoters or other enhancers (73,117). In addition, knockdown of eRNA of the estrogen receptor-α enhancer, concomitantly resulted in the reduction of both enhancer-promoter interaction and gene expression (71), suggesting that eRNA may initiate or stabilise enhancer looping likely by interacting with Mediator and the cohesin complex (36,60). Other knockdown studies of eRNAs have also demonstrated that eRNAs may facilitate RNA polII recruitment specifically to the gene promoter, as has been demonstrated for the MyoD gene (89). The effect of eRNAs on RNA polII are likely to be multifactorial, since it has also been shown that eRNAs can facilitate the release of RNA polII at poised genes into productive elongation (119). Most recently, eRNAs were also shown to interact with the transcriptional activators CBP/p300 to stimulate modifications in histone acetylation, that are characteristic of active enhancers (7).

Our knowledge and understanding of eRNA has at large followed advances in high-throughput transcriptomic analysis and it is likely to improve with new tools and methods that detect spatial and temporal changes with greater sensitivity, as has recently been demonstrated (85). These may change our view of eRNA function and strengthen the evidence that supports a functional role for eRNAs in transcriptional activation.

PIWI-associated RNA in chromatin activation

PIWI-associated RNAs (piRNAs) are small ncRNAs that are traditionally considered to act in silencing transposons. Especially during spermatogenesis (100) they bind AGO/Piwi family of proteins and direct the methylation of DNA. In addition, piRNAs have also been shown to regulate Heterochromatin Protein 1 (HP1) activity and H3K9 methylation (151). Besides transcriptional gene silencing, piRNAs have been associated with miRNA-like functions in PTGS, specifically during late spermatogenesis (33).

Already in 2007, piRNAs in association with Piwi-proteins were shown to transcriptionally activate gene expression by inducing epigenetic changes in the chromatin of *D. melanogaster* (163). This study showed that also piRNAs may have a dual role in gene regulation, where they act as a guide to chromatin for either activating or repressing enzyme complexes. piRNAs can be processed from other longer non-coding RNA transcripts and a study by He et al (35) showed that lncRNA and snoRNA –derived piRNAs activated TNF-related apoptosis-inducing ligand (TRAIL) gene expression. In this study, the piRNA pi-sno75 induced H3K4 methylation while decreasing H3K27 methylation at the promoter of the proapoptotic factor TRAIL, in addition recruitment of the histone methyltransferases macromolecular MLL/COMPASS-like complex to the piRNA site on chromatin was observed. Some other studies have also suggested that 22G-RNAs, that are derived from the piRNA genes, activate gene expression by association with AGO protein CSR-1 and bind to nascent RNA transcripts in *C. elegans*, affecting the epigenetic status of the chromatin (19,123). This function is similar to piRNA-mediated silencing of transposons and other elements in the genome and was suggested that the pathway mediates trans-generational ON/OFF-expression memory for germline genes (153).

MicroRNAs: Biogenesis, function and proposed mechanism for activation

miRNAs are small (20-24 nt) ncRNAs which in spite of their small size have notable roles in numerous cellular functions (138), such as cell proliferation and cell apoptosis (9,159). After the first miRNA, let-7, was discovered in *C.elegans* in 1993 (65), many different miRNAs have been annotated and some of their regulatory roles have been studied both in plants and animals.

miRNA genes are spread across the genome, they can be found in intronic and exonic sequences or be intergenic. They can be found either in the sense or antisense orientation (Figure 1). Furthermore, miRNAs are often found in clusters and from one RNA transcript, one or more mature miRNAs can be generated (6). miRNA biogenesis (Figure 2) can be divided into two main steps: the formation of the miRNA transcripts in the nucleus and the maturation of miRNAs in the cytoplasm (57). Most often, miRNA biogenesis starts with RNA polII mediated transcription which leads to the formation of primary miRNA (pri-miRNA). pri-miRNA is then processed into precursor miRNA (pre-miRNA) by Drosha ribonuclease III (Drosha) enzyme. This is not absolutely required since some of the intronic miRNAs are so small that they bypass Drosha-mediated processing (116) and are instead cleaved by spliceosomes into pre-miRNAs (57). The hairpin pre-miRNA is then transported from the nucleus to the cytoplasm by Ran-GTP-dependant exportins (6). In the cytoplasm, DICR enzyme cleaves pre-miRNAs into double-stranded structures which are then further processed into single-stranded mature miRNAs.

To become functional, mature miRNAs must be loaded into the RNA-induced silencing complex (RISC). RISC contains many different proteins including AGOs. The AGOs are vital for miRNA-mediated regulation as they form a suitable environment for miRNA-mRNA binding and therefore support miRNA function. Zamudio et al. (167) showed that the loss of AGOs in mouse embryonic stem cells leads to the destabilization of miRNAs. In the context of RISC, miRNAs act in the cytoplasm to induce PTGS. Generally, this is initiated through binding of miRNAs to the 3'untranslated regions (3'UTRs) of their target mRNAs. miRNAs recognize their target mRNAs based on the seed sequence which is the first 2-8 nt from the 5'end of the mature miRNA. Depending on the extent of the complementary, target mRNAs are either degraded or inhibited (114).

In addition to their role in PTGS, miRNAs were also found to activate gene translation in the cytoplasm. Vasudevan et al. showed in 2007 that miR-369-3 can activate tumor necrosis factor–a (TNFa) expression by

recruiting AGOs and other proteins to 3'UTRs of target mRNAs (136). There is also some evidence that miRNAs can regulate gene expression by binding the 5'UTR of the mRNAs. In mouse ES cells, miR-10a was shown to bind to the 5'UTR of the mRNA encoding ribosomal protein leading to the induction of ribosomal protein expression (97). Similar regulation was found in human liver cells, where miR-122 enhanced viral replication by binding the 5'end of viral genome (45).

Although the canonical view of miRNAs is that they function in the cytoplasm, recent research has also suggested a role for miRNAs in the nucleus. This idea has been supported by studies that have found mature miRNAs in the nuclear fractions of the cells. For example, miR-29b was shown to preferentially localize in the nuclear fractions of human Hela cells (41). It has been proposed that nuclear miRNAs are processed in the same way as canonical miRNAs and only after maturation in the cytoplasm, they are transported back to the nucleus by importins (155). In addition to mature miRNAs, AGOs and other RISC components are also found in the nuclear fraction of the cells and are functionally active in the nucleus (31).

As mature miRNAs were found in the nucleus of the cells, it raised the question of the function of the nuclear miRNAs. Small RNAs had been known to repress gene expression at the gene promoter level in *C.elegans* (30). Mette et al. also showed in plants that small dsRNAs can silence their target gene expression by inducing DNA methylation on gene promoters (84). miRNA-mediated silencing at the transcriptional level was shown by Omato and Fujii in 2004, as human immunodeficiency virus (HIV)-1 transcription in human T-cells was reduced by miR-N367 induction (94). In another study, miR-320 was linked with epigenetic modifications of the RNA polIII subunit D (POLR3D) gene promoter leading to the downregulation of that gene (52). However, the newest studies suggest that some miRNAs can actually also activate gene expression in the nucleus. Potential miRNA target sites have also been found in the introns of nascent mRNAs, non-coding RNAs, and coding sequences (17).

Bioinformatic studies showed that there are 800 000 potential miRNA target sites (seed matches) in over 27 000 gene promoters (166). A functional activity of miRNA activating gene expression by targeting gene promoters has been demonstrated for a number of promoters. miR-373 was shown to have target sites on E-cadherin and CSDC2 gene promoters, and over-expression of miR-373 or its precursor form induced the expression of corresponding genes (103). Likewise, Wang et al. also showed that p21 expression can be induced by miR-370-5p which targets its promoter, in human bladder cancer cells and tissues (142). Equally, miR-205 was demonstrated to have target sites on promoters of interleukins-24 and -32 (IL-24 and IL-32) in human prostate cancer cells (77). After transfection of pre-miR-205, IL-24 and IL-32 expression levels multiplied, compared to control transfection levels, both at the mRNA and protein levels. Further evidence that supports a functional role for miRNA targeting gene promoters was provided by Huang et al. who not only demonstrated that miR-744 targets the cyclin B1 (Ccnb1) promoter to activate gene expression, but also demonstrated the decrease in Ccnb1 expression following siRNA knockdown of either enzymes needed for miRNA biogenesis (such as DICER and DROSHA Ribonuclease III) or RISC components (including AGO1 and AGO2) (38).

As miRNA target sites are found also in non-coding RNA transcripts, miRNA-mediated regulation of genes could also happen indirectly via regulation of the gene-associated ncRNAs. Indeed, this was demonstrated in lung cancer cells where two target sites for miR-589 were found on lncRNA that overlap the COX-2 promoter (78). When miR-589 was bound to lncRNA, COX-2 expression was induced. Interestingly, miR-589 binding sites were not perfectly complementary to lncRNA as in mRNA targeting by miRNAs.

Bioinformatic studies have shown a correlation between miRNAs expression and histone modifications characteristic of an open chromatin (128). miRNA may activate gene promoters by altering the promoter histone marks. In support of this, Majid et al. have demonstrated enrichment of open histone marks on IL-24 and IL-32 promoter areas following activation by miRNA-205 (77). Likewise, overexpression of miR-744 that targets the Ccnb1 promoter lead to enrichment of open histone mark H3K4me3 at the TSS of Ccnb1 (38). The evidence for additional functional mechanisms for miRNA activity in the nucleus is still sparse, however this will be fueled by the increasing interest in nuclear miRNAs.

