Received: 9 May 2019 | Revised: 13 June 2019 | Accepted: 17 June 2019

DOI: 10.1111/cas.14107

REVIEW ARTICLE

Cancer Science WILEY

Noncoding RNA transcription at enhancers and genome folding in cancer

Takeshi Isoda | Tomohiro Morio | Masatoshi Takagi

Department of Pediatrics and Developmental Biology, Tokyo Medical and Dental University, Tokyo, Japan

Correspondence
Takeshi Isoda, Department of Pediatrics and Developmental Biology, Tokyo Medical and Dental University, Tokyo, Japan.
Email: tisoda.ped@tmd.ac.jp

Funding information
The Japanese Society of Hematology Research, Grant/Award Number: 2889; Japan Society for the Promotion of Science, Grant/Award Number: 18K15661; Japan Foundation for Pediatric Research, Grant/Award Number: 18-003

Abstract

Changes of nuclear localization of lineage-specific genes from a transcriptionally inert to permissive environment are a crucial step in establishing the identity of a cell. Noncoding RNA transcription-mediated genome folding and activation of target gene expression have been found in a variety of cell types. Noncoding RNA *ThymoD* (thymocyte differentiation factor) transcription at superenhancers is essential for mouse T-cell lineage commitment. The cessation of *ThymoD* transcription abolishes transcription-mediated demethylation, recruiting looping factors such as the cohesin complex, CCCTC-binding factor (CTCF), ultimately leading to the phenotype of severe combined immunodeficiency and T-cell leukemia/lymphoma. In this review, we describe the functional role of RNA polymerase II-mediated transcription at enhancers and in genome folding. We also highlight the involvement of faulty activation or suppression of enhancer transcription and enhancer-promoter interaction in cancer development.

KEYWORDS
cancer, genome folding, noncoding RNA, ThymoD, transcription

1 | INTRODUCTION

During cell development, lineage-specific genes are repositioned from the nuclear lamina to the nuclear interior to establish the cell's identity. The immunoglobulin heavy chain locus, the *EBF1* gene for pre-pro-B cell to pro-B cell transition, and the *BCL11b* gene involved at the T-cell commitment stage are prominent examples of such changes of nuclear localization (Figure 1).[1-3] B cell and T cell development is controlled by a series of well-characterized TFs: E2A (TCF3), EBF1, PAX5, and FOXO1 for early B cell differentiation[2,4-6]; and Notch1, GATA3, TCF-1 (TCF7), RUNX1, and BCL11b for early T cell development.[7-10] The binding of these TFs and global changes of methylation status on genomic DNA define the transformation of accessible chromatin and 3-D structure of the genome to orchestrate the cell fate.[11,12] Such large-scale nuclear repositioning is tightly regulated by associated cognate enhancers. In the early T cell commitment stage, noncoding RNA *ThymoD* is transcribed from a promoter at one of the superenhancer domains of *BCL11b* (Figure 1A).[3] The *ThymoD* transcribed region allows the recruitment of insulator protein CTCF to the demethylated CpG residues. *ThymoD* transcription also facilitates the recruitment of

Abbreviations: 5hmC, 5-methylcytosine to 5-hydroxymethylcytosine; AFF4, AF4/FMR2 family member 4; BCL11b, B-cell lymphoma/leukemia 11B; BRD4, bromodomain-containing protein 4; CBP, CREB-binding protein; CDK, cyclin-dependent kinase; CTCF, CCCTC-binding factor; CTD, carboxyl terminal domain; CYCT1, Cyclin T1; DNA-PKcs, DNA-dependent protein kinase, catalytic subunit; DRB, 5,6-dichloro-1-*beta*-D-ribofuranosylbenzimidazole; EBF1, early B-cell factor 1; ELL, eleven-nineteen lysine-rich leukemia; EP400, E1A-binding protein p400; eRNA, enhancer RNA; ETS, E-twenty-six; ETV6, ETS variant 6; FOXO1, forkhead box O1; H3K27ac, acetylation of H3 lysine 27; H3K4me1, mono-methylation of H3 lysine 4; Igk, immunoglobulin kappa chain; LMO1, LIM domain only 1; LMO2, LIM domain only 2; pAS, polyadenylation signal; PAX5, paired box protein 5; Pcdhα, protocadherin α; P-TEFb, positive transcription elongation factor; RNAPII, RNA polymerase II; RUNX1, runt-related transcription factor 1; SEC, super elongation complex; Sfmbt2, Scm-like with four MBT domains 2; STAG1, cohesion subunit SA-1; TAD, topologically associating domain; T-ALL, T-cell acute lymphoblastic leukemia; TAL1, T-cell acute lymphocytic leukemia protein 1; TCF, transcription factor; TERT, telomerase reverse transcriptase; TET, ten-eleven translocation methylcytosine dioxygenase; TF, transcription factor; TOP, topoisomerase; TSS, transcription start site; WAPL, Wings apart-like.

a cohesin complex to the transcribed region to form cohesin-dependent looping and juxtaposition with the enhancer and promoter into a single-loop domain (Figure 2). Through this step-by-step process, *ThymoD* transcription repositions *BCL11b* superenhancer from a heterochromatic to a euchromatic environment and modulates epigenetic marks across the loop domain to promote phase separation. The cessation of *ThymoD* nascent transcription by the insertion of a pAS disrupts enhancer-promoter communication, leading to combined immune deficiency and T-cell leukemia/lymphoma through a reduction of functional activity of lineage-specific TF and tumor suppressor function of BCL11b (Figure 1B).[3] In this review, we introduce a mechanism of RNAPII-mediated transcription, and then mainly focus on the upregulation or downregulation of RNAPII-mediated noncoding transcription at enhancers, which modulates enhancer-promoter interaction and ultimately leads to a change in the genome structure in cancer.

2 | NONCODING TRANSCRIPTION PRODUCES ERNA, LNCRNA, AND ENHANCER ACTIVATION

Long noncoding RNAs are defined as those having a length greater than 200 nucleotides.[13] Enhancer RNAs generally show low stability and abundance and are infrequently spliced.[14] Both lncRNAs and eRNAs lack an ORF. They are transcribed from enhancers with various histone marks, such as H3K4me1 and H3K27ac, and the occupancy of lineage-specific enhancer-binding proteins. They can be detected from the overlapping locus control region, and their expression correlates with enhancer activity. These noncoding RNAs, like other protein-coding genes, are transcribed by RNAPII. Several functional roles of eRNAs and lncRNAs at enhancers have been proposed, such as colorectal-specific lncRNA *CCAT1-L* (CCAT1, the long isoform) transcribed from a superenhancer region of *MYC* and p53-induced lncRNA named *LED* (lncRNA activator of enhancer domains).[14-16] However, tens of thousands of eRNAs and lncRNAs have been detected in the nucleus, the exact functions of which remain largely unclear.

