Cell. Mol. Life Sci. (2015) 72:2361–2375
DOI 10.1007/s00018-015-1871-9

Cellular and Molecular Life Sciences

REVIEW

Eukaryotic enhancers: common features, regulation, and participation in diseases

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Received: 22 December 2014 / Revised: 7 February 2015 / Accepted: 20 February 2015 / Published online: 26 February 2015

Abstract Enhancers are positive DNA regulatory sequences controlling temporal and tissue-specific gene expression. These elements act independently of their orientation and distance relative to the promoters of target genes. Enhancers act through a variety of transcription factors that ensure their correct match with target promoters and consequent gene activation. There is a growing body of evidence on association of enhancers with transcription factors, co-activators, histone chromatin marks, and lncRNAs. Alterations in enhancers lead to misregulation of gene expression, causing a number of human diseases. In this review, we focus on the common characteristics of enhancers required for transcription stimulation.

Keywords Chromatin loop · Gene regulation · Epigenetics · Transcription · Regulatory elements

Introduction

Cells establish individual patterns of gene expression during differentiation and development. The spatiotemporal control of transcription by RNA polymerase II (RNAPII) depends on enhancers, DNA regulatory elements that activate gene transcription [1, 2]. The first known enhancer was identified in 1981 [3, 4] in the SV40 virus genome, but subsequently such sequences have been found to be widespread among higher eukaryotes.

Enhancers are modular elements that lack stereotyped sequence composition and are located at a distance from transcription start sites of genes. The distance from an enhancer to its target promoter can vary from a few kb to 1 Mb. For example, this distance in human CD4+ T cells is ~50 kb [5]. Enhancers can be located in intra- and intergenic regions, introns, and even exons of genes [6, 7]. Enhancers usually activate transcription independently of their orientation and position relative to the target gene. Multiple enhancers can control the activity of a certain gene or group of genes [8, 9].

Enhancers are usually several hundred base pairs long and contain clusters of different 4- to 8-bp degenerate DNA sequences that are recognized by multiple transcription factors [10]. As a consequence of transcription factors binding to the enhancer DNA, these elements are characterized by low nucleosome occupancy and could be detected by their hypersensitivity to DNaseI [11–13]. Nucleosome destabilization at enhancers is facilitated by the presence of a highly dynamic H3.3/H2A.Z combination of histone variants [14–16] that were shown to form less stable contacts with DNA [17].

Binding of transcription factors to enhancers leads to a subsequent activation of transcription by recruitment of co-activators, releasing RNAPII pausing and stimulation of elongation. The activity of each enhancer is restricted to a definite spatiotemporal window by a specific set of DNA-binding transcription factors that control their specificity to promoters [10, 18–20]; by enhancer-blocking elements named insulators [21–24] and by chromosome separators

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termed TADs (Topologically Associated Domains) boundaries [25, 26].

In recent years, our knowledge of enhancers and their features has greatly expanded due to application of genome-wide technologies. Different features of enhancers, including transcription factors, co-factors, histone marks, were mapped throughout the genomes and used to predict novel enhancers [27, 28]. These data have changed our views on the prevalence of enhancers. For example, it has been predicted that the human genome contains approximately one million enhancers [29, 30]. Notwithstanding this progress, there are a lot of gaps and challenges in identification of features significant for enhancer activity.

In the present review, we summarize common features of enhancers that are important for stimulation of transcription: basic steps of enhancer–protein complex formation, factors essential for the interaction of enhancer with the promoter, enhancer-associated histone modifications, and implications of lncRNAs in enhancer activity. We discuss the recent studies that provide evidence for the relationship between mutations in enhancers and various human diseases.

Principles of enhancer-dependent transcription

Enhancers act through protein factors that are assembled on their DNA. At the first step of enhancer complex formation, so-called pioneer factors are recruited (Fig. 1a, b) [31, 32]. These factors control the accessibility of enhancer DNA by displacing nucleosomes and opening chromatin locally at inactive enhancers, thereby facilitating the binding of developmentally regulated transcription factors [10, 32, 33]. The recruitment of pioneer factors is accompanied by DNA demethylation [34–36] and can be followed by active histone modification [37, 38]. However, the binding of pioneer factor is usually insufficient to allow enhancers to stimulate transcription [36, 37, 39, 40]. Then a set of developmentally regulated transcription factors, whose activity is restricted to certain spatiotemporal windows, bind to each enhancer sequence in a specific pattern, usually in cooperation with each other. Several transcription factors may compete for the binding to the same or partially overlapping binding sites.

