Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Genetics & Development

Gene regulation by non-coding RNAs in the 3D genome architecture

Hiroaki Tachiwana, Tatsuro Yamamoto and Noriko Saitoh

Appropriate gene expression is essential for producing the correct amount of proteins at the right time, which is critical for living organisms. In the three-dimensional (3D) space of the nucleus, genomes are folded into higher order chromatin structures that are intimately associated with epigenetic factors, including histone modifications and nuclear long non-coding RNAs (lncRNAs). LncRNAs regulate transcription for both activation and repression, either in *cis* or in *trans*. Many ncRNAs are expressed in development-specific, differentiation-specific, and disease-specific manners, suggesting that they are critical regulators for organ generation and maintenance. In this review, we mainly describe the following ncRNAs: *Xist*, involved in X chromosome inactivation, *Firre*, which serves as a platform for trans-chromosomal associations, and *UMLILO* and *ELEANORS*, which co-regulate genes involved in the immune response and breast cancer, respectively. These ncRNAs are gene regulators in the context of the 3D genome structure.

Address
Division of Cancer Biology, The Cancer Institute of JFCR, 3-8-31 Ariake, Koto-ku, Tokyo 135-8550, Japan

Corresponding author: Saitoh, Noriko (noriko.saito@jfcr.or.jp)

Current Opinion in Genetics & Development 2020, **61**:69–74

This review comes from a themed issue on **Genome architecture and expression**

Edited by **Kerstin Bystricky** and **Matthias Merkenschlager**

For a complete overview see the Issue and the Editorial

Available online 5th May 2020

https://doi.org/10.1016/j.gde.2020.03.002

Introduction

In eukaryotes, the genomic DNA is packaged into chromatin, in which the fundamental repeating unit is a nucleosome consisting of 180 bp DNA and four histone proteins, H2A, H2B, H3, and H4 [1]. The array of nucleosomes are folded into multiple layers, from lower to higher order, involving hundreds of kbs of chromatin loops, approximately 1 Mb of TADs (topologically associated domains), mega-bases of A and B compartments, and individual chromosome territories [2–8]. Chromatin loops contain long-range chromosomal contacts and local chromatin loops, such as in enhancer–promoter interactions. TAD is a self-interacting chromatin region that compartmentalizes genomes. Enhancers can co-regulate genes within the TAD, but not outside of it. Disruption of the TAD boundaries leads to impaired gene expression, and corresponds to certain diseases [9,10]. The A and B compartments are much larger chromatin domains, and roughly correspond to euchromatin with active histone marks and heterochromatin with repressive histone marks, respectively [5,11]. This finding implies that the 3D genome structures originating from chromatin interactions play a key role in the regulation of gene expression.

Over 40 years ago, chromatin was found to cofractionate with RNAs, thus suggesting the presence of chromatin-associated RNAs [12–14]. More recent experiments with the Drosophila cell line have demonstrated that chromatin is increasingly endonuclease-resistant when cellular RNAs are hydrolyzed with RNaseA [15]. In this case, small nucleolar RNAs bind to chromatin though their associated proteins, and this is responsible for the chromatin inaccessibility. The possible involvement of other less-abundant RNAs remains to be investigated. These indicate that nuclear RNAs may facilitate the formation of an open euchromatin structure, and regulate gene expression under certain circumstances.

Recent high throughput sequence analyses have revealed that the genome is pervasively transcribed [16]. It is estimated that over 100 000 RNAs lacking protein coding potential, referred to as non-coding RNAs (ncRNAs), exist in cells [16]. ncRNAs with lengths longer than 200 nt are long ncRNAs (lncRNAs), and some play key roles in development. One of the best-studied examples is the *Xist* RNA, which is involved in X-chromosome inactivation (XCI) in mammalian females, as described below. *Xist* is produced from the unique locus, *Xic* (X chromosome inactivation center), which contains a cluster of ncRNA genes including *RepA*, *Tsix*, *Xite*, *Jpx*, *Ftx*, and *Tsx*. These ncRNAs are involved in the regulation of *Xist* expression and function, as well as XCI. This implies that ncRNAs are important cellular regulatory factors.

In this review article, we discuss the recent work on the ncRNAs that are involved in gene regulation, mainly through modulating higher order chromatin structures and epigenetic marks. We also consider the significance of ncRNAs in mammalian development, immunity, and cancer.