What controls the spatial organization of the genome during cell development? Two models of nuclear repositioning regarding the correlation between noncoding transcription and nuclear architecture have been proposed.[17] One possibility is that changes in the nuclear organization by lineage-specific TFs, associated cofactors, and other modifications in chromatin could influence and regulate noncoding RNA transcription. However, it is also possible that RNAPII-mediated transcription of noncoding regions, such as lncRNAs and eRNAs, itself could activate enhancer regions and reorganize the

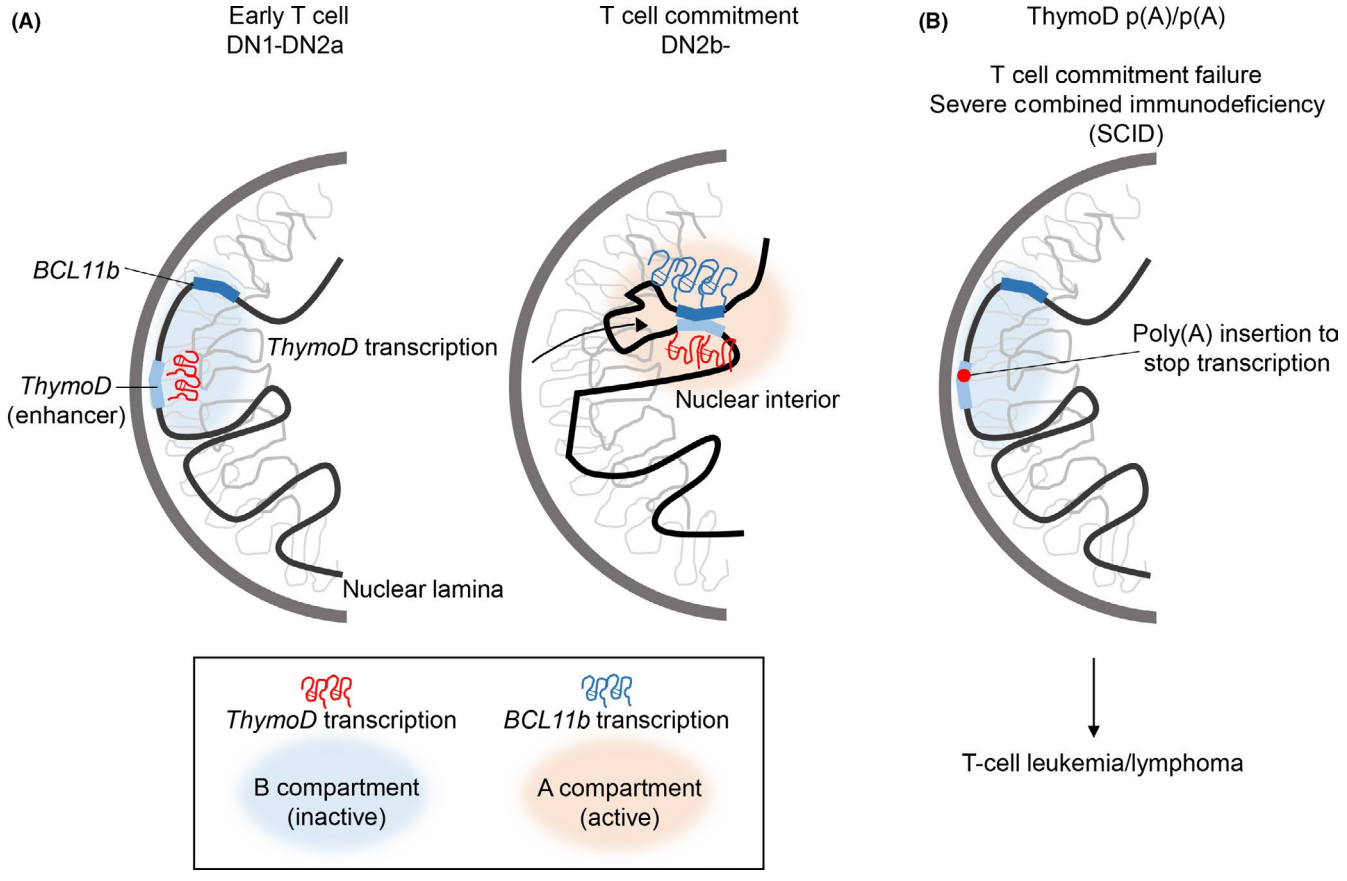

FIGURE 1 Large-scale changes in nuclear architecture in early T cell development. A, The B-cell lymphoma/leukemia 11B (BCL11b) intergenic region is repositioned from the nuclear lamina to the nuclear interior over the course of development. B, Forced cessation of thymocyte differentiation factor (*ThymoD*) transcription abolishes nuclear repositioning and leads to T cell commitment failure

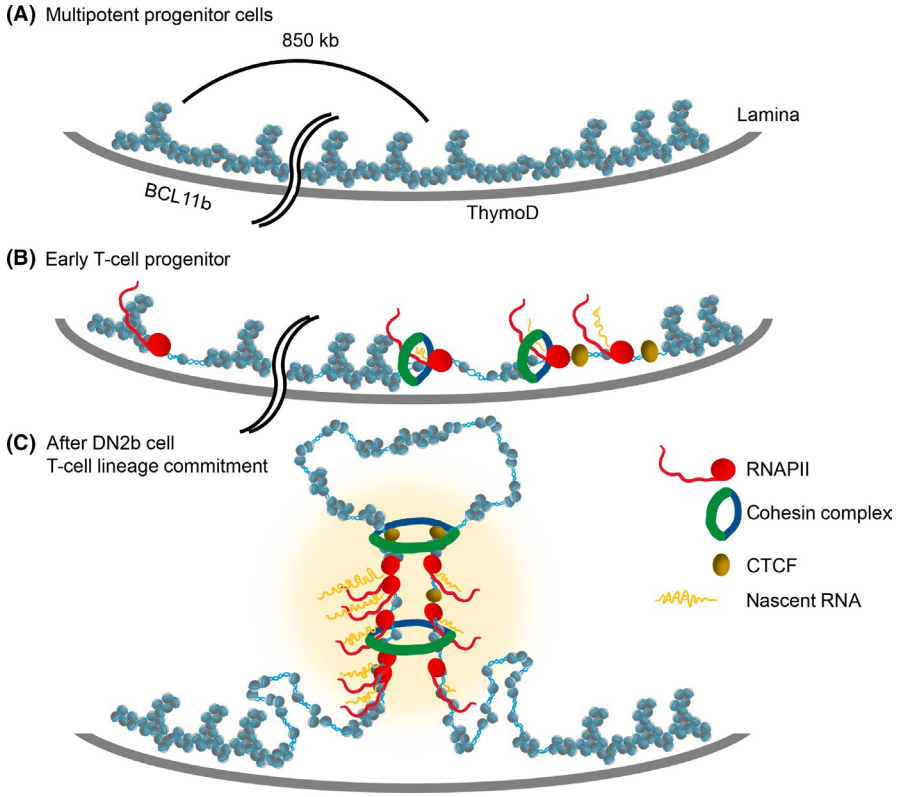

FIGURE 2 Noncoding RNA thymocyte differentiation factor (*ThymoD*) transcription facilitates loop extrusion and enhancer-promoter communication. A, Chromatin organization of the B-cell lymphoma/leukemia 11B (BCL11b) and enhancer locus in multipotent progenitors. B, *ThymoD* transcription recruits the cohesin complex and CCCTC-binding factor (CTCF) to release the BCL11b intergenic region from the lamina. C, *ThymoD* transcription facilitates the formation of de novo loops to bring BCL11b superenhancer to the BCL11b promoter and promote histone exchange and DNA modification

nucleus. These mechanisms possibly synchronize and partially overlap to facilitate the relocalization of genomic loci.[17,18]