The binding of developmentally regulated factors (Fig. 1c) is followed by the recruitment of co-activators (Fig. 1d) that lack the DNA-binding capacity, resulting in the formation of an active enhancer complex. These co-activators can mediate contacts between enhancers and general transcription factors at the promoters and/or function as chromatin remodelers (Fig. 1e).

Mediator is a large multiprotein co-activator complex conserved from yeast to humans. In mammals, Mediator consists of about 30 polypeptides (named MED1–MED31), CDK8, and cyclin C [41]. Mediator subunits associate with enhancers and active promoters; knockdown of Mediator subunits reduces the transcription of enhancer-controlled genes [42–45]. Mediator interacts with transcription factors, including general transcription factors bound to promoters, as well as with RNAPII and elongation factors [46, 47]. Mediator transfers the activating signal from enhancer to promoter, stimulating preinitiation complex assembly, activating paused RNAPII, and regulating transcription elongation [48, 49].

As shown recently, Mediator occupies in murine ESC large enhancer domains, or super-enhancers that have an average size of about 8.5 kb, and are enriched in the key transcription factors (master regulators) Oct4, Sox2, Nanog, Klf4, and Esrrb that control the pluripotent state of ESC cells [45]. A significant proportion of human super-enhancers and their target genes are tissue- and cell type-specific [50]. Several super-enhancers correspond to previously identified locus control regions (LCRs): a long cis-regulatory elements consisting of multiple functional enhancers [50].

Other common enhancer co-activators include two histone acetyltransferases: the CREB-binding protein (CBP) and the related E1A-interacting 300-kDa protein p300 [51, 52]. In mammals, these proteins are paralogs that have more than 90 % sequence identity in the HAT domain [53]; therefore, they are often referred to as p300/CBP. The majority of p300/CBP-bound genome regions overlap [54, 55] and tend to localize to active enhancers and promoters [56–59]. *Drosophila* has only the CBP homologue (named dCBP, or nejire), which also binds to active enhancers and promoters [60, 61]. Several studies suggest that the p300/CBP enrichment on DNA accurately predicts enhancers [56–59, 62, 63].

The p300/CBP protein has at least 400 interacting protein partners. The lysine 27 of histone 3 is one of its main targets in vivo [64, 65]. Genome-wide studies have shown that active enhancers associate with histone 3 acetylated at lysine 27 (H3K27ac) [62, 66, 67]. Similar to p300/CBP, H3K27ac marks both enhancers and active promoters [16]. In addition to histones, p300/CBP acetylates more than 70 non-histone proteins [53], including GATA3/4 [68, 69] and PU.1 [70] enhancer pioneer factors.

Recently, Krebs et al. [71] reported the association of an ATAC histone acetyltransferase complex with enhancers and promoters in two human cell lines. Furthermore, the ATAC complex was found to bind to a group of enhancers deprived of p300/CBP. This is evidence of a novel class of p300/CBP-independent enhancers that waits to be studied.

Current models suggest that the activity of histone acetyltransferases and ATP-dependent chromatin remodelers reduces the affinity of histones to enhancer DNAs and