Xist functions in X chromosome inactivation during female early embryonic development

During early development in female mammals, one of the two X chromosomes (XX) is silenced as dosage compensation, relative to males with only one X chromosome (XY).

This is referred to as X-chromosome inactivation (XCI). The X chromosome carries over 1000 genes essential for development and cell viability, and their overexpression due to XCI failure is potentially harmful [17,18]. The key regulator of XCI is the *Xist* (*X-inactive–specific transcript*) RNA, a 17 kb lncRNA expressed from the inactive X chromosome (Xi) [19–22]. The depletion of *Xist* results in the failure of XCI initiation [23,24], while the forced *Xist* expression on autosomes leads to silencing of the neighboring genes [25,26].

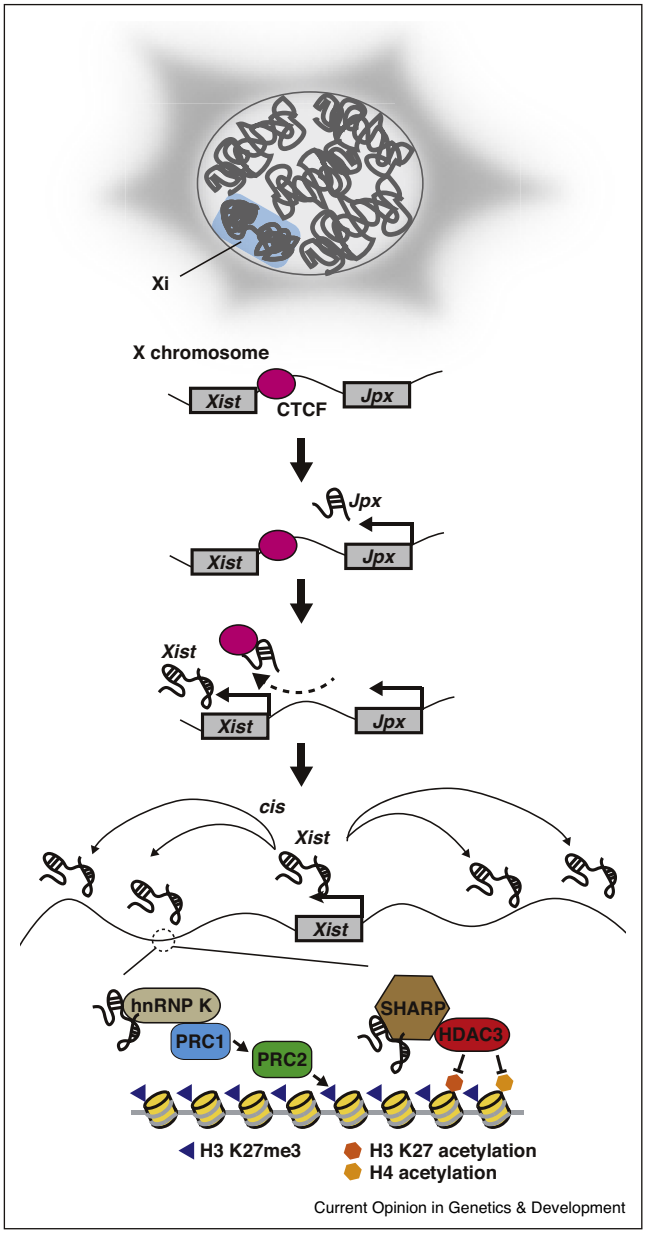
Xist is produced and spread in *cis* along the X chromosome to highly condense the chromosome, leading to silencing of the X-linked genes. Eventually, *Xist* covers the entire Xi and forms an RNA cloud, which is often found near the nuclear membrane or one of the nuclear substructures, the nucleolus. At the beginning of XCI, *Xist* expression is repressed by the binding of the CTCF (CCCTC-binding factor) protein to the *Xist* promoter (Figure 1), and the CTCF protein is evicted by another lncRNA, *Jpx*, which acts as the activator for the *Xist* expression [27] (Figure 1). The produced *Xist* then interacts with hnRNP K (heterogeneous nuclear ribonucleic protein K) and recruits PRC1 (Polycomb group protein complex 1) leading to accumulation of PRC2 [28••,29–32] for the trimethylation of histone H3 at lysine 27 (H3K27me3). *Xist* also interacts directly with SHARP (SMRT/HDAC1 associated repressor protein) to silence nearby transcription, through histone deacetylation by HDAC3 (histone deacetylase 3) (Figure 1) [33]. These combinations of lncRNAs and epigenetic modifiers contribute to the constitutive heterochromatin formation of Xi. Although *Xist* deletion from the previously established Xi disrupts the heterochromatin conformation, it has little effect on X-linked gene silencing [34,35]. This suggests that *Xist* is essential for the Xi-specific chromosome structure, but dispensable for the established Xi, perhaps due to the existence of other epigenetic marks.

