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Invited Mini Review

Functions of DEAD box RNA helicases DDX5 and DDX17 in chromatin organization and transcriptional regulation

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RNA helicases DDX5 and DDX17 are multitasking proteins that regulate gene expression in different biological contexts through diverse activities. Special attention has long been paid to their function as coregulators of transcription factors, providing insight about their functional association with a number of chromatin modifiers and remodelers. However, to date, the variety of described mechanisms has made it difficult to understand precisely how these proteins work at the molecular level, and the contribution of their ATPase domain to these mechanisms remains unclear as well. In light of their association with long noncoding RNAs that are key epigenetic regulators, an emerging view is that DDX5 and DDX17 may act through modulating the activity of various ribonucleoprotein complexes that could ensure their targeting to specific chromatin loci. This review will comprehensively describe the current knowledge on these different mechanisms. We will also discuss the potential roles of DDX5 and DDX17 on the 3D chromatin organization and how these could impact gene expression at the transcriptional and post-transcriptional levels. [BMB Reports 2018; 51(12): 613-622]

INTRODUCTION

DDX5 (p68) and DDX17 (p72) belong to the large family of evolutionarily conserved DEAD box RNA helicases (1). Because of their multiple molecular activities, these two proteins have several partially redundant functions in the regulation of gene expression, particularly in cell differentiation. Some of their most described functions include their control of nuclear microRNA processing and pre-mRNA alternative splicing, which is consistent with their ability to remodel ribonucleoprotein complexes. DDX5 and DDX17 share a highly homologous ATPase domain (90% homology in human proteins), and ATP binding and hydrolysis are necessary to catalyze the unwinding of local RNA secondary structures, as well as for their RNA annealing activity (2-4).

DDX5 and DDX17 are also important coregulators of various transcription factors, and they interact with a number of chromatin-associated factors (5). This may seem to conflict with their enzymatic activity, and it remains uncertain whether their helicase activity is required for this particular function. In animals, it is not known whether these proteins have an affinity for double-stranded DNA or RNA-DNA hybrids, which could be relevant for chromatin and transcription-related functions. However, note that the ortholog of DDX5 in peas (*Pisum sativum* Psp68) exhibits bidirectional DNA helicase activity (6). In this review, we will first update the different mechanisms by which DDX5 and DDX17 coregulate transcription factors, as they have been reviewed elsewhere (5), then we will specifically highlight the contribution of long noncoding RNA (lncRNAs) to DDX5/DDX17-mediated functions, as they help elucidate how RNA helicases could work in the context of ribonucleoprotein complexes. Finally, we will replace these mechanisms in a tridimensional genomic context and propose that DDX5/DDX17 may regulate specific aspects of gene expression by locally modulating the spatial and dynamic gene organization.

DDX5 AND DDX17 REGULATE THE TRANSCRIPTIONAL ACTIVITY OF SEVERAL TRANSCRIPTION FACTORS IN VARIOUS CELLULAR CONTEXTS

DDX5 and DDX17 regulate transcription through their direct interactions with several transcription factors (e.g. ERα, P53) (Table 1). As this has been reviewed previously (5), we will mostly focus below on the biological consequences of these interactions in normal or pathological situations.

The presence of DDX5 or DDX17 within transcription factor complexes may partly explain how the aberrant expression of DDX5 and/or DDX17 contributes to pathologies such as cancer (7-12). In addition to its well-described interaction with ERα and its involvement in breast cancer (13-15), DDX17 also interacts with SOX2 in a subset of ERα-positive breast cancer

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Table 1. Protein and noncoding RNA partners of DDX5 and DDX17