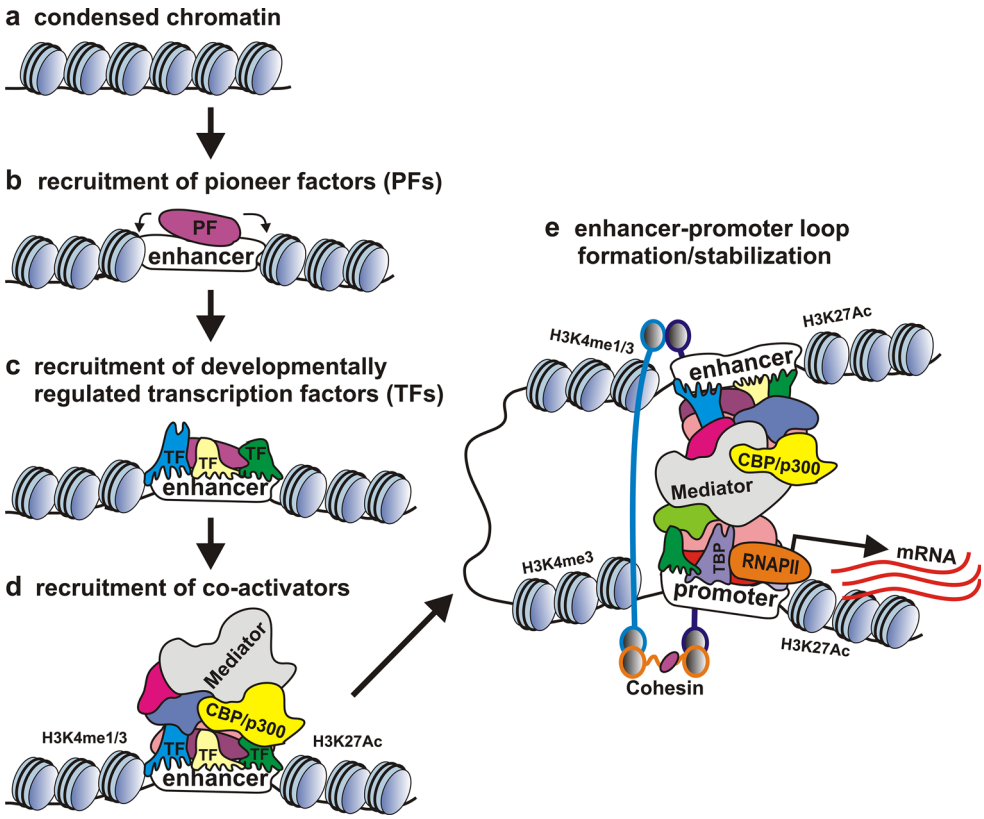

Fig. 1 Crucial steps of enhancer complex formation and long-range interaction of enhancer with promoter. Condensed chromatin at inactive enhancers opens up upon recruitment of pioneer factors (PFs) capable to displace nucleosomes. PFs facilitate the binding of developmentally regulated transcription factors (TFs) to the enhancer DNA, and they, in turn, recruit co-activators of transcription. The Mediator complex binds to transcription factors at enhancers and to general transcription factors, RNAPII at promoters, and links the enhancer and promoter together. The cohesin complex supports enhancer–promoter contact, forming a ring-like structure around DNA

leads to chromatin decompaction, facilitating the binding of transcription factors. However, several studies indicate that acetylation of non-histone proteins may also be important for enhancer-dependent activation of transcription [68, 69].

Long-distance contacts between enhancers and promoters

A critical step required for enhancer-dependent transcription is the establishment of functional contacts between enhancers and target promoters. The dominant "looping" model suggests that active enhancers form direct physical contacts with promoters, while the intervening DNA is looped out (Fig. 1e). Data obtained by 3C and derivative technologies support this model [2, 19, 72, 73]. The first 3C study in mammals was performed on the β-globin LCR located 40–60 kb away from the globin genes [74]. Interactions between LCR and the target promoter were observed in fetal liver cells expressing β-globin but not in brain cells, in which β-globin gene is inactive, suggesting that enhancer–promoter interactions are important for promoter stimulation.

To date, the existence of enhancer–promoter loops has been confirmed for various enhancers [23, 72, 75–77]. Moreover, enhancer–promoter interactions have proved to differ between cell types and correlate with target gene transcription [78, 79]. There is some evidence that enhancer–promoter contacts are not constant but can be established prior to gene activation.

In particular, GR [80], FOXO3 [81] and TNFα [82] dependent enhancers interact with their target promoters prior to signaling. Likewise, long-range interactions involving Oct4 enhancer are established only in a subpopulation of cells prior to activation of Oct4 gene [83]. Specialized proteins might be responsible for the establishment of enhancer–promoter contacts prior to activation. Insulator proteins binding to the promoter regions are possible candidates for this role [84].

The physical contacts between the enhancer and promoter are sensitive to the loss of several DNA-binding transcription factors. For example, EKLF and GATA-1 are sufficient for the establishment of the enhancer–promoter contact at the β-globin locus [85, 86], and Oct4 factor is required for the enhancer–promoter contact at the *Nanog* locus [87].

The enhancer–promoter loops are further controlled by co-factors such as Mediator and cohesin complexes. The cohesin complex is composed of four core subunits: Smc1(A/B), Smc3, Scc1, and Scc3 (SA1/SA2) [88, 89]. The long coiled-coil polypeptides Smc1(A/B) and Smc3

interact with each other through the hinge domain and are additionally connected through the Scc1 subunit, forming a ring-like structure around DNA, and Scc3 (SA1/SA2) links to the central part of Scc1 [88, 89].

Cohesin acts synergistically with Mediator: these complexes could be co-purified [42, 90, 91]. Cohesin is enriched at Mediator-bound enhancers and promoters [42, 50, 92], and, as in the case of Mediator, knockdown of cohesin reduces the transcription of the enhancer-controlled genes [42]. Depletion of cohesin influences the RNAPII occupancy at predicted enhancers and promoters [93]. Depletion of Mediator [42, 43, 94] and cohesin subunits [42, 91, 94–96] results in a decreased frequency of interaction between enhancers and promoters, suggesting their direct contribution to enhancer–promoter communication.