Other ncRNAs that recruit repressive and active factors to chromatin

Several genes are expressed from only the maternal or paternal chromosome, in a phenomenon referred to as genomic imprinting. In addition to DNA methylation and histone modifications, ncRNAs are involved in this process. The *Airn* (*Antisense Igf2r RNA non-coding*) ncRNA is expressed only from the paternal allele, and required for the paternal-specific silencing of the multiple neighboring imprinted genes, *Slc22a3*, *Slc22a2*, and *Igf2r*, in the mouse placenta [36–38]. As with *Xist*, *Airn* forms an RNA cloud in the nucleus, covers the paternal *Slc22a3*, and recruits the histone methyltransferase G9a, for the repressive histone mark (H3K9me3).

Unlike *Xist* and *Airn*, the *HOTAIR* (*HOX transcript antisense RNA*) ncRNA functions in *trans*. It is produced from the *HOXC* locus on chromosome 12, and functions on the *HOXD* locus on chromosome 2 [39]. *HOTAIR* demarcates the silent and active chromatin domains in the *HOXD* locus, by recruiting PRC2 to accumulate the repressive histone mark (H3K27me3), and LSD1 (Lysine-specific demethylase 1) to demethylate and erase the active histone mark (H3K4me1).

Figure 1



Xist lncRNA is required for X chromosome inactivation.
During early female embryonic development, *Xist* is produced from one of the two X chromosomes, and spread along the chromosome to form the highly condensed and inactive X chromosome (Xi). *Xist* expression is repressed by CTCF protein binding to the promoter, and CTCF is evicted by another lncRNA, *Jpx*. The produced *Xist* then recruits PRC1, PRC2 and HDAC3, through hnRNPK and SHARP, respectively, resulting in the accumulation of repressive histone modifications.

Firre serves as a platform for trans-chromosomal associations

The long-range chromatin interaction analyses identified a genomic region that interacts with the X-linked macro-satellite region, *DXZ4*. It is the *Firre* (*Functional intergenic repeating RNA element*) locus that abundantly produces the *Firre* lncRNA, primarily from the active X chromosome [40,41•]. *Firre* forms RNA clouds in the nucleus, and serves as a platform for trans-chromosomal associations. *Firre* has 156 nt repeats, termed the repeating RNA domain (RRD), and they bind to the nuclear-matrix protein hnRNP U, which may connect *Firre* with other genomic loci, including *Ppp1r10*, *Slc25a12*, and *Ype14* on other chromosomes [40] (Figure 2). The *Firre* locus deletion changes gene expression in a hematopoietic progenitor cell type, which can be rescued by expressing *Firre* RNAs from an autosomal transgene [41•]. *Firre* also functions in anchoring Xi to the nucleolus, and maintains H3K27me3 for silencing genes [42]. Taken together, *Firre* is a trans-acting RNA molecule that constructs the 3D genome architecture.

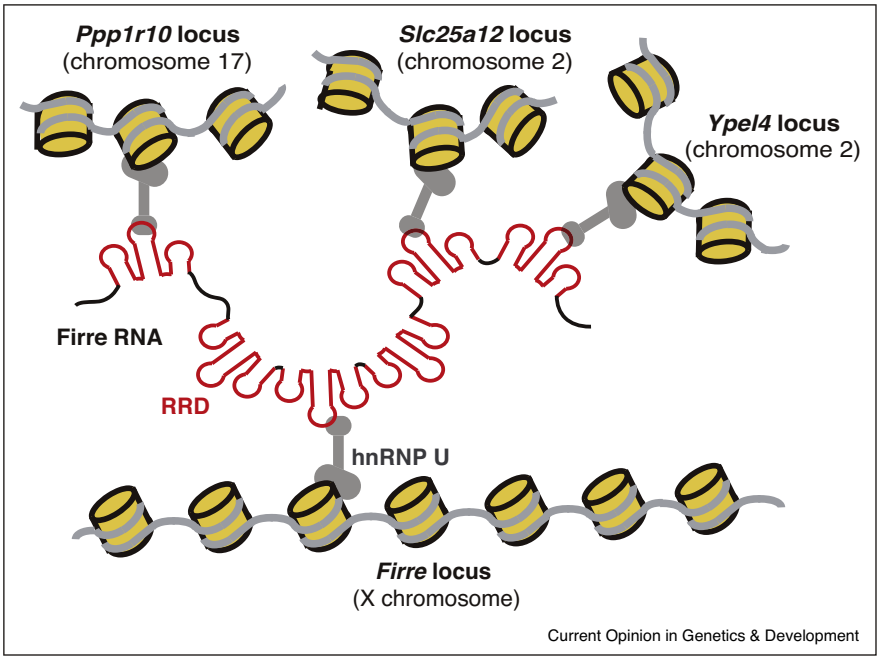
UMLILO primes immune-genes for robust transcription in trained immunity