Transcription factor	Other protein partner	ncRNA	DDX partner	Function - biological context	Reference
TRP53			DDX5/DDX17	Apoptosis	(5)
VDR			DDX5	n.d.	
ERα	CBP, p300	*SRA*	DDX5/DDX17	Cell proliferation	
AR			DDX5/DDX17		
NF-KB p50			DDX5		(8)
SMADs	CBP		DDX5/DDX17		(18, 20, 21)
SOX2			DDX17		(16)
NOTCH1/RBP-J	p300, MAML1	*SRA*	DDX5		(53, 94)
STAT3			DDX5		(95)
E2F1			DDX5		(5)
Beta-Catenin			DDX5		(9, 12)
		mrhl	DDX5	Cell differentiation	(59)
MYOD	BRG1, p300	*SRA*	DDX5/DDX17	Myoblast differentiation	(21, 22)
RUNX2			DDX5	Osteoblast differentation	(23)
REST	EHMT2		DDX17	Neuronal differentiation	(24)
Chromatin modifiers					
HDAC1			DDX5/DDX17	n.d.	(26-28)
PRC2 - TrxG		*SRA*	DDX5	Pluripotency - HBV infection - Reprogramming inhibition	(30, 55)
SU(VAR)3-9			Rm62	Heat shock response	(44)
Insulators					
CTCF / Cohesin		*SRA*	DDX5	Gene insulation - 3D genome organization	(69, 96)
CP190		RNAi	Rm62	Gene insulation	(67)
		MeXis	DDX17	Lipid Homeostasis	(62)
		LOC284454	DDX5	Cell Migration - Breast cancer	(63)

Only transcription factors and chromatin-associated factors are indicated.

cells that respond to this transcription factor (16). In these stem cell-like cells, DDX17 contributes to the activation of SOX2-responsive genes by stabilizing SOX2 binding to its target promoters. DDX17 depletion is associated with a decrease of tumor characteristics (e.g. colony formation), highlighting its importance in breast tumorigenesis (16). Both RNA helicases were identified as SOX2 binding proteins in glioblastoma cells, suggesting that DDX5 may also be involved in SOX2 transcriptional activity (17).

DDX5 may also contribute to cancer development by modulating various signaling pathways. For example, DDX5 interacts with β-catenin in non-small-cell lung cancer cells as well as colorectal cancer cells, and it also promotes its nuclear translocation, which is associated with the coactivation of Wnt-responsive genes such as *MYC* or *CCND1* (12, 18). DDX5 and β-catenin are also involved together in the regulation of androgen receptor (AR) transcriptional activity in prostate cancer cells, where DDX5 promotes the recruitment of both transcription factors to AR target genes (9). Finally, the interaction between DDX5 and β-catenin contributes to the epithelial to mesenchymal transition (EMT), a process involved in the formation of metastases (19). Interestingly, some function of DDX5 (and DDX17) in EMT is also supported by its interaction with SMAD proteins, which are mediators of the EMT-inducing TGF-β pathway. DDX5 has been shown to enhance SMAD3 transcriptional activity in response to TGF-β (20), and more recently, our group has shown that DDX5 and DDX17 directly control the SMAD4-dependent expression of master EMT factors *SNAI1* and *SNAI2* upon TGF-β treatment (21).

This involvement of DDX5 and DDX17 in EMT, an embryonic trans-differentiation process, illustrates their contributions toward controlling cell fate transitions and cell differentiation. Accordingly, murine Ddx5 and Ddx17 are essential for the early stages of myoblast or osteoblast differentiation through their interaction with master transcription factors Myod or Runx2, respectively (21-23). In both cases, Ddx5 is recruited to Myod and Runx2 responsive promoters, and it enhances their transcriptional activity. During myogenesis, one consequence is the induced expression of myogenic microRNAs, myogenic transcription factors (Myog or Mef2c), as well as muscle specific genes (21, 22).

Recently, we have shown on a genome-wide scale that an interaction between an RNA helicase and a transcription factor can also lead to gene repression (24). DDX17 and DDX5 are necessary for repressing the expression of a large subset of neuronal genes in undifferentiated neuroblastoma cells, in cooperation with the REST transcription factor. The bindings of both REST and DDX17 to their target promoters are mutually dependent on each other, but the way in which RNA helicases stabilize REST complex binding at the chromatin remains unknown.

DDX5 AND DDX17 INTERACT WITH CHROMATIN MODIFIERS AND REMODELERS

As described above, the contributions of DDX5 and DDX17 to physiological or cancer-related processes may be mediated largely by their coregulator activity of transcription factors. As we will see below, DDX5 and DDX17 function by modulating chromatin accessibility at different levels, in an active or repressive way.