Mediator proteins may mediate contacts between enhancer- and promoter-bound transcription factors, while cohesin supports chromatin looping by forming a ring-like structure around the interacting elements [42].

Histone marks and enhancers

Histones are subject to covalent modifications (such as acetylation, methylation, phosphorylation and ubiquitination) that occur mainly at their N-terminal tails and may correlate with the transcriptional status of genes. The existence of distinct histone modifications provided a basis for the 'histone code' hypothesis, according to which specific histone modification patterns affect binding of proteins to chromatin and determine the active and inactive regions of the genome [97]. For example, H3K27ac (see above) is associated with active gene transcription sites [62, 66, 67], and histone H3 mono-, di-, or trimethylated at lysine 4 (H3K4Me1, H3K4me2, and H3K4Me3, respectively) marks active chromatin [14, 16, 56, 98, 99].

The results of most studies suggest that both active enhancers and promoters are marked by nucleosomes containing H3K4me2 [14, 56, 100], while data concerning the distribution of H3K4Me1 and H3K4Me3 between active enhancers and promoters are contradictory. Some authors report that in human cells (e.g., HeLa, K562, and GM06990) H3K4Me1 is a hallmark of active enhancers, while H3K4Me3 is associated with active promoters [56, 62, 98, 99]. Indeed, the DNA sequences predicted as enhancers by enrichment of H3K4me1 and p300 and depletion of H3K4me3 gives over 75 % of positives in a functional test [62, 101].

Other researchers were not able to detect any significant difference between the presence of H3K4Me3 and H3K4Me1 at active enhancers and promoters in human CD4+ T cells [14, 16]. Moreover, Pekowska et al. [102] reported that H3K4me3 is enriched at active enhancers, while H3K4me1 is distributed independently of enhancer activity. The level of H3K4me3 at enhancers is lower than at promoters. Likewise, experiments with *Drosophila* embryos have shown that H3K4me1 modification takes place regardless of the functional activity of mesodermal enhancers [103].

The supposed enhancer-specific histone modifications may involve a limited recruitment of specific methyltransferases to enhancers. Therefore, additional information can be extracted from the distribution of methyltransferase proteins. For example, mammalian MLL3/MLL4 proteins—the main regulators of H3K4me1 [104]—are enriched at enhancer regions [104–106]. Similar results have been obtained in *Drosophila*: Trr (a homologue of mammalian MML3/MML4) and Trx, which are responsible for bulk H3K4me1 [105, 107], are associated with enhancers and co-localize with H3K4me1 and dCBP [107]. However, the distribution of the main H3K4 trimethylases Set1a and Set1b (dSet1) proteins [108–111] relative to enhancers has not yet been analyzed.

To date, the analysis of alternative histone modifications in mammals and *Drosophila* has failed to reveal their correlation with the majority of enhancers, suggesting that enhancers are heterogeneous [16, 28, 103]. It has also been found that over 20 % of human enhancers are associated with acetylation of histone H3 lysine 18 (H3K18Ac) chromatin hallmark [16]; in *Drosophila,* enhancers also tend to associate with H3K18Ac [112]; and trimethylated histone H3 lysine 79 (H3K79me3) marks about 15 % of intergenic enhancers [103].

Thus, currently available information about the relationship between histone modifications and enhancer activity is rather limited. The analysis of additional histone marks and an accurate comparison of different signatures in individual cell types are necessary to capture a complete picture of active enhancers.

Long non-coding RNAs and enhancers

Early evidence that transcription could be associated with enhancers came from studies of the human beta-globin locus where a non-coding RNA (ncRNA) is transcribed from the HS2 enhancer within the LCR [113] only in cells where enhancer is active [114, 115]. This ncRNA is transcribed mainly in one direction and from multiple sites of the enhancer [116]. The generated ncRNA were polyadenylated and spliced; however it did not appear to contain the normal cap-structure at 5′-ends [117]. HS2 was found to be associated with RNAPII [118] that seems to be bound to enhancer independently of the promoter-bound RNAPII [118, 119].

Further systematic analysis indicated that a major portion of the genome is being transcribed and that the bulk of genome transcripts account for long (>200 bp) non-

protein-coding RNAs (lncRNAs) [120–123], which can have a positive or negative effect on gene transcription [124].

LncRNAs originating from enhancers were named enhancer RNAs (eRNAs) (Fig. 2). Based on the genome-wide studies, they are ~0.5- to 5-kb ncRNAs derived from DNA regions that share enhancer-associated features [57, 120, 125–128]. The eRNAs could be transcribed uni- or bidirectionally; they may contain or lack poly(A) tails [57, 120, 125–127, 129, 130].