Conclusion

Already various species of ncRNAs have been identified and characterized, and the research is increasingly focused on the mechanisms of action of these new types of gene expression regulators. The biological importance of the ncRNAs is increasingly appreciated, and what is clear is that these RNA transcripts are not just noise, but play an important role in gene regulation. As research of the ncRNAs continues, the classification of ncRNA types may need to be refined. Already we see that classification of ncRNAs by size is overly simplistic as is the case for the lncRNAs that serve multiple and different roles in the cells and act by distinct mechanisms. The function and the mechanisms of ncRNA activity, in particular activation by ncRNA, have not yet been defined for all the ncRNA types. However, despite the paucity in detailed molecular mechanisms, what is clear is that ncRNA can activate the chromatin, either by inducing histone modifications to open up the chromatin, by increasing the recruitment of transcriptional elements, or by looping the chromatin to alter transcription. The net yield of these modifications is increased transcriptional activity. Transcription as an event takes place more rapidly than protein production, offering the cells an option for a more instant response to changes in the environment. When environmental variation induces cellular signaling, transcription is the faster reaction to the stimulus and ncRNAs may be more potent and rapid regulators of genes that are required for environmental responses. From the evolutionary respect, the ncRNAs are more prone to mutations or new variations of the sequence since they are not restricted by codon rules as the protein-coding sequences. This means that the ncRNAs are more poised to sequence diversity and modifications can be achieved quickly for the fine-tuning of gene expression.

For the lncRNAs, the mechanisms of action in gene activation have so far been mainly associated with either guiding proteins and factors to the transcription site (cis-acting) and sequence dependent loci (trans-acting), or binding miRNAs and proteins to preventing them from binding their target sequences. lncRNAs are transcribed and processed in a similar way as mRNAs so it is not surprising that already some lncRNAs have been observed to be translated into peptides, and also some mRNAs have been associated with lncRNA-like functions in the nucleus. Processing of lncRNA, such as splicing, also creates variability in the transcript sequences that may direct different secondary structure formation. This can impact on the lncRNA interactions with proteins, other RNAs or the genome, and can increase the potential roles for any one lncRNA. Transcriptional regulation is not the only function identified for these RNAs, as they seem to have even structural roles for example in the maintenance of the nuclear matrix, cytoskeleton and extracellular matrix structure. In a similar manner, lncRNAs may play a part in coactivator and/or corepressor complexes and act potentially to guide the protein complex to its target site, or purely to maintain the correct structure or to affect the assembly of the transcription factor complex.

The usefulness of saRNA and miRNA that target promoters and thereby activate gene expression is clear both as a research tool and for therapeutic potential. In particular, for saRNA that can modify simultaneously different isoforms of a single gene, the therapeutic potential for potent molecules that would benefit from temperate regulation is evident. Likewise, miRNA that are able to simultaneously target multiple promoters or other ncRNAs that participate in the regulation of gene expression, may be valuable. An additional attractive feature of these ncRNA is not only their sequence specificity but also their differential activity in varying cell types. To date much of the evidence on saRNA has been derived from exogenous saRNA with few examples of endogenous saRNA. However, immunoprecipitation studies of AGO-associated sRNAs have revealed fragments of diverse origin (11) including many promoter-proximal RNA (167), therefore it is likely that we are yet to realise the significance of nuclear saRNAs and miRNAs in activating gene expression. Whether by inducing histone modifications to open the chromatin, or by increased recruitment of transcriptional elements, or by looping of the chromatin to alter transcription, ncRNA play an important role in the regulation and activation of the chromatin.

Laham-Karam N.

Acknowledgements

We would like to sincerely thank Jyrki Äikäs for his kind assistance in the preparation of the figures. This work was supported by the Finnish Academy Centre of Excellence and the ERC Advance grant.

List of Abbreviations

AGO Argonaute

CRISPR Clustered regularly interspaced short palindromic repeats

dsRNA double-stranded RNA

eRNA enhancer RNA

HIV human immunodeficiency virus

hnRNPs heterogeneous nuclear ribonucleoproteins

iNOS inducible nitric oxide synthase

lncRNA long non-coding RNA

mRNA messenger RNA

miRNA micro RNA

ncRNA non-coding RNA

nt nucleotides

paRNA promoter-associated RNA

piRNA PIWI-interacting RNA

PRC2 polycomb repressor complex

PTGS Post-transcriptional gene silencing

RISC RNA-induced silencing complex

RNAi RNA interference

RNA pol RNA polymerase

sRNA small RNA

saRNA small activating RNA

siRNA small interfering RNA

TGS transcriptional gene silencing

TSS transcriptional start sites

UTR untranslated region

Laham-Karam N.

References

1. Aavik E, Lumivuori H, Leppanen O, Wirth T, Hakkinen SK, Brasen JH, Beschorner U, Zeller T, Braspenning M, van Criekinge W, Makinen K and Yla-Herttuala S. Global DNA methylation analysis of human atherosclerotic plaques reveals extensive genomic hypomethylation and reactivation at imprinted locus 14q32 involving induction of a miRNA cluster. *Eur Heart J* 36: 993-1000, 2015.

2. Ackley A, Lenox A, Stapleton K, Knowling S, Lu T, Sabir KS, Vogt PK and Morris KV. An Algorithm for Generating Small RNAs Capable of Epigenetically Modulating Transcriptional Gene Silencing and Activation in Human Cells. *Mol Ther Nucleic Acids* 2: e104, 2013.

3. Ahlenstiel CL, Lim HG, Cooper DA, Ishida T, Kelleher AD and Suzuki K. Direct evidence of nuclear Argonaute distribution during transcriptional silencing links the actin cytoskeleton to nuclear RNAi machinery in human cells. *Nucleic Acids Res* 40: 1579-1595, 2012.

4. Anderson DM, Anderson KM, Chang CL, Makarewich CA, Nelson BR, McAnally JR, Kasaragod P, Shelton JM, Liou J, Bassel-Duby R and Olson EN. A micropeptide encoded by a putative long noncoding RNA regulates muscle performance. *Cell* 160: 595-606, 2015.

5. Ballarino M, Morlando M, Fatica A and Bozzoni I. Non-coding RNAs in muscle differentiation and musculoskeletal disease. *J Clin Invest* 126: 2021-2030, 2016.

6. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116: 281-297, 2004.

7. Bose DA, Donahue G, Reinberg D, Shiekhattar R, Bonasio R and Berger SL. RNA Binding to CBP Stimulates Histone Acetylation and Transcription. *Cell* 168: 135-149.e22, 2017.

8. Bramsen JB, Laursen MB, Nielsen AF, Hansen TB, Bus C, Langkjaer N, Babu BR, Hojland T, Abramov M, Van Aerschot A, Odadzic D, Smicius R, Haas J, Andree C, Barman J, Wenska M, Srivastava P, Zhou C, Honcharenko D, Hess S, Muller E, Bobkov GV, Mikhailov SN, Fava E, Meyer TF, Chattopadhyaya J, Zerial M, Engels JW, Herdewijn P, Wengel J and Kjems J. A large-scale chemical modification screen identifies design

rules to generate siRNAs with high activity, high stability and low toxicity. *Nucleic Acids Res* 37: 2867-2881, 2009.

9. Brennecke J, Hipfner DR, Stark A, Russell RB and Cohen SM. bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. *Cell* 113: 25-36, 2003.

10. Brockdorff N, Ashworth A, Kay GF, Cooper P, Smith S, McCabe VM, Norris DP, Penny GD, Patel D and Rastan S. Conservation of position and exclusive expression of mouse Xist from the inactive X chromosome. *Nature* 351: 329-331, 1991.

11. Burroughs AM, Ando Y, de Hoon MJ, Tomaru Y, Suzuki H, Hayashizaki Y and Daub CO. Deep-sequencing of human Argonaute-associated small RNAs provides insight into miRNA sorting and reveals Argonaute association with RNA fragments of diverse origin. *RNA Biol* 8: 158-177, 2011.

12. Calviello L, Mukherjee N, Wyler E, Zauber H, Hirsekorn A, Selbach M, Landthaler M, Obermayer B and Ohler U. Detecting actively translated open reading frames in ribosome profiling data. *Nat Methods* 13: 165-170, 2016.

13. Cernilogar FM, Onorati MC, Kothe GO, Burroughs AM, Parsi KM, Breiling A, Lo Sardo F, Saxena A, Miyoshi K, Siomi H, Siomi MC, Carninci P, Gilmour DS, Corona DF and Orlando V. Chromatin-associated RNA interference components contribute to transcriptional regulation in Drosophila. *Nature* 480: 391-395, 2011.

14. Cesana M, Cacchiarelli D, Legnini I, Santini T, Sthandier O, Chinappi M, Tramontano A and Bozzoni I. A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. *Cell* 147: 358-369, 2011.

15. Chen L, Feng P, Zhu X, He S, Duan J and Zhou D. Long non-coding RNA Malat1 promotes neurite outgrowth through activation of ERK/MAPK signalling pathway in N2a cells. *J Cell Mol Med* 20: 2102-2110, 2016.

Laham-Karam N.

16. Chen Z, Place RF, Jia ZJ, Pookot D, Dahiya R and Li LC. Antitumor effect of dsRNA-induced p21(WAF1/CIP1) gene activation in human bladder cancer cells. *Mol Cancer Ther* 7: 698-703, 2008.

17. Chi SW, Zang JB, Mele A and Darnell RB. Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps. *Nature* 460: 479-486, 2009.