First, DDX5 and DDX17 have often been reported to modulate the post-translational modifications of histones. Both factors regulate histone acetylation positively or negatively by interacting with histone acetyltransferases or deacetylases. DDX5 and DDX17 interact and synergize with acetyltransferases CBP (CREB-binding protein) and p300 to activate transcription, such as in the context of SMAD3-mediated transcriptional activation (20, 25). In contrast, the interaction between RNA helicases and histone deacetylase 1 (HDAC1) contributes *in vitro* to the DDX5/DDX17-dependent corepression of specific promoters (26). Interestingly, this interaction is reinforced by the sumoylation of DDX5 and DDX17, which stabilizes both proteins but differentially affects their activities as transcriptional coregulators, depending on the tested promoter or transcription factor (27, 28).

DDX5 and DDX17 also have effects on histone methylation. In undifferentiated neuronal precursors, DDX17 is recruited to some of the REST-responsive promoters along with the histone methyltransferase EHMT2, and it is required for the binding of EHMT2 to chromatin (24). EHMT2 catalyzes the mono and dimethylation of histone H3 lysine 9 (H3K9me1/2) and contributes to REST-mediated repression (29). Our results suggest that a DDX17-controlled deposition of H3K9me2 mark to REST-responsive promoters could contribute to the REST- and DDX17-mediated repression of neuronal genes (24). In hepatocytes, DDX5 also interacts with several subunits of the Polycomb-repressive complex 2 (PRC2), including the histone methyltransferase EZH2, which catalyzes the trimethylation of histone H3 lysine 27 (30). Upon Hepatitis B virus (HBV) infection, DDX5 helps stabilize another PRC2 subunit, SUZ12, and hence allows for PRC2-mediated gene repression, particularly at key pluripotency genes. However, whether DDX5 is directly recruited to PRC2 target genes is yet to be tested (30).

Second, DDX5 and DDX17 interact with the BRG1 chromatin remodeler. In muscle cells, DDX5 and DDX17 recruit BRG1 to MYOD target genes, increasing the chromatin accessibility for the transcription machinery, which helps coactivate MYOD-dependent transcription (22).

Finally, several reports have suggested that DDX5 may be involved in the control of DNA methylation and/or demethylation of CpG dinucleotides, as it interacts with DNA methyltransferase 3 proteins (DNMT3A and B) as well as with thymine DNA glycosylase (TDG) (31-34). The exact role of DDX5 in the control of these processes is unclear. DDX5 is recruited to chromatin along with both DNMT3A/B and TDG proteins at the beginning of each transcription cycle of an ERα-responsive promoter, suggesting some function in the subsequent DNA demethylation of this promoter (34). Interestingly, TDG-mediated demethylation activity requires its association with CpG-rich ncRNAs that serve as guides for the enzyme (35). This raises the possibility that DDX5 and DDX17 could control this process by modulating RNA-DNA rearrangements. Importantly, the DDX5/DDX17-mediated modulation of local DNA demethylation would likely impact the binding of CTCF, another DDX5/DDX17-interacting factor that controls chromatin 3D architecture (see below).

All of these results reinforce the idea that these factors act at different levels for the fine epigenetic control of gene expression. The consequences of DDX5 and DDX17 activity on the chromatin environment and on transcription depend on the nature of the complex that is recruited along with their transcription factor partner.

THE REGULATORY ACTIVITY OF DDX5 AND DDX17 IN TRANSCRIPTION IS CONSERVED THROUGHOUT EVOLUTION

Dbp2, the DDX5 yeast ortholog, is associated to chromatin in *Saccharomyces Cerevisiae* and contributes to the fine-tuning control of the *GAL* gene cluster, which is required for galactose metabolism (36-38). In a glucose-rich environment, Dbp2 corepresses *GAL* genes with Cyc8 (38). In the absence of Dbp2 or upon a glucose-to-galactose switch, long antisense ncRNAs overlapping the repressed genes accumulate and form RNA-DNA hybrids (R-loops) that evict Cyc8 from the promoters and contribute to gene activation (36). Dbp2 may prevent the formation of these R-loops, either by promoting the assembly of ribonucleoprotein complexes onto the ncRNAs or by stabilizing them (37, 39). Genome-wide studies have suggested that Dbp2 and Cyc8 control many genes involved in sugar transport and energetic metabolism through a similar mechanism (40). This indicates that Dbp2, which is rapidly exported to the cytoplasm upon glucose deprivation (41), integrates nutritional signals in order to control energy homeostasis, a function that may be conserved in higher eukaryotes (42, 43).