For instance, Kim and colleagues [57] have found in mouse neuronal cells ~12,000 of CBP-bound regions enriched in H3K4Me1 and located distally from known TSSs of protein-coding genes. Among these regions, 25 % recruited RNAPII, and 16.7 % were transcribed, resulting in the production of RNAs with a length of <2 kb. Most of transcripts identified in this study were transcribed bidirectionally and were non-poly(A)+ [57].

Several studies have revealed eRNAs derived from extragenic RNAPII bound sites [125, 127] and from activator binding sites: estrogen receptor [126, 130], p53 [131], and MYOD1 [132].

Orom et al. [128] performed a search of enhancer-associated transcripts based on the functional test. They selected ≈0.1- to 9-kb lncRNAs from intergenic regions whose knockdown by siRNAs resulted in down-regulation of nearby protein-coding genes. The selected transcripts were predominantly spliced and poly(A)+. Unlike in the majority of eRNAs, H3K4me3 was present at their 5′-ends, and H3K36me3 marked their bodies [128]. Based on this difference, the authors classified them into a distinct group of lncRNAs, named ncRNA-a (ncRNA-activating). However, it is difficult to discriminate some of them from unidirectionally transcribed and poly(A)+ eRNAs. Indeed, due to nonexclusive conditions of search for eRNAs and ncRNA-a, transcripts of both groups may overlap.

A number of studies indicate a positive role of eRNAs in the enhancer function. For example, transcription of eRNAs positively correlates with the expression of nearby genes [57, 125, 133, 134], and targeted degradation of a major part of eRNAs leads to reduction of the expression of nearby protein-coding genes, as in the case of ncRNA-a [131–133, 135, 136]. Furthermore, enhancer activation upon stimulation correlates with eRNA production

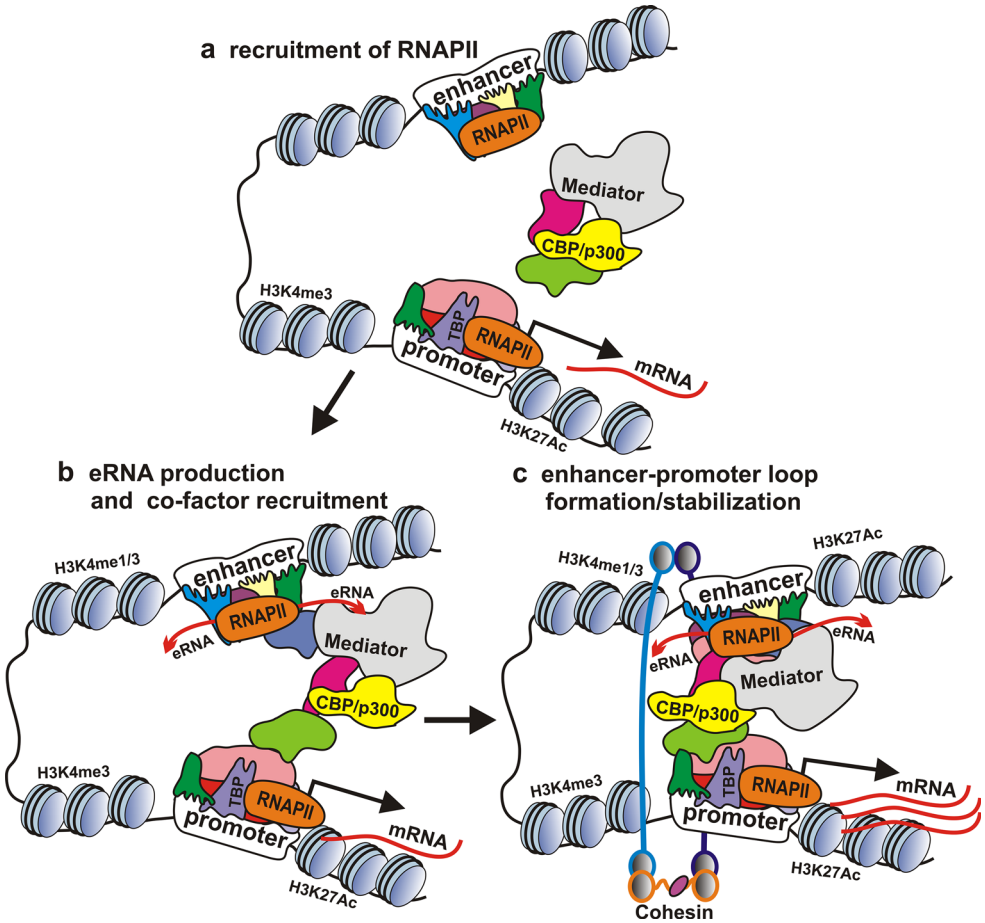

Fig. 2 The hypothesized positive role of eRNAs in the activity of transcribed enhancers. Enhancer activation by developmentally regulated transcription factors leads to RNAPII recruitment and to production of eRNAs that help to recruit co-activators and facilitate enhancer–promoter loop formation

[136, 137]. The expression of eRNAs enhances transcription of the reporter gene in an RNA tethering assay [131, 136]. However, the selected eRNAs were ineffective in *trans*-stimulation experiments [136].