18. Chu Y, Yue X, Younger ST, Janowski BA and Corey DR. Involvement of argonaute proteins in gene silencing and activation by RNAs complementary to a non-coding transcript at the progesterone receptor promoter. *Nucleic Acids Res* 38: 7736-7748, 2010.

19. Claycomb JM, Batista PJ, Pang KM, Gu W, Vasale JJ, van Wolfswinkel JC, Chaves DA, Shirayama M, Mitani S, Ketting RF, Conte D,Jr and Mello CC. The Argonaute CSR-1 and its 22G-RNA cofactors are required for holocentric chromosome segregation. *Cell* 139: 123-134, 2009.

20. Clemson CM, Hutchinson JN, Sara SA, Ensminger AW, Fox AH, Chess A and Lawrence JB. An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. *Mol Cell* 33: 717-726, 2009.

21. Cloutier SC, Wang S, Ma WK, Al Husini N, Dhoondia Z, Ansari A, Pascuzzi PE and Tran EJ. Regulated Formation of lncRNA-DNA Hybrids Enables Faster Transcriptional Induction and Environmental Adaptation. *Mol Cell* 62: 148, 2016.

22. Cloutier SC, Wang S, Ma WK, Petell CJ and Tran EJ. Long noncoding RNAs promote transcriptional poising of inducible genes. *PLoS Biol* 11: e1001715, 2013.

23. Czech B and Hannon GJ. One Loop to Rule Them All: The Ping-Pong Cycle and piRNA-Guided Silencing. *Trends Biochem Sci* 41: 324-337, 2016.

24. da Rocha ST, Boeva V, Escamilla-Del-Arenal M, Ancelin K, Granier C, Matias NR, Sanulli S, Chow J, Schulz E, Picard C, Kaneko S, Helin K, Reinberg D, Stewart AF, Wutz A, Margueron R and Heard E. Jarid2 Is

Laham-Karam N.

Implicated in the Initial Xist-Induced Targeting of PRC2 to the Inactive X Chromosome. *Mol Cell* 53: 301-316, 2014.

25. De Santa F, Barozzi I, Mietton F, Ghisletti S, Polletti S, Tusi BK, Muller H, Ragoussis J, Wei CL and Natoli G. A large fraction of extragenic RNA pol II transcription sites overlap enhancers. *PLoS Biol* 8: e1000384, 2010.

26. Deng C, Li Y, Zhou L, Cho J, Patel B, Terada N, Li Y, Bungert J, Qiu Y and Huang S. HoxBlinc RNA Recruits Set1/MLL Complexes to Activate Hox Gene Expression Patterns and Mesoderm Lineage Development. *Cell Rep* 14: 103-114, 2016.

27. Diaz-Beya M, Brunet S, Nomdedeu J, Pratcorona M, Cordeiro A, Gallardo D, Escoda L, Tormo M, Heras I, Ribera JM, Duarte R, de Llano MP, Bargay J, Sampol A, Nomdedeu M, Risueno RM, Hoyos M, Sierra J, Monzo M, Navarro A, Esteve J and Cooperative AML group CETLAM. The lincRNA HOTAIRM1, located in the HOXA genomic region, is expressed in acute myeloid leukemia, impacts prognosis in patients in the intermediate-risk cytogenetic category, and is associated with a distinctive microRNA signature. *Oncotarget* 6: 31613-31627, 2015.

28. Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, Tanzer A, Lagarde J, Lin W, Schlesinger F, Xue C, Marinov GK, Khatun J, Williams BA, Zaleski C, Rozowsky J, Roder M, Kokocinski F, Abdelhamid RF, Alioto T, Antoshechkin I, Baer MT, Bar NS, Batut P, Bell K, Bell I, Chakrabortty S, Chen X, Chrast J, Curado J, Derrien T, Drenkow J, Dumais E, Dumais J, Duttagupta R, Falconnet E, Fastuca M, Fejes-Toth K, Ferreira P, Foissac S, Fullwood MJ, Gao H, Gonzalez D, Gordon A, Gunawardena H, Howald C, Jha S, Johnson R, Kapranov P, King B, Kingswood C, Luo OJ, Park E, Persaud K, Preall JB, Ribeca P, Risk B, Robyr D, Sammeth M, Schaffer L, See LH, Shahab A, Skancke J, Suzuki AM, Takahashi H, Tilgner H, Trout D, Walters N, Wang H, Wrobel J, Yu Y, Ruan X, Hayashizaki Y, Harrow J, Gerstein M, Hubbard T, Reymond A, Antonarakis SE, Hannon G, Giddings MC, Ruan Y, Wold B, Carninci P, Guigo R and Gingeras TR. Landscape of transcription in human cells. *Nature* 489: 101-108, 2012.

Laham-Karam N.

29. ENCODE Project Consortium. The ENCODE (ENCyclopedia Of DNA Elements) Project. *Science* 306: 636-640, 2004.

30. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE and Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* 391: 806-811, 1998.

31. Gagnon KT, Li L, Chu Y, Janowski BA and Corey DR. RNAi factors are present and active in human cell nuclei. *Cell Rep* 6: 211-221, 2014.

32. Gorski SA, Vogel J and Doudna JA. RNA-based recognition and targeting: sowing the seeds of specificity. *Nat Rev Mol Cell Biol* 18: 215-228, 2017.

33. Gou LT, Dai P, Yang JH, Xue Y, Hu YP, Zhou Y, Kang JY, Wang X, Li H, Hua MM, Zhao S, Hu SD, Wu LG, Shi HJ, Li Y, Fu XD, Qu LH, Wang ED and Liu MF. Pachytene piRNAs instruct massive mRNA elimination during late spermiogenesis. *Cell Res* 24: 680-700, 2014.

34. Guo X, Feng L, Jia J, Chen R and Yu J. Upregulation of VEGF by small activating RNA and its implications in preeclampsia. *Placenta* 46: 38-44, 2016.

35. He X, Chen X, Zhang X, Duan X, Pan T, Hu Q, Zhang Y, Zhong F, Liu J, Zhang H, Luo J, Wu K, Peng G, Luo H, Zhang L, Li X and Zhang H. An Lnc RNA (GAS5)/SnoRNA-derived piRNA induces activation of TRAIL gene by site-specifically recruiting MLL/COMPASS-like complexes. *Nucleic Acids Res* 43: 3712-3725, 2015.

36. Hsieh CL, Fei T, Chen Y, Li T, Gao Y, Wang X, Sun T, Sweeney CJ, Lee GS, Chen S, Balk SP, Liu XS, Brown M and Kantoff PW. Enhancer RNAs participate in androgen receptor-driven looping that selectively enhances gene activation. *Proc Natl Acad Sci U S A* 111: 7319-7324, 2014.

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

Laham-Karam N.

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

39. Huang V, Qin Y, Wang J, Wang X, Place RF, Lin G, Lue TF and Li LC. RNAa is conserved in mammalian cells. *PLoS One* 5: e8848, 2010.

40. Husso T, Turunen MP and Yla-Herttuala S. Regulation of VEGF-A expression in endothelial cells by transcriptional gene activation or transcriptional gene silencing: Analysis of genome wide transcriptional response. *Gene Technology* 4: 10000122, 2015.

41. Hwang HW, Wentzel EA and Mendell JT. A hexanucleotide element directs microRNA nuclear import. *Science* 315: 97-100, 2007.

42. Jackson AL and Linsley PS. Recognizing and avoiding siRNA off-target effects for target identification and therapeutic application. *Nat Rev Drug Discov* 9: 57-67, 2010.

43. Janowski BA, Huffman KE, Schwartz JC, Ram R, Nordsell R, Shames DS, Minna JD and Corey DR. Involvement of AGO1 and AGO2 in mammalian transcriptional silencing. *Nat Struct Mol Biol* 13: 787-792, 2006.

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

45. Jopling CL, Yi M, Lancaster AM, Lemon SM and Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science* 309: 1577-1581, 2005.

46. Judge AD, Bola G, Lee ACH and MacLachlan I. Design of Noninflammatory Synthetic siRNA Mediating Potent Gene Silencing in Vivo. *Molecular Therapy* 13: 494-505, 2006.

47. Kagey MH, Newman JJ, Bilodeau S, Zhan Y, Orlando DA, van Berkum NL, Ebmeier CC, Goossens J, Rahl PB, Levine SS, Taatjes DJ, Dekker J and Young RA. Mediator and cohesin connect gene expression and chromatin architecture. *Nature* 467: 430-435, 2010.

Laham-Karam N.

48. Kaikkonen MU, Lam MT and Glass CK. Non-coding RNAs as regulators of gene expression and epigenetics. *Cardiovasc Res* 90: 430-440, 2011.

49. Kalantari R, Chiang CM and Corey DR. Regulation of mammalian transcription and splicing by Nuclear RNAi. *Nucleic Acids Res* 44: 524-537, 2016.

50. Kang MR, Yang G, Place RF, Charisse K, Epstein-Barash H, Manoharan M and Li LC. Intravesical delivery of small activating RNA formulated into lipid nanoparticles inhibits orthotopic bladder tumor growth. *Cancer Res* 72: 5069-5079, 2012.

51. Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, Thomas K, Presser A, Bernstein BE, van Oudenaarden A, Regev A, Lander ES and Rinn JL. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. *Proc Natl Acad Sci U S A* 106: 11667-11672, 2009.

52. Kim DH, Saetrom P, Snove O,Jr and Rossi JJ. MicroRNA-directed transcriptional gene silencing in mammalian cells. *Proc Natl Acad Sci U S A* 105: 16230-16235, 2008.