Our attempts at inhibiting *ThymoD* transcripts by shRNAs or locked nucleic acids did not result in a change of *ThymoD* expression, *BCL11b* expression, or nuclear localization of the *BCL11b* enhancer. However, forced cessation by inserting a pAS into the region immediately downstream of the *ThymoD* control region 2 affected subsequent steps to generate efficient enhancer-promoter looping and a change of nuclear localization (Figure 1B).[3] Similar studies also supported this finding. For example, Engreitz et al[19] revealed pAS insertions at 5 different locations at lncRNA *Bluster* from immediately adjacent to the TSS to 15 kb downstream of it and found that increasing the length of the *Bluster* transcribed region by RNAPII allowed an increase of expression of the target gene *Sfmbt2*. The blocking of the lncRNA upperhand (*Uph*), but not knockdown of the transcript, abrogated expression of the target gene *Hand2*, leading to right ventricular hypoplasia and embryonic lethality in mice. Thus, we mainly focus on how the RNAPII-mediated transcription contributes to enhancer activation and de novo looping formation between enhancer and promoter.

3 | NONCODING TRANSCRIPTION BY RNAPII AT ENHANCERS

The aforementioned lncRNAs and eRNAs at enhancers are uni- or bidirectionally transcribed by RNAPII. RNA polymerase II is involved in the transcription of protein-coding and many noncoding genes.[20] Transcription consists of 4 organized stages: initiation, promoter clearance, elongation, and termination (Figure 3). RNA polymerase II requires a set of general TFs to be positioned correctly at the promoter inside of the enhancer. The general TFs consist of a set of transcription initiation factors, such as TFIIA, TFIIB, TFIIC, and TFIID (Figure 3A). These factors are required for transcription initiation from the promoter to start the elongation mode. The P-TEFb is a multiprotein complex and a cyclin-dependent kinase that phosphorylates the DRB-sensitivity-inducing factor, negative elongation factor, and the CTD of RNAPII. DRB-sensitivity-inducing factor and negative elongation factor are required for the stalling of RNAPII downstream of the TSS (Figure 3B). Transcriptional initiation is also affected by transcriptional elongation and RNAPII pausing, suggesting that the feedback mechanism regulates new initiation.[21] Lu et al[22] showed that, after initiation, cyclin T1 of P-TEFb and dual specific tyrosine-phosphorylation-regulated kinase 1A promotes hyperphosphorylation of the CTD and induces phase separation in vitro and in vivo (Figure 3B,D). Prephosphorylation by CDK7 of TFIIH strengthens the hyperphosphorylation of the CTD. In contrast to the conventional model, in which the CTD picks up passenger molecules, the mechanism through the hyperphosphorylation of CTD recruits RNAPII into the functional phase-separated compartment to establish the efficient elongation of RNAPII (Figure 3D).[22,23] Bidirectional transcription at enhancers has been proposed to control initiation through phase separation.[24]

Super elongation complex includes ELL, P-TEFb, AFF4, and several other factors (Figure 3C).[25] ELL is one of the main subunits of SEC and the first translocation partner of the *MLL* (*KMTA2*) gene.[26] Super elongation complex increases the rate of transcriptional

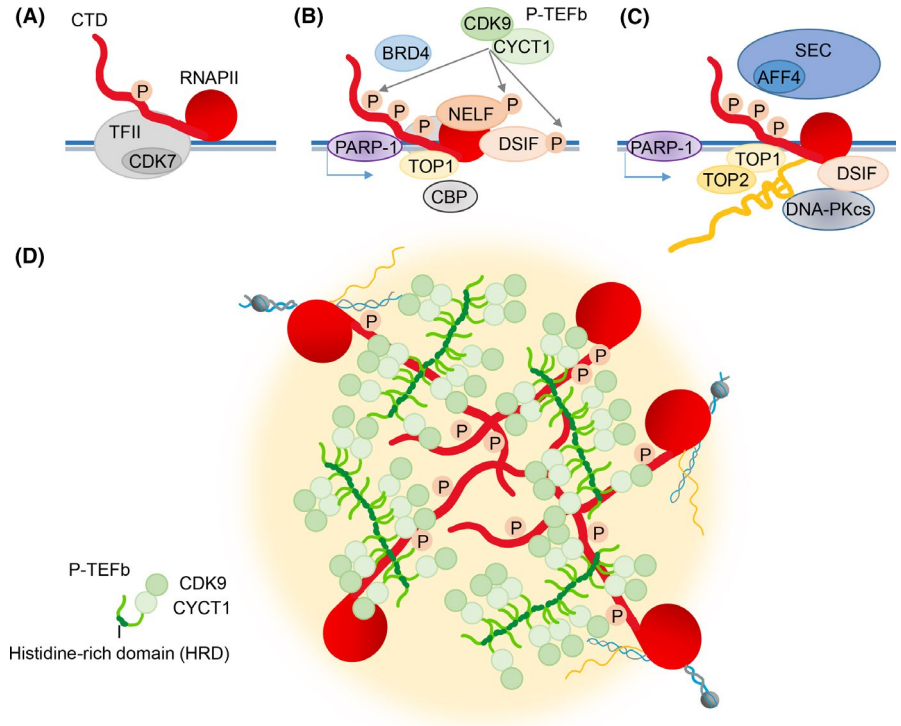

FIGURE 3 Stages of transcription and RNA polymerase II clustering. A, General transcription factors (TFII) are required for transcription initiation. TFIIH phosphorylates (P) Ser5 of the RNA polymerase C-terminal domain (CTD). B, RNA polymerase II (RNAPII) during promoter-proximal pausing regulates the transition into elongation. At this stage, RNAPII is phosphorylated at Ser5 and Ser7 downstream of the transcription start site. RNAPII is bound by negative elongation factor (NELF) and DRB-sensitivity-inducing factor (DSIF). Positive elongation factor b (P-TEFb) phosphorylates NELF, DSIF, and Ser2 of RNAPII. C, During elongation, the CTD contains lower levels of Ser5P and Ser7P and a higher level of Ser2P, which facilitates super elongation complex (SEC), chromatin modifiers, and RNA-processing factors. D, CYCT1 histidine-rich domain in P-TEFb recruits the RNAPII CTD into a phase-separated compartment to facilitate the phosphorylation of CTD. AFF4, AF4/FMR2 family member 4; BRD4, bromodomain-containing protein 4; CBP, CREB-binding protein; CDK, cyclin-dependent kinase; CYCT1, Cyclin T1; DNA-PKcs, DNA-dependent protein kinase, catalytic subunit; PARP1, poly(ADP-ribose) polymerase-1; TOP, topoisomerase