In *Drosophila melanogaster*, several reports have also

highlighted a transcriptional silencing activity of the DDX5 ortholog, Rm62. Upon heat shock, Rm62 is recruited to the *hsp70* promoter, along with the histone methyltransferase SU(VAR)3-9. This allows the level of H3K9 methylation to increase in order to re-silence *hsp70* expression rapidly after heat shock (44). In line with this report, Rm62 promotes the clearance of transcripts from the chromatin, which allows for the deactivation of gene expression (45).

An intriguing possibility is that these repressive functions of DDX5 contribute to the formation of heterochromatin in connection with the RNA interference (RNAi) machinery. The RNAi pathway, which involves small RNA complexed with members of the Argonaute (AGO) protein family, controls mRNA translation and degradation in the cytoplasm, but it also has major nuclear functions to modify chromatin structure and control transcription, from yeast to mammals (46). Interestingly, DDX5 interacts with Ago2-containing complexes both in drosophila and in human cells, and Rm62/Ddx5 is required for RNAi in flies (47, 48). This suggests that DDX5 may be involved in the double-stranded RNA-mediated epigenetic control of gene expression, a fascinating question which should be further explored in future studies.

DDX5 AND DDX17 FORM COMPLEXES WITH LONG NON-CODING RNAS

Long noncoding RNAs (lncRNAs) are key epigenetic regulators of gene expression that employ a variety of mechanisms to enhance or silence promoter activity; these include recruiting and/or guiding chromatin modifiers or remodelers to specific regions, repressing specific factors by acting as decoys or via allosteric inhibition, or regulating the 3D organization of the genome (49, 50). As mentioned above, lncRNAs have also been found to regulate transcription in yeast, underlining the conservation of their function throughout evolution. DDX5 and DDX17 interact with various lncRNAs in several cell types, which impacts the chromatin state and the transcription of their target genes.

SRA

The most studied DDX5/DDX17-associated lncRNA is the steroid nuclear receptor activator RNA (*SRA*) that has been identified as a coactivator of several steroid nuclear receptors (51), although it also interacts with other transcription factors. *SRA* plays critical roles in diverse physiological and pathological processes, such as myogenesis, adipogenesis and cancer. Notably, alternative splicing generates several *SRA* isoforms that are expressed differently depending on the biological context, as during myogenesis, and some *SRA* variants code for a protein (SRAP) that antagonizes the cotranscriptional function of the lncRNA (52). This bifaceted aspect of the function of *SRA* should be accounted for when studying this lncRNA.

As with DDX5 and DDX17, *SRA* interacts with ERα, AR, Notch1, and MyoD (22, 51, 53). *SRA* coactivates the Notch pathway with Ddx5 in mouse Beko cells through its interaction with both the Notch intracellular domain and the downstream transcription factor RBP-J, as well as by promoting p300 recruitment to Notch target genes (53). During myogenic differentiation of mouse C2C12 cells, both RNA helicases and *SRA* coactivate the transcription factor MyoD, and the joint overexpression of *SRA* and Ddx5 stimulates the MyoD-induced conversion of mouse embryonic fibroblasts in skeletal muscle cells (22). Whether *SRA* contributes to the Ddx5/Ddx17-dependent recruitment of Brg1 to MyoD-activated promoters is unknown, and the function of *SRA* in the coactivation process remains unclear as well.