53. Kim TK, Hemberg M, Gray JM, Costa AM, Bear DM, Wu J, Harmin DA, Laptewicz M, Barbara-Haley K, Kuersten S, Markenscoff-Papadimitriou E, Kuhl D, Bito H, Worley PF, Kreiman G and Greenberg ME. Widespread transcription at neuronal activity-regulated enhancers. *Nature* 465: 182-187, 2010.

54. Kornienko AE, Guenzl PM, Barlow DP and Pauler FM. Gene regulation by the act of long non-coding RNA transcription. *BMC Biol* 11: 59-7007-11-59, 2013.

55. Kosaka M, Kang MR, Yang G and Li LC. Targeted p21WAF1/CIP1 activation by RNAa inhibits hepatocellular carcinoma cells. *Nucleic Acid Ther* 22: 335-343, 2012.

56. Krishnan J and Mishra RK. Emerging trends of long non-coding RNAs in gene activation. *FEBS J* 281: 34-45, 2014.

Laham-Karam N.

57. Krol J, Loedige I and Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet* 11: 597-610, 2010.

58. Kuwabara T, Hsieh J, Nakashima K, Taira K and Gage FH. A small modulatory dsRNA specifies the fate of adult neural stem cells. *Cell* 116: 779-793, 2004.

59. Laham-Karam N, Lalli M, Leinonen N and Yla-Herttuala S. Differential Regulation of Vascular Endothelial Growth Factors by Promoter-targeted shRNAs. *Mol Ther Nucleic Acids* 4: e243, 2015.

60. Lai F, Orom UA, Cesaroni M, Beringer M, Taatjes DJ, Blobel GA and Shiekhattar R. Activating RNAs associate with Mediator to enhance chromatin architecture and transcription. *Nature* 494: 497-501, 2013.

61. Lam MT, Cho H, Lesch HP, Gosselin D, Heinz S, Tanaka-Oishi Y, Benner C, Kaikkonen MU, Kim AS, Kosaka M, Lee CY, Watt A, Grossman TR, Rosenfeld MG, Evans RM and Glass CK. Rev-Erbs repress macrophage gene expression by inhibiting enhancer-directed transcription. *Nature* 498: 511-515, 2013.

62. Lam MT, Li W, Rosenfeld MG and Glass CK. Enhancer RNAs and regulated transcriptional programs. *Trends Biochem Sci* 39: 170-182, 2014.

63. Lam MTY, Li W, Rosenfeld MG and Glass CK. Enhancer RNAs and regulated transcriptional programs. *Trends Biochem Sci* 39: 170-182, 2014.

64. Lee JT, Davidow LS and Warshawsky D. Tsix, a gene antisense to Xist at the X-inactivation centre. *Nat Genet* 21: 400-404, 1999.

65. Lee RC, Feinbaum RL and Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 75: 843-854, 1993.

66. Li C, Jiang W, Hu Q, Li LC, Dong L, Chen R, Zhang Y, Tang Y, Thrasher JB, Liu CB and Li B. Enhancing DPYSL3 gene expression via a promoter-targeted small activating RNA approach suppresses cancer cell motility and metastasis. *Oncotarget* 7: 22893-22910, 2016.

Laham-Karam N.

67. Li JQ, Hu SY, Wang ZY, Lin J, Jian S, Dong YC, Wu XF, Dai-Lan and Cao LJ. Long non-coding RNA MEG3 inhibits microRNA-125a-5p expression and induces immune imbalance of Treg/Th17 in immune thrombocytopenic purpura. *Biomed Pharmacother* 83: 905-911, 2016.

68. Li LC. Chromatin remodeling by the small RNA machinery in mammalian cells. *Epigenetics* 9: 45-52, 2014.

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

70. Li M, Peng Z, Ren W and Wang Z. Small activating ribonucleic acid reverses tyrosine kinase inhibitor resistance in epidermal growth factor receptor-mutant lung cancer by increasing the expression of phosphatase and tensin homolog. *Thorac Cancer* 7: 481-485, 2016.

71. Li W, Notani D, Ma Q, Tanasa B, Nunez E, Chen AY, Merkurjev D, Zhang J, Ohgi K, Song X, Oh S, Kim HS, Glass CK and Rosenfeld MG. Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature* 498: 516-520, 2013.

72. Li W, Notani D and Rosenfeld MG. Enhancers as non-coding RNA transcription units: recent insights and future perspectives. *Nat Rev Genet* 17: 207-223, 2016.

73. Lin YC, Benner C, Mansson R, Heinz S, Miyazaki K, Miyazaki M, Chandra V, Bossen C, Glass CK and Murre C. Global changes in the nuclear positioning of genes and intra- and interdomain genomic interactions that orchestrate B cell fate. *Nat Immunol* 13: 1196-1204, 2012.

74. Liu GY, Zhao GN, Chen XF, Hao DL, Zhao X, Lv X and Liu DP. The long noncoding RNA Gm15055 represses Hoxa gene expression by recruiting PRC2 to the gene cluster. *Nucleic Acids Res* 44: 2613-2627, 2016.

75. Liu SJ, Horlbeck MA, Cho SW, Birk HS, Malatesta M, He D, Attenello FJ, Villalta JE, Cho MY, Chen Y, Mandegar MA, Olvera MP, Gilbert LA, Conklin BR, Chang HY, Weissman JS and Lim DA. CRISPRi-based

genome-scale identification of functional long noncoding RNA loci in human cells. *Science* 355: 10.1126/science.aah7111. Epub 2016 Dec 15, 2017.

76. Liu X, Xiao ZD, Han L, Zhang J, Lee SW, Wang W, Lee H, Zhuang L, Chen J, Lin HK, Wang J, Liang H and Gan B. LncRNA NBR2 engages a metabolic checkpoint by regulating AMPK under energy stress. *Nat Cell Biol* 18: 431-442, 2016.

77. Majid S, Dar AA, Saini S, Yamamura S, Hirata H, Tanaka Y, Deng G and Dahiya R. MicroRNA-205-directed transcriptional activation of tumor suppressor genes in prostate cancer. *Cancer* 116: 5637-5649, 2010.

78. Matsui M, Chu Y, Zhang H, Gagnon KT, Shaikh S, Kuchimanchi S, Manoharan M, Corey DR and Janowski BA. Promoter RNA links transcriptional regulation of inflammatory pathway genes. *Nucleic Acids Res* 41: 10086-10109, 2013.

79. Matsui M, Sakurai F, Elbashir S, Foster DJ, Manoharan M and Corey DR. Activation of LDL receptor expression by small RNAs complementary to a noncoding transcript that overlaps the LDLR promoter. *Chem Biol* 17: 1344-1355, 2010.

80. Mayrose I, Stern A, Burdelova EO, Sabo Y, Laham-Karam N, Zamostiano R, Bacharach E and Pupko T. Synonymous site conservation in the HIV-1 genome. *BMC Evol Biol* 13: 164-2148-13-164, 2013.

81. Melo CA, Drost J, Wijchers PJ, van de Werken H, de Wit E, Oude Vrielink JA, Elkon R, Melo SA, Leveille N, Kalluri R, de Laat W and Agami R. eRNAs are required for p53-dependent enhancer activity and gene transcription. *Mol Cell* 49: 524-535, 2013.

82. Meng X, Jiang Q, Chang N, Wang X, Liu C, Xiong J, Cao H and Liang Z. Small activating RNA binds to the genomic target site in a seed-region-dependent manner. *Nucleic Acids Res* 44: 2274-2282, 2016.

83. Mette MF, Aufsatz W, Kanno T, Daxinger L, Rovina P, Matzke M and Matzke AJ. Analysis of double-stranded RNA and small RNAs involved in RNA-mediated transcriptional gene silencing. *Methods Mol Biol* 309: 61-82, 2005.

Laham-Karam N.

84. Mette MF, Aufsatz W, van der Winden J, Matzke MA and Matzke AJ. Transcriptional silencing and promoter methylation triggered by double-stranded RNA. *EMBO J* 19: 5194-5201, 2000.

85. Michel M, Demel C, Zacher B, Schwalb B, Krebs S, Blum H, Gagneur J and Cramer P. TT-seq captures enhancer landscapes immediately after T-cell stimulation. *Mol Syst Biol* 13: 920, 2017.

86. Morris KV. RNA-mediated transcriptional gene silencing in human cells. *Curr Top Microbiol Immunol* 320: 211-224, 2008.

87. Morris KV, Chan SW, Jacobsen SE and Looney DJ. Small interfering RNA-induced transcriptional gene silencing in human cells. *Science* 305: 1289-1292, 2004.

88. Moses J, Goodchild A and Rivory LP. Intended transcriptional silencing with siRNA results in gene repression through sequence-specific off-targeting. *RNA* 16: 430-441, 2010.

89. Mousavi K, Zare H, Dell'orso S, Grontved L, Gutierrez-Cruz G, Derfoul A, Hager GL and Sartorelli V. eRNAs promote transcription by establishing chromatin accessibility at defined genomic loci. *Mol Cell* 51: 606-617, 2013.

90. Mukherjee N, Calviello L, Hirsekorn A, de Pretis S, Pelizzola M and Ohler U. Integrative classification of human coding and noncoding genes through RNA metabolism profiles. *Nat Struct Mol Biol* 24: 86-96, 2017.

91. Nelson BR, Makarewich CA, Anderson DM, Winders BR, Troupes CD, Wu F, Reese AL, McAnally JR, Chen X, Kavalali ET, Cannon SC, Houser SR, Bassel-Duby R and Olson EN. A peptide encoded by a transcript annotated as long noncoding RNA enhances SERCA activity in muscle. *Science* 351: 271-275, 2016.