elongation of PolII in vitro. Disruption of the elongation stage of transcription has been reported to be involved in the pathogenesis of human diseases, including cancer.[27] The elongation of enhancer transcription involves common regulators, including BRD4 (Figure 3B). Bromodomain-containing protein 4 is a member of the bromodomain and extraterminal family of TFs, which is a coactivator and binds to acetylated histone H3 and H4 on chromatin to promote RNAPII.[28] Through this interaction, BRD4 recruits P-TEFb, mediators, and other TFs to promote the activation at enhancers.[29,30] Bromodomain-containing protein 4 and cofactors preferentially associate with superenhancers. Inhibition of BRD4 leads to the loss of BRD4, mediators, and P-TEFb at superenhancers and causes the preferential loss of transcription as superenhancers regulate tumor oncogenes.[30]

RNA polymerase II works as a powerful molecular motor and must transcribe supercoiled DNA against the torsional state. RNA polymerase II can generate torque, which regulates the transcription rate and pausing.[31] The excessive accumulation of torque is associated with transcription stalling and DNA structure. Transcription-generated supercoiling also changes or releases bound proteins.[32,33] Torsion generated by RNAPII can evict histones.[32-34] To aid the progression of RNAPII transcription, RNAPII is associated with factors including energy-dependent chromatin remodelers, modifiers adding histone posttranslational modifications, and histone chaperones.[35] To protect against the spontaneous destruction of genomic DNA during transcription, topoisomerase I is recruited to functional enhancers to relax both negative and positive supercoils (Figure 3B,C).[36] DNA damage response machinery including DNA ligase IV, ataxia telangiectasia mutated, KU80, exonuclease 1, bloom syndrome protein, and DNA ligase I also work at transcriptional enhancers.[36] Histone H1 and poly(ADP-ribose) polymerase-1 bind exclusively at RNAPII-transcribed promoters. Poly(ADP-ribose) polymerase-1 is enriched and H1 is depleted at these promoters (Figure 3B,C).[37]

4 | ENRICHMENT OF OXIDIZED METHYLCYTOSINE AT ENHANCERS

Nascent transcription at enhancers is associated with hypomethylated DNA and a prominent hypomethylated superanchor was found to be located downstream of the Ig heavy chain locus.[11] The TET

catalyzes the modification of the DNA base 5hmC, 5-formylcytosine, or 5-carboxylcytosine. The former is enriched on TF binding sites.[38] Moreover, the loss of Tet2 and Tet3 in murine early B cells was shown to block the pro- to pre-B differentiation in bone marrow.[39] However, it is still unclear whether methylcytosine oxidation recruits unknown factors to open chromatin and then a TF binds there, whether TFs recruit TET proteins, or whether both of these mechanisms cross-talk with each other.

The enrichment of 5hmC at enhancers is mediated by TET proteins, but also the blocking of access of DNA methyltransferase by the physical presence of a TF. TET2 binds to enhancers and facilitates the recruitment of estrogen receptor-α.[40] The loss of TET2 in native hematopoiesis and transformed acute myeloid leukemia attenuates the binding of basic helix-loop-helix TFs on enhancers.[41] Lineage-specific TF PU.1 coimmunoprecipitates with TET2. Knockdown of E2A or PU.1 was also shown to decrease chromatin accessibility and increase CpG methylation at the Igk3′ and distal enhancers.[39] In contrast, histone exchange contributes to the prevention of DNA methylation at CpG islands. Recruited chromatin remodelers and EP400 promote the stable incorporation of histone variants H2A.Z and H3.3 into enhancers and promoters and facilitate transcription.[42] H2A.Z antagonizes DNA methylation to protect promoters from DNA methylation and activate genes.[43] H2A.Z is enriched in euchromatic regions and prevents the spread of heterochromatin.[44] *ThymoD* transcription facilitates the demethylation and incorporation of H3.3 onto the transcribed region at superenhancers to recruit CTCF/cohesin for efficient loop extrusion.[3] Canzio et al[45] recently showed that noncoding transcription-mediated demethylation also contributes to the binding of CTCF on Pcdhα promoters. An R-loop consists of a DNA : RNA hybrid and nontemplate single-stranded DNA. Antisense lncRNA *TARID* (TCF21 antisense RNA inducing promoter demethylation) generates an R-loop at the *TCF21* promoter. GADD45A (growth arrest and DNA damage protein 45A) binds to R-loops and recruits TET1 to promote local DNA demethylation.[46,47] These reports suggest that RNAPII-mediated transcription controls demethylation at enhancers.

5 | FACTORS ASSOCIATED WITH NONCODING RNA TRANSCRIPTION ON ENHANCERS TO FORM A DE NOVO ENHANCER-PROMOTER DOMAIN

CTCF is a zinc-finger protein and main regulator of transcriptional insulation and loop-anchors in a convergent orientation in which sequence motifs are facing each other.[48,49] RNA polymerase II-mediated transcriptional activity occurs within the CTCF/cohesin-mediated chromatin structure, and most RNAPII-mediated loops are smaller than CTCF loops.[50] Cohesin localization preferentially takes place on either convergent or transcriptionally active genes, suggesting that cohesin movement is associated with RNAPII (Figure 4A).[51,52] A component of the cohesin complex, STAG1, interacts with SEC components including AFF4, ELL2, cyclin T1, and CDK9. Additionally, STAG1 interacts with RNAPII (Figure 3A). Reduction of RNAPII Serine 2 and 5 phosphorylation by the addition of DRB and flavopiridol decreased precipitated STAG1, indicating the direct molecular interaction of SEC, cohesin complex, and RNAPII.[53] The distribution of cohesin depends on transcription, the position of CTCF, and the cohesin-releasing factor WAPL (Figure 4A). Following the knockdown of CTCF and WAPL, cohesin complex accumulates at the central position between transcriptionally convergent genes, referred to as a cohesin island, indicating that cohesin sliding is dependent on RNAPII activity (Figure 4B).[54] Cohesin depletion decreases transcription on the gene body and frequently increases the level of paused RNAPII on cohesin-binding genes, indicating that the cohesin complex also contributes to the promotion of RNAPII activity from pausing to the elongation step.[55] *ThymoD* transcription facilitates cohesin loading and sliding on its transcribed region. The forced cessation of *ThymoD* nascent transcription by the insertion of a pAS led to loss of enhancer activation, leading to disruption of the efficient interaction between the enhancer and *BCL11b* promoter (Figures 1 and 2).[3] Transcription can affect the genome 3-D structure on the influenza A virus infection model. Influenza A virus NS1 protein globally inhibits transcription termination, leading to readthrough transcription (Figure 4C,D).[56] Readthrough transcription can allow TF binding on the heterochromatic region and convert the chromatin status from a transcriptionally inert to a permissive environment (Figure 4D).[56] Taking these findings together, cohesin recruitment and relocalization are also regulated by RNAPII transcription.