Importantly, *SRA* interacts with several factors involved in transcriptional silencing (54). Co-immunoprecipitation experiments have indicated that *SRA* associates with both TrxG and PRC2 complexes, which catalyze the trimethylation of histones H3 on lysine 4 (H3K4me3, active chromatin) and lysine 27 (H3K27me3, repressed chromatin), respectively (55). Members of a particular class of transcriptionally poised promoters carry both marks (bivalent promoters) and often correspond to the genes involved in differentiation. A significant fraction of *SRA* binding sites on chromatin carry the bivalent mark, suggesting that SRA could simultaneously guide both complexes (55). Remarkably, DDX5 facilitates the interaction of *SRA* only with the TrxG complex, and not with the PRC2 complex, perhaps depending on the capacity of DDX5 to alter the secondary structure of the lncRNA. Interestingly, in human pluripotent stem cells, the chromatin sites co-occupied by both *SRA* and DDX5 carry the H3K4me3 mark more frequently than those bound only by *SRA*, and DDX5 promotes *SRA*-mediated H3K4 methylation (55).

These results suggest that the *SRA* lncRNA can act as a multimodal scaffold for several complexes, and that it can be dynamically regulated by RNA helicases. Depending on the genomic context, *SRA* could contribute to the modification of histones in three different ways (mono or bivalent trimethylation) through TrxG and PRC2 activity, but in the presence of DDX5, the *SRA*-containing complex could switch to a more active form, thereby favouring the activation of target genes.

In the same study, the authors showed that *SRA* is required for reprogramming induced pluripotent stem cells (iPSC) (55). One hypothesis is that the lncRNA could be involved in the deposition of bivalent histone modifications, a phenomenon that has been found to increase during cell reprogramming (56). In this context, DDX5 would counteract the production of iPSC by promoting the *SRA*-TrxG association and limiting the number of bivalent sites. Interestingly, DDX5 has recently been identified as a barrier to iPSC reprogramming (57, 58). The mechanism proposed in that study was different, but diverse strategies could be employed by DDX5 to disturb this very complex process.

Mrhl

The meiotic recombination hot spot locus (*mrhl*) lncRNA is involved in the regulation of the Wnt signaling pathway in spermatogonial cells, where it interacts with DDX5 at specific chromatin loci in a manner linked to the regulation of the neighbouring genes (59, 60). *Mrhl* has also been shown to recruit corepressors to the negatively regulated *Sox8* promoter (61), indicating that it could act as a guide for repressive complexes. As most *mrhl*-regulated chromatin loci are bound by the lncRNA in a DDX5-dependent manner (60), DDX5 may allow for the correct folding of the lncRNA through its unwinding activity, although this hypothesis was not demonstrated in that study. Another hypothesis is that the lncRNA may retain the RNA helicase in the nucleus. Consequently, the activation of the Wnt pathway induced by *mrhl* downregulation may result at least in part from the relocation of DDX5 to the cytoplasm and from the DDX5-dependent nuclear translocation of β-catenin, as has been previously shown (19, 59).

MeXis

MeXis (macrophage-expressed LXR-induced sequence) is a chromatin-associated liver X receptor (LXR)-responsive lncRNA that regulates *in vivo* the expression of several LXR-responsive genes involved in cholesterol homeostasis, including the *Abca1* gene (62). Mechanistically, *MeXis* helps open the chromatin at the *Abca1* locus. Ddx17 has been identified as one protein partner of *MeXis*, and it is involved in the activation of the *Abca1* gene through its *MeXis*-dependent recruitment to chromatin. This suggests that the complex *MeXis*/Ddx17 contributes to the activation of LXR-responsive genes (62).

Altogether, these examples highlight the essential role of lncRNAs in the transcriptional corepression or coactivation by DDX5 and DDX17. It is likely that many lncRNAs interact with these proteins, and these interactions likely differ depending on the cell type or biological context. For example, 73 new DDX5-interacting lncRNAs have been recently identified in HEK 293T cells, including the chromatin-associated lncRNA *LOC284454*, which has a global impact on gene expression (63). It will therefore be important to determine whether the DDX5- and/or DDX17-dependent regulation of other transcription factors involves lncRNAs, as many of these factors (or their coregulators) have known RNA partners, like REST (64) or SOX2 (65).