92. Niazi F and Valadkhan S. Computational analysis of functional long noncoding RNAs reveals lack of peptide-coding capacity and parallels with 3' UTRs. *RNA* 18: 825-843, 2012.

93. Ohhata T, Matsumoto M, Leeb M, Shibata S, Sakai S, Kitagawa K, Niida H, Kitagawa M and Wutz A. Histone H3 Lysine 36 Trimethylation Is Established over the Xist Promoter by Antisense Tsix Transcription and Contributes to Repressing Xist Expression. *Mol Cell Biol* 35: 3909-3920, 2015.

Laham-Karam N.

94. Omoto S and Fujii YR. Regulation of human immunodeficiency virus 1 transcription by nef microRNA. *J Gen Virol* 86: 751-755, 2005.

95. Ordog T, Syed SA, Hayashi Y and Asuzu DT. Epigenetics and chromatin dynamics: a review and a paradigm for functional disorders. *Neurogastroenterol Motil* 24: 1054-1068, 2012.

96. Orom UA, Derrien T, Beringer M, Gumireddy K, Gardini A, Bussotti G, Lai F, Zytnicki M, Notredame C, Huang Q, Guigo R and Shiekhattar R. Long noncoding RNAs with enhancer-like function in human cells. *Cell* 143: 46-58, 2010.

97. Orom UA, Nielsen FC and Lund AH. MicroRNA-10a binds the 5'UTR of ribosomal protein mRNAs and enhances their translation. *Mol Cell* 30: 460-471, 2008.

98. Pal-Bhadra M, Bhadra U and Birchler JA. RNAi related mechanisms affect both transcriptional and posttranscriptional transgene silencing in Drosophila. *Mol Cell* 9: 315-327, 2002.

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

100. Peng JC and Lin H. Beyond transposons: the epigenetic and somatic functions of the Piwi-piRNA mechanism. *Curr Opin Cell Biol* 25: 190-194, 2013.

101. Peng W and Fan H. Long noncoding RNA CCHE1 indicates a poor prognosis of hepatocellular carcinoma and promotes carcinogenesis via activation of the ERK/MAPK pathway. *Biomed Pharmacother* 83: 450-455, 2016.

102. Pirrotta V. The Necessity of Chromatin: A View in Perspective. *Cold Spring Harb Perspect Biol* 8: a019547, 2016.

103. Place RF, Li LC, Pookot D, Noonan EJ and Dahiya R. MicroRNA-373 induces expression of genes with complementary promoter sequences. *Proc Natl Acad Sci U S A* 105: 1608-1613, 2008.

Laham-Karam N.

104. Place RF, Noonan EJ, Foldes-Papp Z and Li LC. Defining features and exploring chemical modifications to manipulate RNAa activity. *Curr Pharm Biotechnol* 11: 518-526, 2010.

105. Place RF, Wang J, Noonan EJ, Meyers R, Manoharan M, Charisse K, Duncan R, Huang V, Wang X and Li LC. Formulation of Small Activating RNA Into Lipidoid Nanoparticles Inhibits Xenograft Prostate Tumor Growth by Inducing p21 Expression. *Mol Ther Nucleic Acids* 1: e15, 2012.

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

107. Portoso M, Ragazzini R, Brencic Z, Moiani A, Michaud A, Vassilev I, Wassef M, Servant N, Sargueil B and Margueron R. PRC2 is dispensable for HOTAIR-mediated transcriptional repression. *EMBO J* 36: 981-994, 2017.

108. Qin Q, Lin YW, Zheng XY, Chen H, Mao QQ, Yang K, Huang SJ and Zhao ZY. RNAa-mediated overexpression of WT1 induces apoptosis in HepG2 cells. *World J Surg Oncol* 10: 11-7819-10-11, 2012.

109. Reebye V, Saetrom P, Mintz PJ, Huang KW, Swiderski P, Peng L, Liu C, Liu X, Lindkaer-Jensen S, Zacharoulis D, Kostomitsopoulos N, Kasahara N, Nicholls JP, Jiao LR, Pai M, Spalding DR, Mizandari M, Chikovani T, Emara MM, Haoudi A, Tomalia DA, Rossi JJ and Habib NA. Novel RNA oligonucleotide improves liver function and inhibits liver carcinogenesis in vivo. *Hepatology* 59: 216-227, 2014.

110. Ren S, Kang MR, Wang J, Huang V, Place RF, Sun Y and Li LC. Targeted induction of endogenous NKX3-1 by small activating RNA inhibits prostate tumor growth. *Prostate* 73: 1591-1601, 2013.

111. Rinn JL and Chang HY. Genome regulation by long noncoding RNAs. *Annu Rev Biochem* 81: 145-166, 2012.

112. Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E and Chang HY. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. *Cell* 129: 1311-1323, 2007.

Laham-Karam N.

113. Robbins M, Judge A and MacLachlan I. siRNA and innate immunity. *Oligonucleotides* 19: 89-102, 2009.

114. Roberts TC, Andaloussi SE, Morris KV, McClorey G and Wood MJ. Small RNA-Mediated Epigenetic Myostatin Silencing. *Mol Ther Nucleic Acids* 1: e23, 2012.

115. Rothschild G and Basu U. Lingering Questions about Enhancer RNA and Enhancer Transcription-Coupled Genomic Instability. *Trends Genet* 33: 143-154, 2017.

116. Ruby JG, Jan CH and Bartel DP. Intronic microRNA precursors that bypass Drosha processing. *Nature* 448: 83-86, 2007.

117. Sanyal A, Lajoie BR, Jain G and Dekker J. The long-range interaction landscape of gene promoters. *Nature* 489: 109-113, 2012.

118. Sas-Chen A, Srivastava S and Yarden Y. The short and the long: non-coding RNAs and growth factors in cancer progression. *Biochem Soc Trans* 45: 51-64, 2017.

119. Schaukowitch K, Joo JY, Liu X, Watts JK, Martinez C and Kim TK. Enhancer RNA facilitates NELF release from immediate early genes. *Mol Cell* 56: 29-42, 2014.

120. Schmitz SU, Grote P and Herrmann BG. Mechanisms of long noncoding RNA function in development and disease. *Cell Mol Life Sci* 73: 2491-2509, 2016.

121. Schwanhausser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, Chen W and Selbach M. Global quantification of mammalian gene expression control. *Nature* 473: 337-342, 2011.

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

123. Seth M, Shirayama M, Gu W, Ishidate T, Conte D,Jr and Mello CC. The C. elegans CSR-1 argonaute pathway counteracts epigenetic silencing to promote germline gene expression. *Dev Cell* 27: 656-663, 2013.

Laham-Karam N.

124. Siomi MC, Sato K, Pezic D and Aravin AA. PIWI-interacting small RNAs: the vanguard of genome defence. *Nat Rev Mol Cell Biol* 12: 246-258, 2011.

125. Sun HJ, Hou B, Wang X, Zhu XX, Li KX and Qiu LY. Endothelial dysfunction and cardiometabolic diseases: Role of long non-coding RNAs. *Life Sci* 167: 6-11, 2016.

126. Sun M, Nie F, Wang Y, Zhang Z, Hou J, He D, Xie M, Xu L, De W, Wang Z and Wang J. LncRNA HOXA11-AS Promotes Proliferation and Invasion of Gastric Cancer by Scaffolding the Chromatin Modification Factors PRC2, LSD1, and DNMT1. *Cancer Res* 76: 6299-6310, 2016.

127. Suzuki K, Ishida T, Yamagishi M, Ahlenstiel C, Swaminathan S, Marks K, Murray D, McCartney EM, Beard MR, Alexander M, Purcell DF, Cooper DA, Watanabe T and Kelleher AD. Transcriptional gene silencing of HIV-1 through promoter targeted RNA is highly specific. *RNA Biol* 8: 1035-1046, 2011.

128. Taguchi YH. Apparent microRNA-Target-specific Histone Modification in Mammalian Spermatogenesis. *Evol Bioinform Online* 11: 13-26, 2015.

129. Tao H, Yang JJ, Hu W, Shi KH, Deng ZY and Li J. Noncoding RNA as regulators of cardiac fibrosis: current insight and the road ahead. *Pflugers Arch* 468: 1103-1111, 2016.

130. Turunen MP, Husso T, Musthafa H, Laidinen S, Dragneva G, Laham-Karam N, Honkanen S, Paakinaho A, Laakkonen JP, Gao E, Vihinen-Ranta M, Liimatainen T and Yla-Herttuala S. Epigenetic upregulation of endogenous VEGF-A reduces myocardial infarct size in mice. *PLoS One* 9: e89979, 2014.

131. Turunen MP, Lehtola T, Heinonen SE, Assefa GS, Korpisalo P, Girnary R, Glass CK, Vaisanen S and Yla-Herttuala S. Efficient regulation of VEGF expression by promoter-targeted lentiviral shRNAs based on epigenetic mechanism: a novel example of epigenetherapy. *Circ Res* 105: 604-609, 2009.

132. Vadaie N and Morris KV. Long antisense non-coding RNAs and the epigenetic regulation of gene expression. *Biomol Concepts* 4: 411-415, 2013.

Laham-Karam N.

133. Valadkhan S and Gunawardane LS. lncRNA-mediated regulation of the interferon response. *Virus Res* 212: 127-136, 2016.

134. Vallot C, Huret C, Lesecque Y, Resch A, Oudrhiri N, Bennaceur-Griscelli A, Duret L and Rougeulle C. XACT, a long noncoding transcript coating the active X chromosome in human pluripotent cells. *Nat Genet* 45: 239-241, 2013.