6 | DYSREGULATED NASCENT TRANSCRIPTION IN CANCER

Based on the mechanism of activation through RNAPII-mediated transcription, single nucleotide variations, mutation, small deletion, inversion, or insertion at the TF binding region at enhancers would potentially decrease or increase the activity of noncoding transcription. These genetic alterations could lead to changes in chromatin topology and the expression level of tumor suppressors or oncogenes involved in any process from the initiation to the final process of tumorigenesis, including metastasis. It has been reported that mutation in the noncoding promoter region of *TERT* changes a consensus binding site for ETS TFs and increases the transcriptional activity by 2- to 4-fold in melanoma, bladder, and hepatocellular cancer cells.[57-59] Somatic noncoding mutations generate de novo TF binding sites near the *TAL1*, *LMO1*, and *LMO2* oncogenes in T-ALL. Insertions in the upstream noncoding region of *TAL1* introduce a de novo MYB binding motif that recruits coactivators and RNAPII, and forms an H3K27ac marked superenhancers that activates oncogenic *TAL1* expression (Figure 4E).[60] Small genomic insertions form enhancers leading to the misregulation of oncogenes in several types of tumor. For example, somatic short insertions in leukemia are frequently observed in an enhancer near the *LMO2* oncogene and activate it, which increases *LMO2* transcription.[61] Heterozygous intronic mutations that create

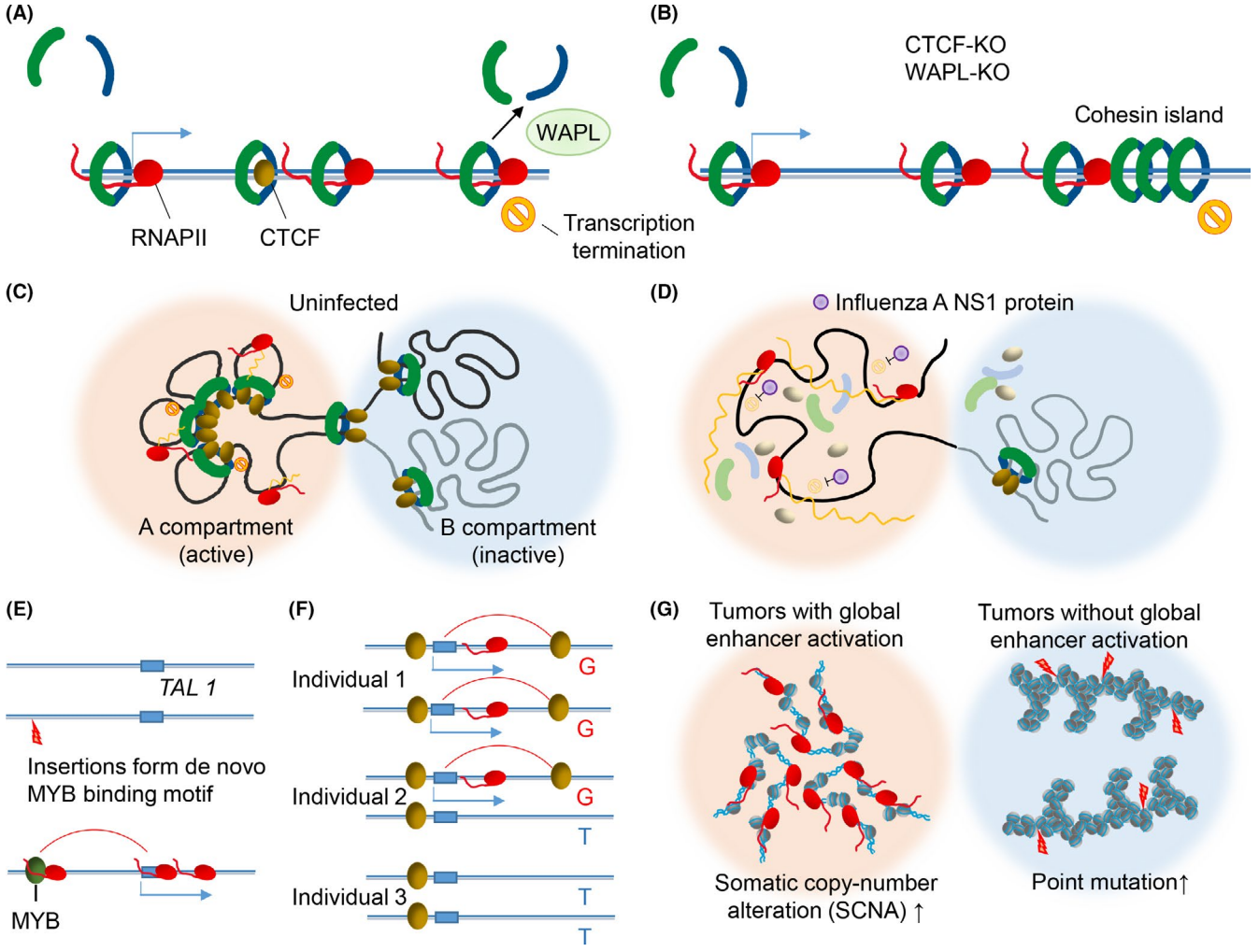

FIGURE 4 Transcription-mediated cohesin translocation and models depicting noncoding mutation in cancer. A, Transcription can relocate cohesin into the CCCTC-binding factor (CTCF) binding site. Wings apart-like (WAPL) can release cohesin at the 3′-ends of transcribed genes. RNAPII, RNA polymerase II. B, Cohesin accumulates at 3′-ends in CTCF and WAPL double-knockout (KO) cells. C, In an uninfected setting, boundaries are maintained by the efficient cessation of transcription. D, Influenza A NS1 protein allows global readthrough transcription beyond 3′-ends. Readthrough transcription disrupts cohesin/CTCF-mediated loops and causes a change of compartment from an inactive to an active state. E, Insertions in the upstream noncoding region form a de novo MYB binding site, which drives *TAL1* expression. F, SNP, deletion, or inversion in CTCF binding site can alter topologically associating domain structure and gene transcription. G, Aneuploidy is associated with global enhancer activation

novel MYB, ETS1, or RUNX1 binding sites to increase monoallelic *LMO2* overexpression are frequently observed in pediatric and adult T-ALL patient samples.[62] Somatic noncoding regulatory mutation has also been discovered in T-ALL, owing to the de novo formation of an MYB binding site linked to the recruitment of coactivators and RNAPII-mediated transcription.[63] These are representative examples of mutations in promoters or enhancers that alter TF binding sites to modulate the looping pattern and activity of oncogenes.