THE REGULATORY FUNCTIONS OF DDX5 AND DDX17 IN A TRIDIMENSIONAL CHROMATIN CONTEXT

Thus far, we have discussed the transcription-related functions of DDX5 and DDX17 from a fairly basic perspective, describing their recruitment and/or stabilization to the chromatin along with a variety of protein and RNA cofactors, and the resulting epigenetic modifications affecting gene expression. However, we must consider these mechanisms in the context of a highly complex and dynamically regulated tridimensional organization of the genes, and more generally, of the nucleus (66). Increasing evidence suggests that DDX5 and DDX17 may have roles in controlling the spatial organization of genes, with direct implications on gene expression.

DDX5/DDX17 and the regulation of gene insulation

This role of DDX5 was first described in Drosophila. Rm62 has been shown to interact with insulator proteins, and with CP190 in particular, in an RNA-dependent manner (67, 68). Phenotypic and immunofluorescence analyses have shown that Rm62 negatively affects the function of the *gipsy* insulator as well as the formation of insulator bodies (67). The authors proposed that CP190-containing higher-order chromatin loops form onto insulator sequences and are stabilized by RNAs that may originate from the RNAi pathway. The recruitment of Rm62 may promote the dissociation of these protein-RNA insulator complexes through direct contacts with CP190 and RNAs. This function of Rm62 may be dependent on the type of insulator complex, as the activity of the *Fab-8* insulator was not affected by mutation of the *Rm62* gene (68).

In mammals, the main insulation factor is the zinc-finger protein CTCF (CCCTC-binding factor), which is also regulated by DDX5 and DDX17 (69). Both RNA helicases interact with CTCF in an *SRA*-dependent manner, but CTCF binding at the *IGF2*/*H19* imprinted insulator locus depends neither on DDX5 nor on *SRA*. In contrast, binding of the Cohesin complex, which often associates with CTCF (see also below), is reduced in the absences of DDX5 and *SRA*, and DDX5 binding reciprocally depends on CTCF, Cohesin, and *SRA*. These results suggest a mechanism in which CTCF is recruited on the insulator, where it combines with Cohesin and DDX5/*SRA* to insulate the *IGF2* gene from the enhancer region on the maternal allele. Supporting this model, the depletion of any of these components decreases *IGF2*/*H19* insulator function and increases chromatin looping between the enhancer and the gene (69).

While those two studies (67, 69) seem to contradict each other, this difference could reflect an evolutionary divergence of the insulation process between drosophila and mammals, or may be the consequence of differential interactions (Rm62 interacts with CP190 and DDX5 with CTCF). Alternatively, DDX5 may have opposite effects on 3D chromatin organization by reinforcing a subset of local contacts while dissociating others, with a global effect on chromatin higher-order organization.

Possible consequences for the regulation of transcription and mRNA processing

Beyond its function in gene insulation, CTCF globally regulates gene expression through its binding to thousands of sites,

which often mark boundaries between genomic regions defined as compartments, topologically associating domains (TADs), or loop domains, on a descending scale from the higher-order 3D organization of chromosomes to local chromatin folding (70). Locally, DNA folding occurs when two DNA-bound CTCF molecules are brought into close spatial proximity through a loop. This process is also mediated by Cohesin, a ring-like multiprotein complex which co-occupies most genomic CTCF sites (70). Accordingly, the depletion of CTCF or Cohesin leads to a profound disorganization of the 3D genome (71-73).

In order to appreciate what the function of DDX5 and DDX17 may be in this context, we will hereafter focus on the regulation of alternative splicing, a largely cotranscriptional process that has multiple connections with chromatin organization (74), and which is regulated by both RNA helicases and by CTCF at the level of hundreds of exons (21, 75, 76). As described by the kinetic model of splicing regulation, RNA Polymerase II (RNAPII) pausing is important for the inclusion of weak, otherwise skipped exons (74). CTCF and/or Cohesin control RNAPII pausing at different transcription steps, like at promoter-proximal early elongation checkpoints and in the control of termination (75, 77-79). CTCF was initially proposed to control exon inclusion by acting as a roadblock for elongating RNAPII at the exon 5 of the *CD45* gene, a DNA methylation-sensitive effect (75, 80). However, a recent report showed that CTCF binding at alternative exons correlates both with DNA loops joining these exons to their cognate promoters and with exon inclusion at the RNA level (81). This is consistent with earlier observations showing that DNA regions corresponding to alternative exons that are enriched in CTCF/Cohesin binding sites are in close spatial proximity to promoters (82).