135. Vallot C, Patrat C, Collier AJ, Huret C, Casanova M, Liyakat Ali TM, Tosolini M, Frydman N, Heard E, Rugg-Gunn PJ and Rougeulle C. XACT Noncoding RNA Competes with XIST in the Control of X Chromosome Activity during Human Early Development. *Cell Stem Cell* 20: 102-111, 2017.

136. Vasudevan S, Tong Y and Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. *Science* 318: 1931-1934, 2007.

137. Voutila J, Saetrom P, Mintz P, Sun G, Alluin J, Rossi JJ, Habib NA and Kasahara N. Gene Expression Profile Changes After Short-activating RNA-mediated Induction of Endogenous Pluripotency Factors in Human Mesenchymal Stem Cells. *Mol Ther Nucleic Acids* 1: e35, 2012.

138. Wahid F, Shehzad A, Khan T and Kim YY. MicroRNAs: synthesis, mechanism, function, and recent clinical trials. *Biochim Biophys Acta* 1803: 1231-1243, 2010.

139. Wan P, Su W and Zhuo Y. The Role of Long Noncoding RNAs in Neurodegenerative Diseases. *Mol Neurobiol* 54: 2012-2021, 2017.

140. Wan Y, Garner J, Wu N, Phillip L, Han Y, McDaniel K, Annable T, Zhou T, Francis H, Glaser S, Huang Q, Alpini G and Meng F. Role of stem cells during diabetic liver injury. *J Cell Mol Med* 20: 195-203, 2016.

141. Wang B, Sun J, Shi J, Guo Q, Tong X, Zhang J, Hu N and Hu Y. Small-Activating RNA Can Change Nucleosome Positioning in Human Fibroblasts. *J Biomol Screen* 21: 634-642, 2016.

142. Wang C, Chen Z, Ge Q, Hu J, Li F, Hu J, Xu H, Ye Z and Li LC. Up-regulation of p21(WAF1/CIP1) by miRNAs and its implications in bladder cancer cells. *FEBS Lett* 588: 4654-4664, 2014.

Laham-Karam N.

143. Wang D, Garcia-Bassets I, Benner C, Li W, Su X, Zhou Y, Qiu J, Liu W, Kaikkonen MU, Ohgi KA, Glass CK, Rosenfeld MG and Fu XD. Reprogramming transcription by distinct classes of enhancers functionally defined by eRNA. *Nature* 474: 390-394, 2011.

144. Wang J and Li LC. Small RNA and its application in andrology and urology. *Transl Androl Urol* 1: 33-43, 2012.

145. Wang J, Place RF, Huang V, Wang X, Noonan EJ, Magyar CE, Huang J and 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* 70: 10182-10191, 2010.

146. Wang J, Place RF, Portnoy V, Huang V, Kang MR, Kosaka M, Ho MK and Li LC. Inducing gene expression by targeting promoter sequences using small activating RNAs. *J Biol Methods* 2: e14, 2015.

147. Wang KC, Yang YW, Liu B, Sanyal A, Corces-Zimmerman R, Chen Y, Lajoie BR, Protacio A, Flynn RA, Gupta RA, Wysocka J, Lei M, Dekker J, Helms JA and Chang HY. A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. *Nature* 472: 120-124, 2011.

148. Wang T, Li M, Yuan H, Zhan Y, Xu H, Wang S, Yang W, Liu J, Ye Z and Li LC. saRNA guided iNOS up-regulation improves erectile function of diabetic rats. *J Urol* 190: 790-798, 2013.

149. Wang X, Wang J, Huang V, Place RF and Li LC. Induction of NANOG expression by targeting promoter sequence with small activating RNA antagonizes retinoic acid-induced differentiation. *Biochem J* 443: 821-828, 2012.

150. Wang XQ and Dostie J. Reciprocal regulation of chromatin state and architecture by HOTAIRM1 contributes to temporal collinear HOXA gene activation. *Nucleic Acids Res*, 2016.

151. Watanabe T, Tomizawa S, Mitsuya K, Totoki Y, Yamamoto Y, Kuramochi-Miyagawa S, Iida N, Hoki Y, Murphy PJ, Toyoda A, Gotoh K, Hiura H, Arima T, Fujiyama A, Sado T, Shibata T, Nakano T, Lin H, Ichiyanagi

Laham-Karam N.

K, Soloway PD and Sasaki H. Role for piRNAs and noncoding RNA in de novo DNA methylation of the imprinted mouse Rasgrf1 locus. *Science* 332: 848-852, 2011.

152. Watts JK, Yu D, Charisse K, Montaillier C, Potier P, Manoharan M and Corey DR. Effect of chemical modifications on modulation of gene expression by duplex antigene RNAs that are complementary to non-coding transcripts at gene promoters. *Nucleic Acids Res* 38: 5242-5259, 2010.

153. Wedeles CJ, Wu MZ and Claycomb JM. Silent no more: Endogenous small RNA pathways promote gene expression. *Worm* 3: e28641, 2014.

154. Weinberg MS, Barichievy S, Schaffer L, Han J and Morris KV. An RNA targeted to the HIV-1 LTR promoter modulates indiscriminate off-target gene activation. *Nucleic Acids Res* 35: 7303-7312, 2007.

155. Weinmann L, Hock J, Ivacevic T, Ohrt T, Mutze J, Schwille P, Kremmer E, Benes V, Urlaub H and Meister G. Importin 8 is a gene silencing factor that targets argonaute proteins to distinct mRNAs. *Cell* 136: 496-507, 2009.

156. Whitson JM, Noonan EJ, Pookot D, Place RF and Dahiya R. Double stranded-RNA-mediated activation of P21 gene induced apoptosis and cell cycle arrest in renal cell carcinoma. *Int J Cancer* 125: 446-452, 2009.

157. Xia W, Li D, Wang G, Ni J, Zhuang J, Ha M, Wang J and Ye Y. Small activating RNA upregulates NIS expression: promising potential for hepatocellular carcinoma endoradiotherapy. *Cancer Gene Ther* 23: 333-340, 2016.

158. Xie D, Shang L, Peng L and Li L. Up-regulation of VEZT by small activating RNA inhibits the proliferation, invasion and migration of gastric cancer cells. *Biochem Biophys Res Commun*, 2016.

159. Xu P, Vernooy SY, Guo M and Hay BA. The Drosophila microRNA Mir-14 suppresses cell death and is required for normal fat metabolism. *Curr Biol* 13: 790-795, 2003.

160. Yan H, Yuan J, Gao L, Rao J and Hu J. Long noncoding RNA MEG3 activation of p53 mediates ischemic neuronal death in stroke. *Neuroscience* 337: 191-199, 2016.

Laham-Karam N.

161. Yang K, Shen J, Xie YQ, Lin YW, Qin J, Mao QQ, Zheng XY and Xie LP. Promoter-targeted double-stranded small RNAs activate PAWR gene expression in human cancer cells. *Int J Biochem Cell Biol* 45: 1338-1346, 2013.

162. Yang L, Lin C, Liu W, Zhang J, Ohgi KA, Grinstein JD, Dorrestein PC and Rosenfeld MG. ncRNA- and Pc2 methylation-dependent gene relocation between nuclear structures mediates gene activation programs. *Cell* 147: 773-788, 2011.

163. Yin H and Lin H. An epigenetic activation role of Piwi and a Piwi-associated piRNA in Drosophila melanogaster. *Nature* 450: 304-308, 2007.

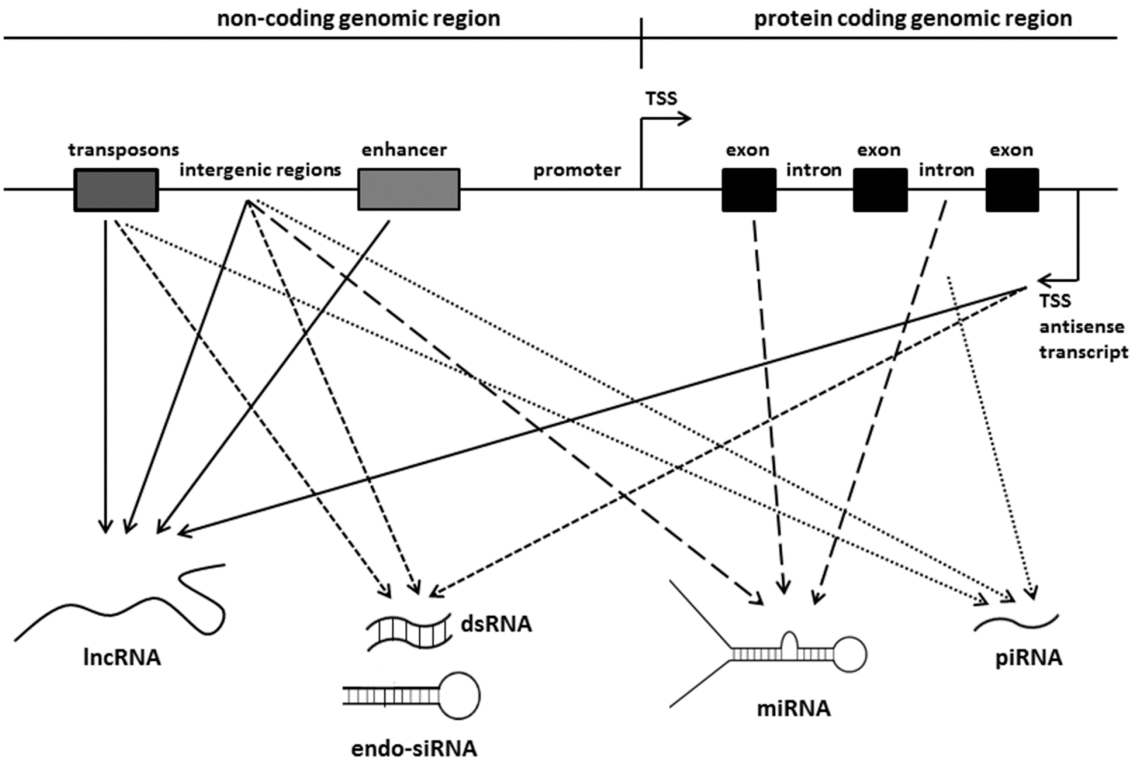
164. Yla-Herttuala S and Kaikkonen M. Does mass balance between sense and antisense transcripts fine-tune the outcome of gene expression? *EMBO Rep* 15: 125-126, 2014.