Deletion and inversion of DNA fragments with CTCF/cohesin-binding sites disrupt the TAD structure and change the transcription of related genes.[64,65] Haplotype variants change monoallelic CTCF-mediated chromatin topology and function, with a link to disease risks (Figure 4F).[50] Cohesin is present in active enhancer regions and colocalizes with CTCF. Cohesin subunits are mutated in cancer. Frequent point mutations at CTCF/cohesin-binding sites, especially accumulating in the CTCF motif, were identified in colorectal cancer as well as multiple other cancer types, indicating that cohesin-binding sites are a major hotspot in noncoding regions in cancer.[66]

Mutation in the B-cell-specific TF *PAX5* enhancer in chronic lymphocytic leukemia, diffuse large B-cell lymphoma, follicular lymphoma, and mantle-cell lymphoma leads to a reduction of *PAX5* expression, possibly acting as a driver event linked to the development of these tumors.[67] Somatic noncoding mutations are detected at enhancer expression quantitative loci in specific cancers. The somatic expression quantitative loci network is disturbed in 88% of tumors, indicating that noncoding mutations are involved in clonal evolution.[68] Notably, global enhancer activation is associated with aneuploidy, not mutation load, which tends to increase the likelihood of DNA rearrangements. In contrast, enhancer silencing is associated with mutation (Figure 4G).[69]

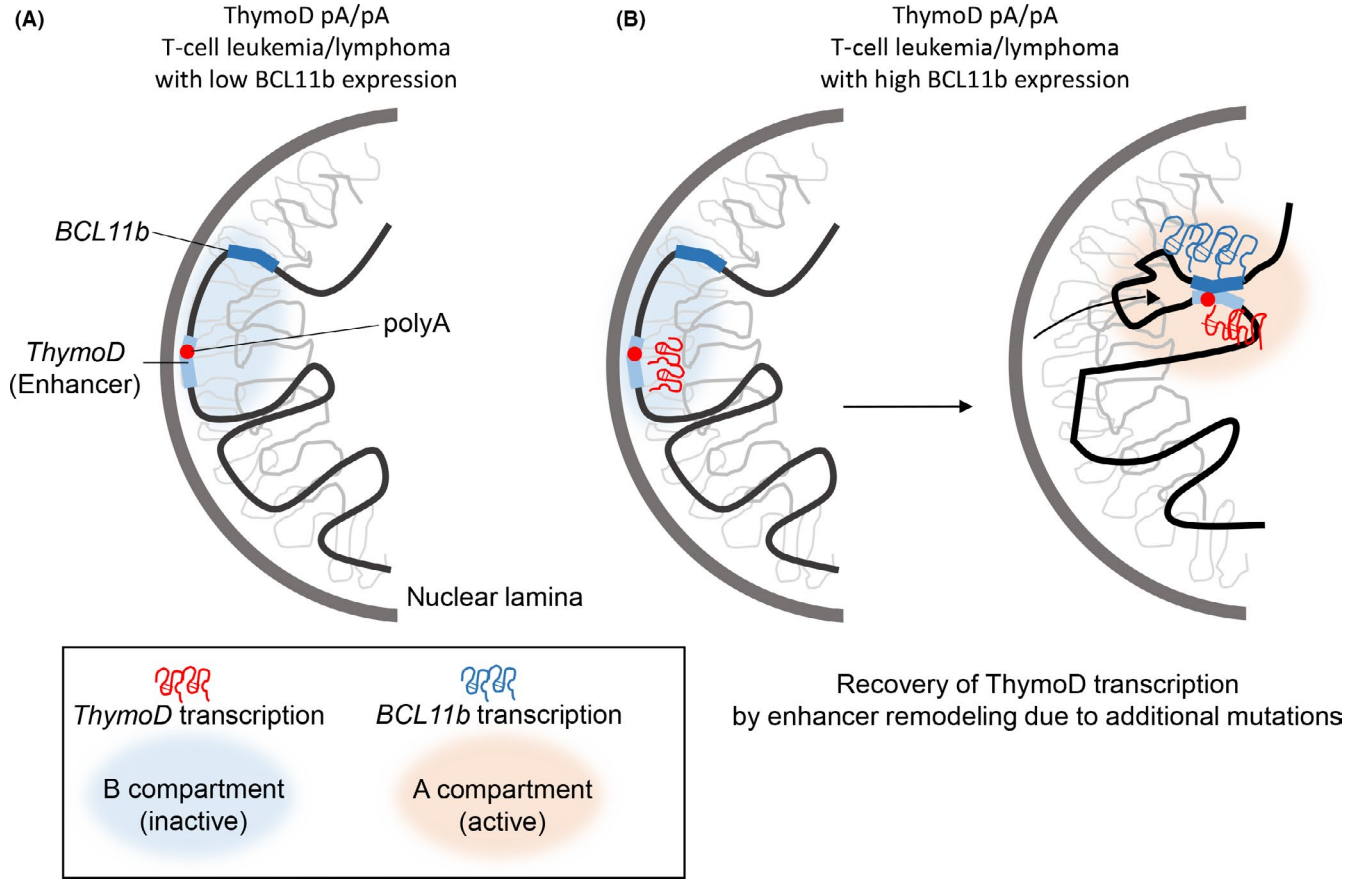

FIGURE 5 Enhancer remodeling in thymocyte differentiation factor (*ThymoD*)-deficient T-cell tumors. A, Development of leukemias and lymphomas in *ThymoD* p(A)/p(A) mice. Tumors had lower *ThymoD* transcription near or on the nuclear lamina. B, The B-cell lymphoma/leukemia 11B (BCL11b) intergenic region was repositioned from the lamina to the nuclear interior in tumors with higher *ThymoD* transcription

Another example is that the reduction of insulator proteins, such as CTCF and cohesin, could lead to transcriptional dysregulation in cancer. Yang et al[70] showed that low expression of CTCF and cohesin in hyperdiploid pediatric B-cell precursor acute lymphoblastic leukemia, compared with the levels in ETV6/RUNX1-positive ALL, tends to be associated with the dysregulation of gene expression linked to a reduction of loss of TAD boundary strength and insulation at TAD borders, possibly involved in a leukemogenic effect.

7 | CONCLUDING REMARKS

We described that genetic alteration potentially affecting RNAPII-mediated transcription at enhancers leads to modulation of the looping pattern and target gene expression in cancer. These candidate genetic alterations identified by next-generation sequencing need to be confirmed by future studies. The prominent feature observed in ThymoD p(A)/p(A) tumors is a variable expression status of *ThymoD* and a change of the nuclear localization of the genome from the nuclear lamina to the nuclear interior. This suggests that acquired mutations can remodel enhancer activity in cancer with the originally same noncoding mutation (Figure 5).