For an enhanced innate immune response, or trained immunity, immune-related gene promoters are primed for robust transcription. The active histone mark H3K4me3 is accumulated at their promoters, before immune stimulations. IPLs (Immune-gene priming lncRNAs) are a collection of lncRNAs expressed from the TAD containing the TNF (tumor-necrosis factor) responsive genes, and regulate them in *cis* [43••]. Among them is the *UMLILO* (*Upstream Master LncRNA of the Inflammatory chemokine Locus*) lncRNA, and it is produced within the TAD where the chemokine genes *IL8*, *CXCL1*, *CXCL2*, and *CXCL3* are transcribed (Figure 3). *UMLILO* interacts with the WDR5 protein (WD repeat-containing protein 5) [44], a component of the MLL1 complex, which catalyzes the methylation of histone H3 at lysine 4 for H3K4me3. *UMLILO* depletion decreases the H3K4me3 level at the *CXCL* promoters. Intriguingly, *HOTTIP* (*HOXA transcript at the distal tip*), another lncRNA, can replace the functions of *UMLILO*, because *HOTTIP* also interacts with WDR5 and promotes the H3K4me3-mediated activation of the *HOXA* genes [44,45]. These findings demonstrate that lncRNAs mediate TAD regulation, which may be central to trained immunology.

ELEANORS delineate the active TAD and the long-range chromatin interactions in breast cancer recurrence

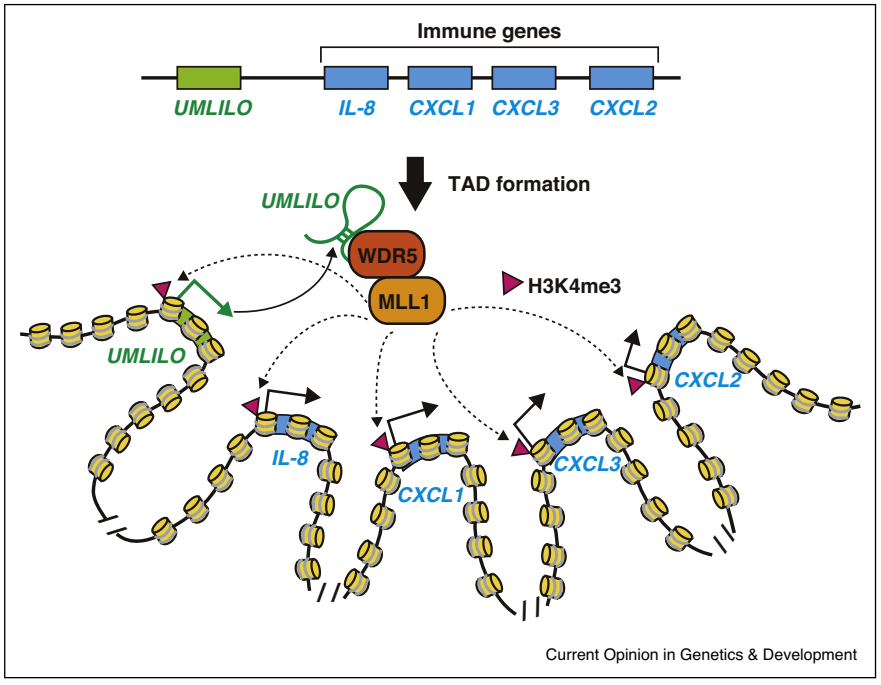
Gene expression profiles are remodeled in cancers. For example, the *ESR1* gene is upregulated when ER (estrogen receptor)-positive breast cancer acquires endocrine therapy resistance. In this recurrence process, estrogen is deprived due to the therapy, and a cluster of lncRNAs, *ELEANORS* (*ESR1 locus enhancing and activating noncoding RNAs*), are produced from the TAD including the *ESR1* gene, termed

Figure 2



Firre serves as a platform for trans-chromosomal associations.
Firre is abundantly produced from the X chromosome. The repeating RNA domain (RRD) in *Firre* binds to the hnRNPU protein, which connects *Firre* with additional genomic loci on other chromosomes.