Recently, the loop extrusion model has been proposed as one mechanism through which Cohesin and CTCF organize genome folding. In this model, Cohesin entraps and extrudes a loop of DNA that expands until it reaches boundaries corresponding to CTCF sites, at which point extrusion pauses (83, 84). Transcription is one of the motors that can promote and orient Cohesin sliding along chromatin fibers (85). Therefore, considering transcription in a context of dynamic loop extrusion has important consequences for our understanding of the regulation of the transcriptional fidelity and cotranscriptional processing of the primary transcript.

We propose that the control of CTCF on splicing should be reconsidered so as to integrate the tridimensional folding of the gene that results from a dynamic loop extrusion process. By altering the kinetics of RNAPII on chromatin at the level of specific exons, this may impact the inclusion or skipping of these exons at the RNA level. As reviewed here, the multiple regulatory activities of DDX5 and DDX17 on epigenetic modifiers and chromatin-associated factors, particularly their effects on CTCF/Cohesin binding, strongly suggest that some of their target exons may be controlled as a result of DDX5/DDX17-regulated modifications of the chromatin state and/or DNA looping.

The mechanism by which DDX5/DDX17 could locally influence the chromatin binding of architectural proteins may involve RNA molecules. CTCF has a high affinity for RNA, and it binds to several lncRNAs involved in dosage compensation and X chromosome inactivation (86). CTCF binding to RNA modulates its binding to DNA, either in *cis* (86) or in *trans* (87). Cohesin binding to chromatin is also regulated by ncRNA, such as eRNA (88) or the lncRNA *Xist*, which inactivates X chromosome (89). *Xist* has been proposed to act both as a Cohesin evictor to prevent the formation of a transcription-favourable chromatin conformation, and as a scaffold to recruit repressive complexes in order to promote X inactivation. The presence of both DDX5 and DDX17 in the *Xist* interactome (89) raises the possibility that both RNA helicases could influence these events through their ability to remodel the *Xist* RNP complex. One could extend this model to other lncRNAs that may specifically regulate CTCF/Cohesin binding to other genomic regions, such as alternative exons.

It is clear that DDX5 and DDX17 can regulate alternative splicing through other mechanisms, via a direct effect on the local folding of their targeted transcripts or via the recruitment of RNA binding cofactors (21, 24, 90, 91), and our intention is not to argue that DDX5/DDX17-mediated splicing regulation relies only on their chromatin-related functions. However, this is a compelling question that should be addressed in the future.

CONCLUSION

As evoked elsewhere in this review, several important issues must be explored further in order to more precisely understand the mechanisms supporting DDX5 and DDX17 functions in chromatin-related gene regulation. This includes, for example, the influence of post-translational modifications, the possible links with the RNAi pathway, or the identification of genes specifically targeted by the 2 RNA helicases. This particular task is difficult to address due to the functional redundancy of the two factors and their cross-regulation, but genome-wide approaches should allow for this objective to be achieved in the near future. Finally, the exact role of their helicase activity also remains only partially addressed. If the RNA-related functions of DDX5 and DDX17, specifically their splicing regulation, require an active ATPase domain (15, 21, 76, 91, 92), luciferase assays have shown that ATPase-dead DDX5 or DDX17 are as efficient as wild type proteins in their activities as transcriptional coregulators (5). However, such experiments have not ruled out the possible recruitment of endogenous RNA helicases to the tested promoters, which would influence the result. Indeed, yeast-two hybrid experiments have shown that both wild type and helicase-dead DDX5 and DDX17 mutants form homo and heterodimers (93), and that both proteins efficiently co-immunoprecipitate from mammalian

extracts (unpublished results). Specific experiments are therefore required to definitively test whether or not ATPase-dead proteins are able to compensate for the absence of the endogenous protein.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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