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Young Scientists (18K15661), The Japan Foundation for Pediatric Research (18-003), and a Research Grant from The Japanese Society of Hematology (2889). We thank Edanz (www.edanzediting.co.jp) for editing the English text of a draft of this manuscript.

DISCLOSURE

The authors declare that they have no conflicts of interest regarding this article.

ORCID

Takeshi Isoda https://orcid.org/0000-0003-3349-3705

REFERENCES

1. Kosak ST, Skok JA, Medina KL, et al. Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development. *Science*. 2002;296(5565):158-162.

2. Lin YC, Benner C, Mansson R, et al. Global changes in the nuclear positioning of genes and intra- and interdomain genomic interactions that orchestrate B cell fate. *Nat Immunol*. 2012;13(12):1196-1204.
3. Isoda T, Moore AJ, He Z, et al. Non-coding transcription instructs chromatin folding and compartmentalization to dictate enhancer-promoter communication and T cell fate. *Cell*. 2017;171(1):103-119.e118.
4. Lin YC, Jhunjhunwala S, Benner C, et al. A global network of transcription factors, involving E2A, EBF1 and Foxo1, that orchestrates B cell fate. *Nat Immunol*. 2010;11(7):635-643.
5. Mansson R, Welinder E, Ahsberg J, et al. Positive intergenic feedback circuitry, involving EBF1 and FOXO1, orchestrates B-cell fate. *Proc Natl Acad Sci USA*. 2012;109(51):21028-21033.
6. Ikawa T, Kawamoto H, Wright LY, et al. Long-term cultured E2A-deficient hematopoietic progenitor cells are pluripotent. *Immunity*. 2004;20(3):349-360.
7. Ikawa T, Kawamoto H, Goldrath AW, et al. E proteins and Notch signaling cooperate to promote T cell lineage specification and commitment. *J Exp Med*. 2006;203(5):1329-1342.
8. Ikawa T, Hirose S, Masuda K, et al. An essential developmental checkpoint for production of the T cell lineage. *Science*. 2010;329(5987):93-96.
9. Li L, Leid M, Rothenberg EV. An early T cell lineage commitment checkpoint dependent on the transcription factor Bcl11b. *Science*. 2010;329(5987):89-93.
10. Kueh HY, Yui MA, Ng KK, et al. Asynchronous combinatorial action of four regulatory factors activates Bcl11b for T cell commitment. *Nat Immunol*. 2016;17(8):956-965.
11. Benner C, Isoda T, Murre C. New roles for DNA cytosine modification, eRNA, anchors, and superanchors in developing B cell progenitors. *Proc Natl Acad Sci USA*. 2015;112(41):12776-12781.
12. Hu G, Cui K, Fang D, et al. Transformation of accessible chromatin and 3D nucleome underlies lineage commitment of early T cells. *Immunity*. 2018;48(2):227-242.e228.
13. Guttman M, Amit I, Garber M, et al. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. *Nature*. 2009;458(7235):223-227.
14. Geisler S, Coller J. RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. *Nat Rev Mol Cell Biol*. 2013;14(11):699-712.
15. Xiang JF, Yin QF, Chen T, et al. Human colorectal cancer-specific CCAT1-L lncRNA regulates long-range chromatin interactions at the MYC locus. *Cell Res*. 2014;24(5):513-531.
16. Leveille N, Melo CA, Rooijers K, et al. Genome-wide profiling of p53-regulated enhancer RNAs uncovers a subset of enhancers controlled by a lncRNA. *Nat Commun*. 2015;6:6520.
17. Mele M, Rinn JL. "Cat's Cradling" the 3D genome by the act of LncRNA transcription. *Mol Cell*. 2016;62(5):657-664.
18. van Steensel B, Furlong EEM. The role of transcription in shaping the spatial organization of the genome. *Nat Rev Mol Cell Biol*. 2019;20:327-337.
19. Engreitz JM, Haines JE, Perez EM, et al. Local regulation of gene expression by lncRNA promoters, transcription and splicing. *Nature*. 2016;539(7629):452-455.
20. Sims RJ 3rd, Mandal SS, Reinberg D. Recent highlights of RNA-polymerase-II-mediated transcription. *Curr Opin Cell Biol*. 2004;16(3):263-271.
21. Shao W, Zeitlinger J. Paused RNA polymerase II inhibits new transcriptional initiation. *Nat Genet*. 2017;49(7):1045-1051.
22. Lu H, Yu D, Hansen AS, et al. Phase-separation mechanism for C-terminal hyperphosphorylation of RNA polymerase II. *Nature*. 2018;558(7709):318-323.
23. Boehning M, Dugast-Darzacq C, Rankovic M, et al. RNA polymerase II clustering through carboxy-terminal domain phase separation. *Nat Struct Mol Biol*. 2018;25(9):833-840.
24. Hnisz D, Shrinivas K, Young RA, et al. A phase separation model for transcriptional control. *Cell*. 2017;169(1):13-23.
25. Mohan M, Lin C, Guest E, et al. Licensed to elongate: a molecular mechanism for MLL-based leukaemogenesis. *Nat Rev Cancer*. 2010;10(10):721-728.
26. Shilatifard A, Lane WS, Jackson KW, et al. An RNA polymerase II elongation factor encoded by the human ELL gene. *Science*. 1996;271(5257):1873-1876.
27. Lin C, Smith ER, Takahashi H, et al. AFF4, a component of the ELL/P-TEFb elongation complex and a shared subunit of MLL chimeras, can link transcription elongation to leukemia. *Mol Cell*. 2010;37(3):429-437.
28. Dey A, Chitsaz F, Abbasi A, et al. The double bromodomain protein Brd4 binds to acetylated chromatin during interphase and mitosis. *Proc Natl Acad Sci USA*. 2003;100(15):8758-8763.
29. Wu SY, Chiang CM. The double bromodomain-containing chromatin adaptor Brd4 and transcriptional regulation. *J Biol Chem*. 2007;282(18):13141-13145.
30. Loven J, Hoke HA, Lin CY, et al. Selective inhibition of tumor oncogenes by disruption of super-enhancers. *Cell*. 2013;153(2):320-334.
31. Ma J, Bai L, Wang MD. Transcription under torsion. *Science*. 2013;340(6140):1580-1583.
32. Kouzine F, Sanford S, Elisha-Feil Z, et al. The functional response of upstream DNA to dynamic supercoiling in vivo. *Nat Struct Mol Biol*. 2008;15(2):146-154.
33. Levchenko V, Jackson B, Jackson V. Histone release during transcription: displacement of the two H2A-H2B dimers in the nucleosome is dependent on different levels of transcription-induced positive stress. *Biochemistry*. 2005;44(14):5357-5372.
34. Lavelle C. Transcription elongation through a chromatin template. *Biochimie*. 2007;89(4):516-527.
35. Venkatesh S, Workman JL. Histone exchange, chromatin structure and the regulation of transcription. *Nat Rev Mol Cell Biol*. 2015;16(3):178-189.
36. Puc J, Kozbial P, Li W, et al. Ligand-dependent enhancer activation regulated by topoisomerase-I activity. *Cell*. 2015;160(3):367-380.
37. Krishnakumar R, Gamble MJ, Frizzell KM, et al. Reciprocal binding of PARP-1 and histone H1 at promoters specifies transcriptional outcomes. *Science*. 2008;319(5864):819-821.
38. Yu M, Hon GC, Szulwach KE, et al. Base-resolution analysis of 5-hydroxymethylcytosine in the mammalian genome. *Cell*. 2012;149(6):1368-1380.
39. Lio CW, Zhang J, Gonzalez-Avalos E, et al. Tet2 and Tet3 cooperate with B-lineage transcription factors to regulate DNA modification and chromatin accessibility. *Elife*. 2016;5:e18290.
40. Wang L, Ozark PA, Smith ER, et al. TET2 coactivates gene expression through demethylation of enhancers. *Sci Adv*. 2018;4(11):eaau6986.
41. Rasmussen KD, Berest I, Kebetaler S, et al. TET2 binding to enhancers facilitates transcription factor recruitment in hematopoietic cells. *Genome Res*. 2019;29(4):564-575.
42. Pradhan SK, Su T, Yen L, et al. EP400 deposits H3.3 into promoters and enhancers during gene activation. *Mol Cell*. 2016;61(1):27-38.
43. Zilberman D, Coleman-Derr D, Ballinger T, et al. Histone H2A.Z and DNA methylation are mutually antagonistic chromatin marks. *Nature*. 2008;456(7218):125-129.
44. Meneghini MD, Wu M, Madhani HD. Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. *Cell*. 2003;112(5):725-736.
45. Canzio D, Nwakeze CL, Horta A, et al. Antisense lncRNA transcription mediates DNA demethylation to drive stochastic protocadherin alpha promoter choice. *Cell*. 2019;177(3):639-653.e615.
46. Arab K, Park YJ, Lindroth AM, et al. Long noncoding RNA TARID directs demethylation and activation of the tumor suppressor TCF21 via GADD45A. *Mol Cell*. 2014;55(4):604-614.