The role of eRNAs in enhancer–promoter loop formation is unclear. The eRNAs level was found to be higher at enhancers that interact with promoters [78, 136, 138]. However, looping between enhancers and target genes remained intact after inhibition of eRNA transcription [126]. The establishment of enhancer–promoter contact prior to eRNAs synthesis was further supported by the fact that eRNAs were lacking at the enhancer of *arc* gene in case of promoter deletion [57]. However, ncRNA-a depletion by siRNA reduced chromosomal looping between the ncRNA-a expressing region and the target gene loci. Furthermore, the tested ncRNA-as were found to associate with Mediator implicated in the loop formation [43], and several authors reported association of lncRNAs with enhancer-bound activators [139–141].

Recent studies suggest that transcription of long non-coding RNAs through enhancer-containing regulatory regions correlates with decrease in target gene transcription. For example, Gummalla et al. [142] reported that the 90-kb *iab-8* ncRNA is transcribed through the regulatory region of the *abd-A* gene and participate in its repression in *Drosophila*. The authors propose that this repression is established in two ways: (1) the *iab*-8 precursor produces a micro-RNA, which targets the *abdominal-A* mRNA, and (2) *iab-8* transcription directly interferes with the expression of *abdominal-A*, which lies just downstream of the *iab-8* ncRNA poly(A) site [142]. In a previous study, Petruk et al. [143] found that lncRNA transcribed through an enhancer-containing regulatory region interferes with the *Ubx* gene promoter. However, likewise interfering with gene promoter these transcripts can directly affect enhancers located in transcribed regulatory regions.

Using an assay in transgenic lines, we found that transcription leads to suppression of enhancers from the regulatory regions of *yellow* and *white* genes [144]. Transcription through the enhancer of the *white* gene resulted in dislodging of Zeste, a protein important for enhancer–promoter communication, suggesting a role for the mechanism of 'transcriptional interference' (Fig. 3b). This mechanism probably acts on intergenic enhancers and controls their maximum activity by a negative feedback loop, with excessive activation of transcription inhibiting the enhancer activity. Similar positive enhancer-associated transcription from one enhancer can negatively affect the activity of nearby enhancers that should be inactive in a given tissue or a group of cells.

Enhancer inactivation by lncRNAs can possibly involve the recruitment of Polycomb group repressors (Fig. 3c) [145]. For example, over 20 % of lincRNAs expressed in various cell types are bound by the PRC2 Polycomb group complex [146].

More information comes from studies on enhancers in human embryonic stem cells (hESCs) [66, 147]. Active enhancers in hESCs cells show canonical enrichment in H3K4me1 and H3K27ac histone modifications and associate with the p300 protein [66, 147]. The authors identified a class of "poised enhancers" that have features of both active and inactive chromatin but are linked to inactive genes. Being also enriched in H3K4me1 and p300, they are distinguished by the absence of H3K27ac and enrichment in H3K27me3 [66, 147] and are bound by Polycomb group repressors [148]. Analysis of PcG and other repressor proteins for association with inactive enhancers and linkage to ongoing transcription at enhancers may provide new insights into enhancer function.

Diseases and enhancers

Great efforts are made to understand the genetic basis of human diseases. In addition to mutations in the coding part of genes, disruption of gene regulatory regions is a major type of disease-associated changes in DNA. Below, we briefly consider only a few examples of enhancer-related diseases.

The first evidence in humans comes from studies of the β-globin locus linked to β-thalassemia, a transfusion-dependent anemia. Several types of thalassemia are characterized by hematological symptoms observed in the absence of β-globin protein, although the β-globin gene in the patients is intact [149–151]. These thalassemias are associated with deletions of DNA regulatory regions. For example, patients with Dutch ($\gamma\delta\beta^{\circ}$) thalassemia have a 100-kb deletion that removes the LCR and almost all sequences upstream of the β-globin gene [151]. A deletion of $\sim$30 kb found in the Hispanic δβ-thalassemia likewise removes the LCR and affects β-globin gene expression [150].