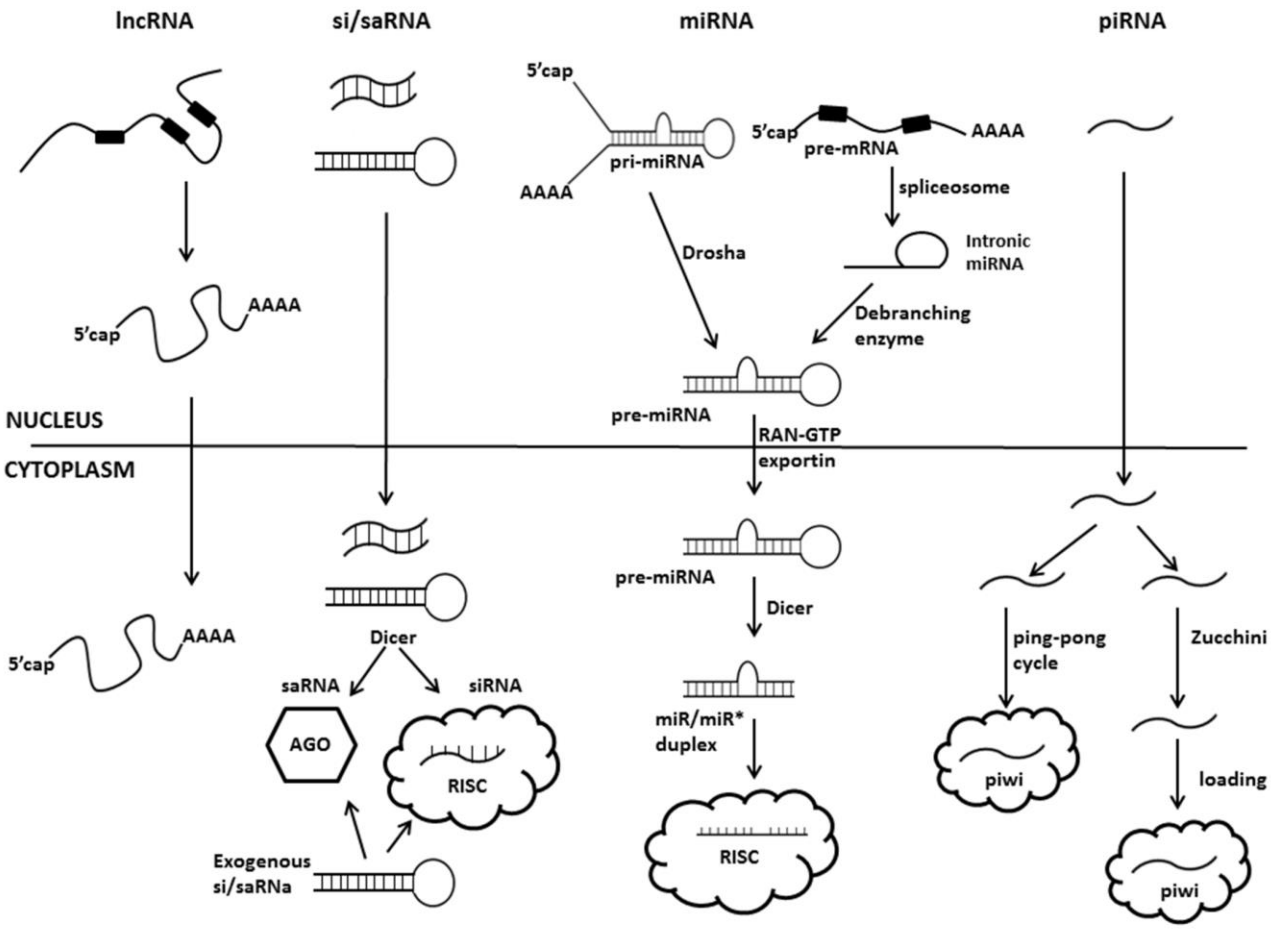
165. Yoon S, Huang KW, Reebye V, Mintz P, Tien YW, Lai HS, Saetrom P, Reccia I, Swiderski P, Armstrong B, Jozwiak A, Spalding D, Jiao L, Habib N and Rossi JJ. Targeted Delivery of C/EBPalpha -saRNA by Pancreatic Ductal Adenocarcinoma-specific RNA Aptamers Inhibits Tumor Growth In Vivo. *Mol Ther* 24: 1106-1116, 2016.

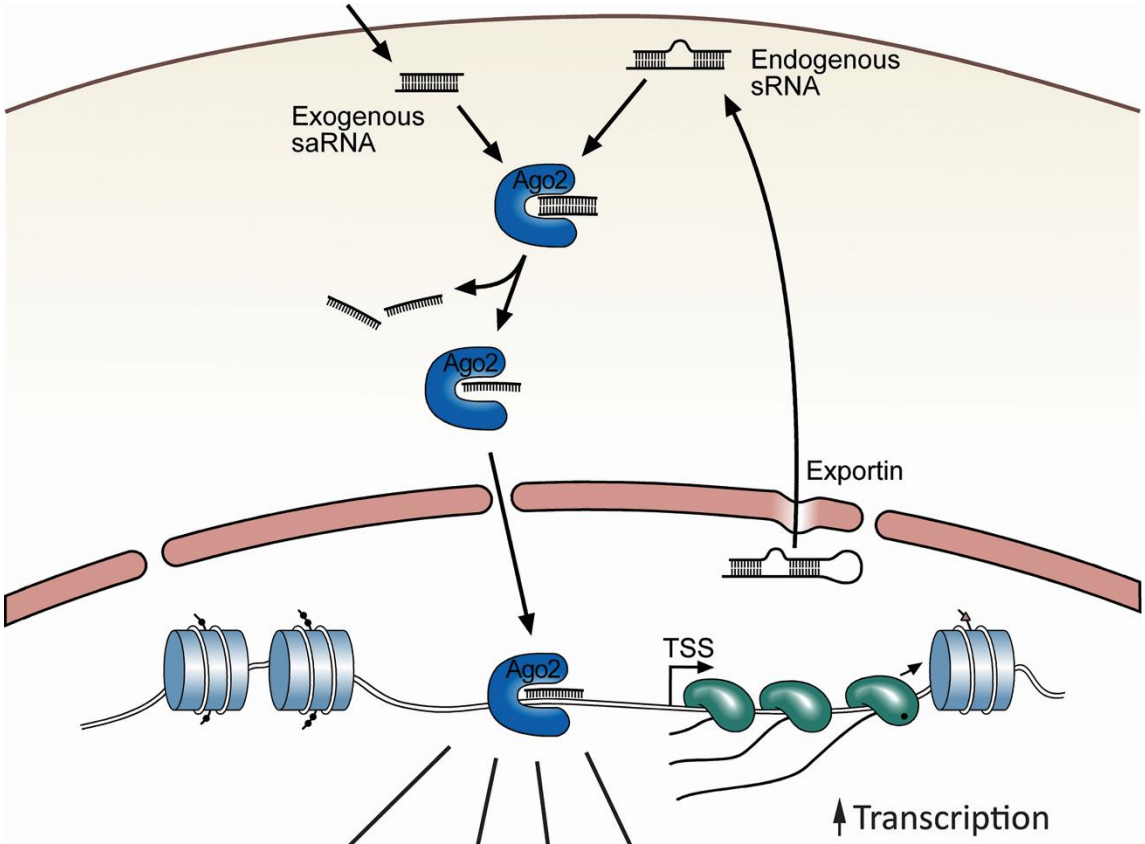
166. Younger ST, Pertsemlidis A and Corey DR. Predicting potential miRNA target sites within gene promoters. *Bioorg Med Chem Lett* 19: 3791-3794, 2009.

167. Zamudio JR, Kelly TJ and Sharp PA. Argonaute-bound small RNAs from promoter-proximal RNA polymerase II. *Cell* 156: 920-934, 2014.