47. Arab K, Karaulanov E, Musheev M, et al. GADD45A binds R-loops and recruits TET1 to CpG island promoters. *Nat Genet*. 2019;51(2):217-223.
48. Wendt KS, Yoshida K, Itoh T, et al. Cohesin mediates transcriptional insulation by CCCTC-binding factor. *Nature*. 2008;451(7180):796-801.
49. Rao SS, Huntley MH, Durand NC, et al. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell*. 2014;159(7):1665-1680.
50. Tang Z, Luo OJ, Li X, et al. CTCF-mediated human 3D genome architecture reveals chromatin topology for transcription. *Cell*. 2015;163(7):1611-1627.
51. Lengronne A, Katou Y, Mori S, et al. Cohesin relocation from sites of chromosomal loading to places of convergent transcription. *Nature*. 2004;430(6999):573-578.
52. Bausch C, Noone S, Henry JM, et al. Transcription alters chromosomal locations of cohesin in *Saccharomyces cerevisiae*. *Mol Cell Biol*. 2007;27(24):8522-8532.
53. Izumi K, Nakato R, Zhang Z, et al. Germline gain-of-function mutations in AFF4 cause a developmental syndrome functionally linking the super elongation complex and cohesin. *Nat Genet*. 2015;47(4):338-344.
54. Busslinger GA, Stocsits RR, van der Lelij P, et al. Cohesin is positioned in mammalian genomes by transcription, CTCF and Wapl. *Nature*. 2017;544(7651):503-507.
55. Schaaf CA, Kwak H, Koenig A, et al. Genome-wide control of RNA polymerase II activity by cohesin. *PLoS Genet*. 2013;9(3):e1003382.
56. Heinz S, Texari L, Hayes MGB, et al. Transcription elongation can affect genome 3D structure. *Cell*. 2018;174(6):1522-1536.e1522.
57. Rutter JL, Mitchell TI, Buttice G, et al. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. *Cancer Res*. 1998;58(23):5321-5325.
58. Huang FW, Hodis E, Xu MJ, et al. Highly recurrent TERT promoter mutations in human melanoma. *Science*. 2013;339(6122):957-959.
59. Horn S, Figl A, Rachakonda PS, et al. TERT promoter mutations in familial and sporadic melanoma. *Science*. 2013;339(6122):959-961.
60. Mansour MR, Abraham BJ, Anders L, et al. Oncogene regulation. An oncogenic super-enhancer formed through somatic mutation of a noncoding intergenic element. *Science*. 2014;346(6215):1373-1377.
61. Abraham BJ, Hnisz D, Weintraub AS, et al. Small genomic insertions form enhancers that misregulate oncogenes. *Nat Commun*. 2017;8:14385.
62. Rahman S, Magnussen M, Leon TE, et al. Activation of the LMO2 oncogene through a somatically acquired neomorphic promoter in T-cell acute lymphoblastic leukemia. *Blood*. 2017;129(24):3221-3226.
63. Hu S, Qian M, Zhang H, et al. Whole-genome noncoding sequence analysis in T-cell acute lymphoblastic leukemia identifies oncogene enhancer mutations. *Blood*. 2017;129(24):3264-3268.
64. Dowen Jill M, Fan Zi P, Hnisz D, et al. Control of cell identity genes occurs in insulated neighborhoods in mammalian chromosomes. *Cell*. 2014;159(2):374-387.
65. Guo Y, Xu Q, Canzio D, et al. CRISPR inversion of CTCF sites alters genome topology and enhancer/promoter function. *Cell*. 2015;162(4):900-910.
66. Katainen R, Dave K, Pitkanen E, et al. CTCF/cohesin-binding sites are frequently mutated in cancer. *Nat Genet*. 2015;47(7):818-821.
67. Puente XS, Bea S, Valdes-Mas R, et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia. *Nature*. 2015;526(7574):519-524.
68. Zhang W, Bojorquez-Gomez A, Velez DO, et al. A global transcriptional network connecting noncoding mutations to changes in tumor gene expression. *Nat Genet*. 2018;50(4):613-620.
69. Chen H, Li C, Peng X, et al. A pan-cancer analysis of enhancer expression in nearly 9000 patient samples. *Cell*. 2018;173(2):386-399.e312.
70. Yang M, Vesterlund M, Siavelis I, et al. Proteogenomics and Hi-C reveal transcriptional dysregulation in high hyperdiploid childhood acute lymphoblastic leukemia. *Nat Commun*. 2019;10(1):1519.

How to cite this article: Isoda T, Morio T, Takagi M. Noncoding RNA transcription at enhancers and genome folding in cancer. *Cancer Sci*. 2019;110:2328-2336. https://doi.org/10.1111/cas.14107