Another example concerns limb abnormalities in humans, mice, cats, and chickens with single-point mutations in ZRS, a highly conserved $\approx$800-bp limb-specific enhancer located 1 Mb from the target *sonic hedgehog* (*SHH*) gene. These mutations affect long-range *SHH* signaling, which plays a central role in patterning numerous embryonic tissues [152].

Hirschsprung (HSCR) disease is a complex genetic disorder attributed to a failure of the enteric neural crest cells to form ganglia in the hindgut. The risk for HSCR is associated with single-nucleotide polymorphism (SNP) in the RET enhancer [153, 154].

Van Buchem (VB) disease is an autosomal skeletal dysplasia characterized by bone overgrowth. This disease

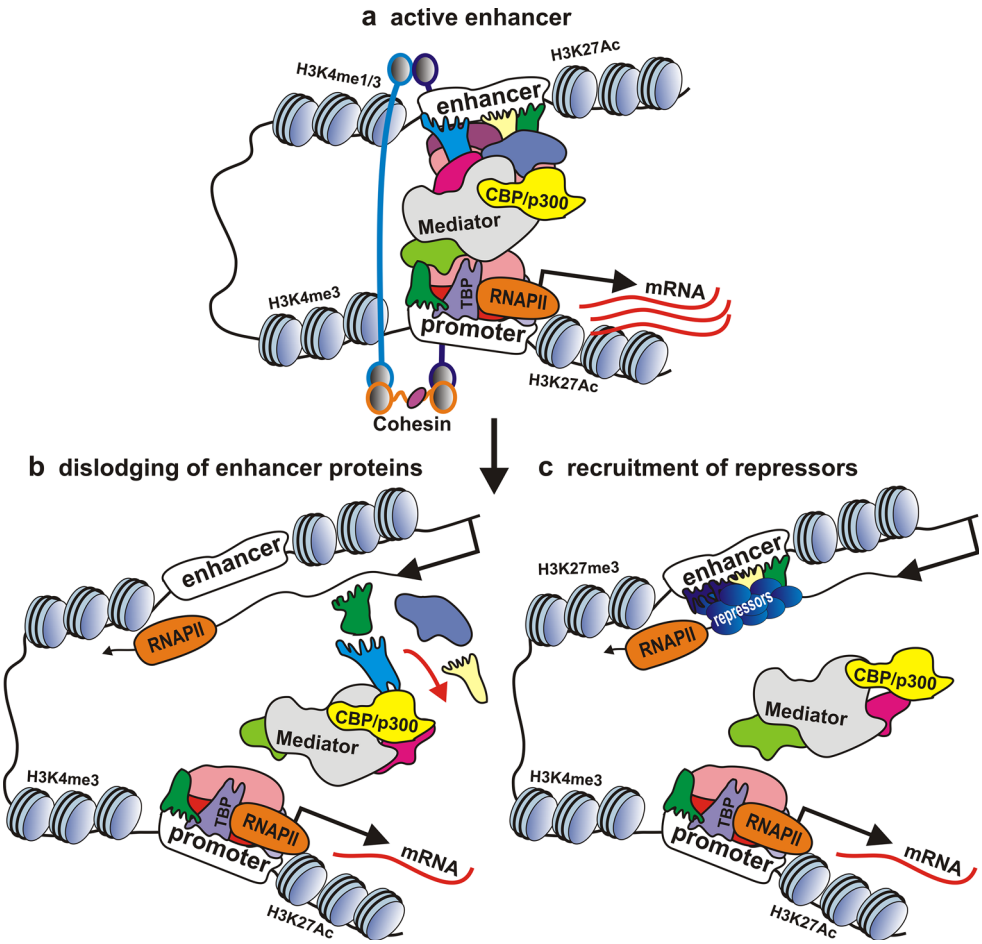

Fig. 3 The potential mechanisms of enhancer inactivation by lncRNAs. Transcription through the enhancer from upstream promoter leads to dislodging of the enhancer-bound protein complex from DNA or/and to the recruitment of repressor proteins onto DNA, resulting in enhancer inactivation

is associated with a 52-kb deletion in the regulatory region 35 kb downstream of the *SOST* gene [155, 156]. The affected region normally contains the ECR5 enhancer [157], deletion of which results in the phenotypes observed in VB disease [158].