168. Zhu B, Mandal SS, Pham AD, Zheng Y, Erdjument-Bromage H, Batra SK, Tempst P and Reinberg D. The human PAF complex coordinates transcription with events downstream of RNA synthesis. *Genes Dev* 19: 1668-1673, 2005.


non-coding genomic region
protein coding genomic region
TSS
transposons
intergenic regions
enhancer
promoter
exon
intron
exon
intron
exon
TSS
antisense
transcript
lncRNA
dsRNA
endo-siRNA
miRNA
piRNA


lncRNA
si/saRNA
miRNA
piRNA
5'cap
AAAA
pri-miRNA
pre-mRNA
spliceosome
Intronic miRNA
Drosha
Debranching enzyme
pre-miRNA
NUCLEUS
CYTOPLASM
RAN-GTP exportin
Dicer
saRNA
siRNA
AGO
RISC
Exogenous si/saRNa
miR/miR* duplex
ping-pong cycle
Zucchini
piwi
loading



Transcription factors

NK3 homeobox1 (NKX3-1; 39)
Krupple-like factor (KLF4; 145)
Myc proto-oncogene (Myc; 137)
Nanog homeobox (NANOG; 149)
CCAAT/enhancer binding protein alpha (CEBPA; 109)

Receptors, transporters and enzymes

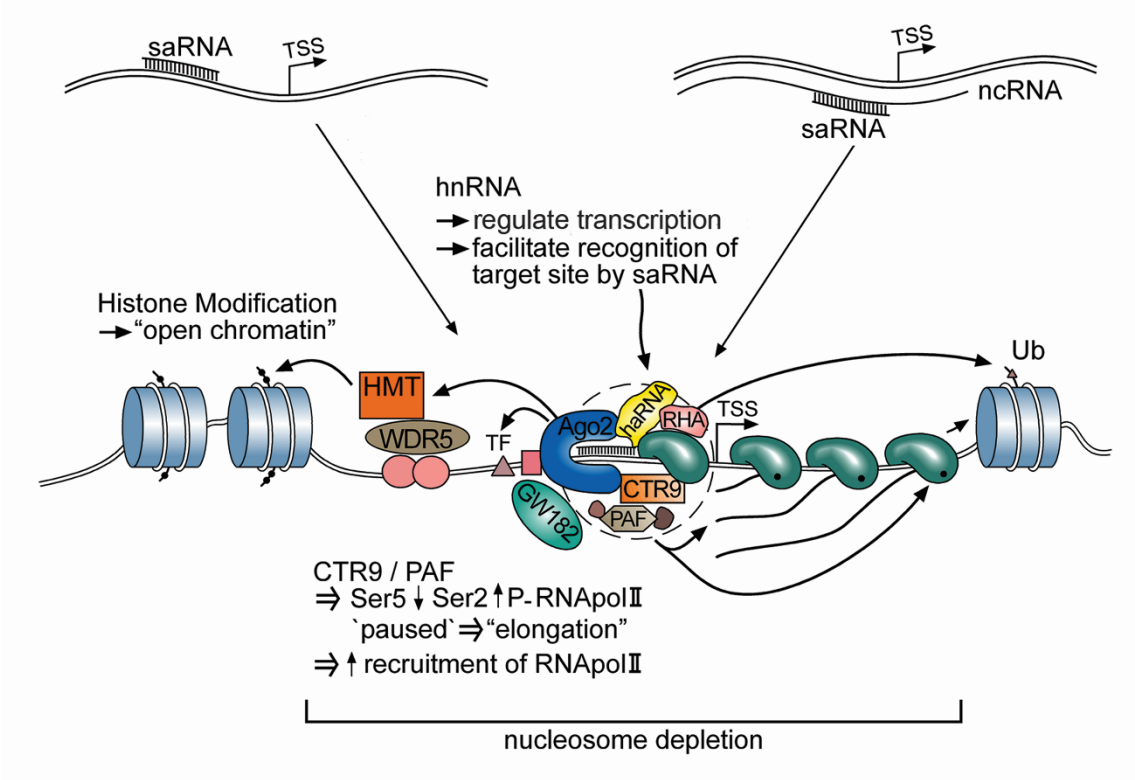
Progesterone receptor (PR; 44)
Major vault protein (MVP; 44)
Chemokine C-X-C receptor 4 (rCSCR4; 39)
Low density lipoprotein receptor (LDLR; 79)
Vascular endothelial growth factor-R1 (FLT1; 59)
Na/I symporter (NIS; 257)

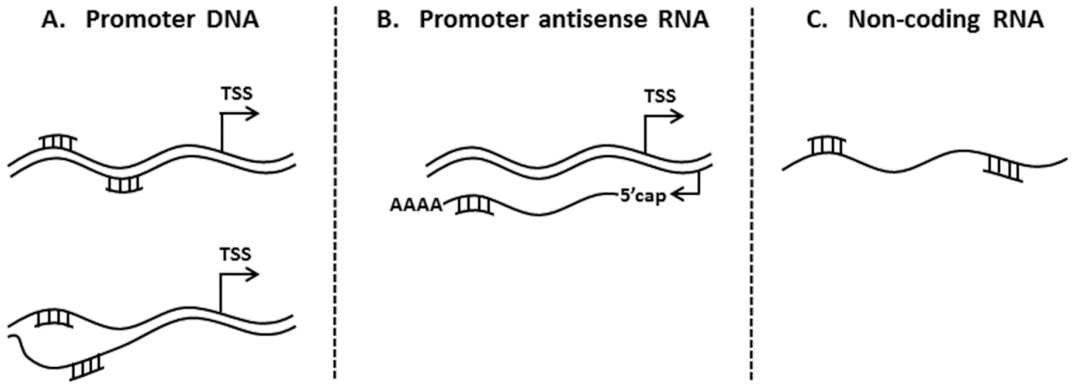
Cell cycle and cancer related genes

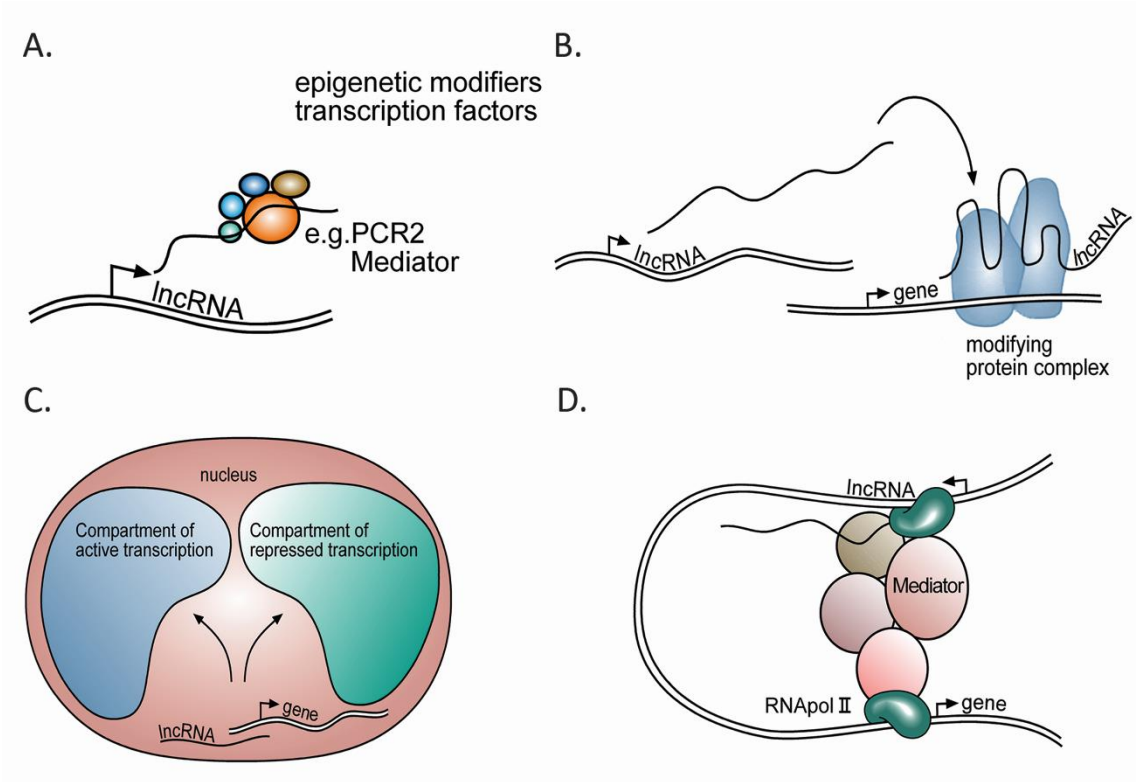
P21 (CDKN1A; 69)
Tumour protein p53 (TP53; 39)
PRKC apoptosis WT1 regulator (PAWR; 39)
Wilms tumour 1 (WT; 39)
Hypermethylated in cancer 1 protein (HIC1; 99)
Phosphatase and tensin homolog (PTEN; 70)
Mouse cyclin B1 (CCNB1; 39)

Growth factors and cellular adhesion

Vascular endothelial growth factor-A (VEGFA; 69, 131)
Vascular endothelial growth factor-C (VEGFC; 59)
E-cadherin (CDH1; 69)
Vezatin (VEST; 158)
Dihydropyrimidinase like 3 (DPYSL3; 66)


saRNA
TSS
TSS
ncRNA
saRNA
hnRNA
→regulate transcription
→facilitate recognition of
target site by saRNA
Histone Modification
→"open chromatin"
Ub
HMT
WDR5
TF
Ago2
haRNA
RHA
TSS
CTR9
PAF
GW182
CTR9 / PAF
⇒ Ser5↓ Ser2↑P-RNApolⅡ
`paused`⇒"elongation"
⇒↑recruitment of RNApolⅡ
nucleosome depletion


A. Promoter DNA
TSS
TSS
B. Promoter antisense RNA
TSS
AAAA
5'cap
C. Non-coding RNA


A.
epigenetic modifiers
transcription factors
e.g.PCR2
Mediator
lncRNA
B.
lncRNA
gene
lncRNA
modifying
protein complex
C.
nucleus
Compartment of
active transcription
Compartment of
repressed transcription
lncRNA
gene
D.
lncRNA
Mediator
RNApol II
gene