Facioscapulohumeral muscular dystrophy (FSHD) is a dominant neuromuscular disease with a prevalence of 1 in 20,000, which leads to weakness and atrophy of specific groups of muscles in the face, shoulder girdle, and lower extremities [159]. FSHD is associated with the subtelomeric region 4q35 containing an array of 3.3-kb-long macrosatellite repeats (D4Z4) [160]. The length of this array varies from 35 to 300 kb in healthy subjects but is consistently lower than 35 kb in FSHD patients [161]. Each D4Z4 repeat contains a potent transcriptional enhancer [162–164], an open reading frame for the double homeobox gene *DUX4* [165, 166], and a number of regulatory elements (for review, see [167]). The maintenance of pathological FSHD phenotype is due to the expression of D4Z4-proximal genes that include *DUX4, DUX4c, FRG1, FRG2,* and *ANT1*. All these genes are upregulated in FSHD (for review, see [167, 168]). The D4Z4 enhancer interacts with the Krüppel-like transcription factor 15 (KLF15) in FSHD patients, thereby activating the *DUX4c* and *FRG2* genes [169]. Recent studies indicate that lncRNA and miRNA are also implicated in transcriptional regulation in FSHD [170, 171].

Aniridia is a panocular malformation associated with haploinsufficiency of PAX6 transcription factor. As shown by Bhatia et al. [172], aniridia can be caused by point mutation in the conserved SIMO enhancer located 150 kb from *PAX6* gene. Another disease causing blindness, nonsyndromic congenital retinal nonattachment (NCRNA), is linked to a deletion of an enhancer 20 kb upstream from the ATOH7 transcription factor gene that is required for retinal ganglion cell and optic nerve development [173].

Alterations in enhancer-containing regulatory regions are also responsible for other development disorders such as Leri–Weill dyschondrosteosis syndrome [174], Axenfeld–Rieger syndrome (ARS) [175], coronary artery disease [176], prostate cancer [177], and MonoMAC syndrome [178].

Many lymphomas, including Bukitt lymphoma (BL), mantle cell lymphoma and follicular lymphoma are caused by translocations that position a strong immunoglobulin heavy chain μ enhancer in a relative proximity (100–1000 kbp) to

proto-oncogenes c-myc, Cyclin D1 (CCNDD1) or bcl2, respectively [179]. The μ enhancer is thought to directly activate the proto-oncogenes [180], although this notion has been challenged in a recent study, where the authors show that activation of CCND1 and CMYC is accomplished by proximal nucleolin-dependent enhancers, following the relocalization of the translocated regions to the proximity of the nucleolus [181]. BL is linked to two human viruses, Epstein–Barr's virus and human immunodeficiency virus [182], while the role of viral enhancers in BL is not known yet; the mutations in the murine Moloney murine leukemia virus enhancer were shown to affect cancer development in mice [183].

Globally, genome-wide association studies (GWAS) localize the majority of disease-associated SNPs to non-coding sequences [29, 184, 185], particularly to enhancers [185–188]. The enhancer-associated SNPs are linked to cancer, diabetes, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, Crohn's disease, celiac disease, Alzheimer's disease, etc. [50, 186, 189, 190]. The above examples are only a small part of long list of enhancer-associated diseases. The relationships between changes in lncRNAs transcription and diseases are also currently under a careful study [191].

Individual genome-wide sequencing and analysis of enhancer-associated SNPs will certainly become an integral part of tests for timely detection of malformations and other abnormalities.

Conclusions and outlook

Genome-wide studies indicate that enhancers are enriched in Mediator and cohesin complexes. A significant proportion of enhancers associate with p300/CBP, ATAC, eRNAs, RNAPII, and active histone marks H3K4me, H3K27Ac, H3K18Ac, and H3K79me3.

However, enhancers show an extreme variability in their DNA-binding transcription factors and most of the known enhancer features are not necessarily required for enhancer activity. Such variability appears to be significant for the plasticity and accuracy of gene expression control. At the same time, we suggest that there should be some general principles and elegant mechanisms of enhancer-dependent gene activation. The mechanisms may also vary depending on the chromosomal context and nuclear compartmentalization.

Many more questions need to be answered. How the connection of cofactors with different combinations of activators is achieved? How the collaboration between diverse cofactors is established? What positive signals are translated from activators to promoter-bound factors: protein modifications, changes of protein conformation, chromatin structure, etc.? What defines lncRNA to be positive/negative? What restricts enhancer activity to certain cells? The answers to these questions will certainly provide a deeper insight into the principles of enhancer action and genetic control.

Acknowledgments We are grateful to N.A. Gorgolyuk for his help in preparing the manuscript. This study was supported by RFBR 15-04-04208-a to D.C., RFBR 15-04-03973-a to M.E., RFBR 13-04-93106-CNRS_a to P.G., and the MEGAFSHD grant from the Association Française contre les Myopathies (AFM) to Y.V.

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