Figure 3

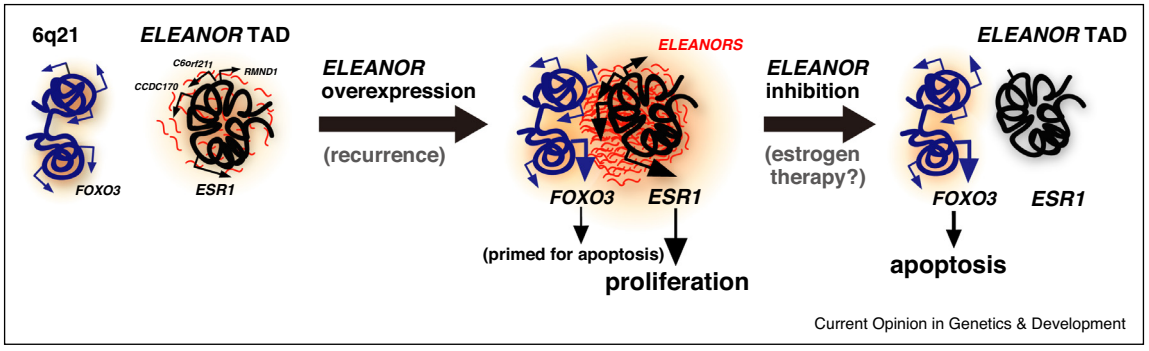


UMLILO mediates immune-gene priming for robust transcription in trained immunity.
UMLILO is produced from the TAD where the immune genes *IL8*, *CXCL1*, *CXCL2*, and *CXCL3* are also transcribed. *UMLILO* interacts with the WDR5 protein, a component of the MLL1 complex, and accelerates H3K4me3 enrichment in the genes before immune stimulation.

the *ELEANOR* TAD. *ELEANORS* remain at their own transcription sites, form the RNA cloud, and activate all of the genes within the TAD [46•,47,48•] (Figure 4, from left to middle).

ER-positive breast cancer patients who relapse after endocrine therapies can be treated with estrogen. This paradoxical therapy may represent the cancer fragility in which the recurrent breast cancer is primed for cell death, before the estrogen treatment. This is explained at least partly by the long-range chromatin interaction. In the recurrent model cells, a subset of apoptotic genes are upregulated, including *FOXO3*. Furthermore, the *ESR1* gene interacts with the *FOXO3* (forkhead box O3) gene, and both are co-upregulated in the A compartment. The two genes are encoded on chromosome 6 and approximately 40 Mb apart,

Figure 4



ELEANORS delineate the active TAD and the long-range chromatin interaction in breast cancer recurrence.
During the acquisition of endocrine-therapy resistance in breast cancer, a cluster of lncRNAs, *ELEANOR* RNAs, is produced from the TAD including the *ESR1* gene (from left to middle). These lncRNAs activate all of the genes within the TAD, and establish the long-range chromatin interaction between *ESR1* and *FOXO3* (middle). Upon inhibition of *ELEANORS*, the chromatin interaction is reduced and the genes in the *ELEANOR* TAD are repressed, while high *FOXO3* expression is maintained (right). This unbalanced gene expression induces apoptosis, which may recapitulate the paradoxical estrogen treatment.

and this long-range interaction is mediated by *ELEANORS*. *ELEANORS* may balance the genes for cell proliferation (*ESR1*) and cell death (*FOXO3*) [46•] (Figure 4, middle). Inhibition of *ELEANORS* by the estrogen-related compound, resveratrol, resolves the chromatin interaction and represses the genes in the *ELEANOR* TAD, while maintaining the high *FOXO3* expression. This unbalanced gene expression induces cell death, which may recapitulate the paradoxical estrogen treatment (Figure 4, right). These findings suggest that lncRNAs may be novel therapeutic targets for cancers.

Conclusion and perspectives

In this review, we have described examples of lncRNAs that are involved in the 3D genome structure and gene regulation. The modes of action for lncRNAs are diverse, and they participate in transcription activation or repression, by recruiting epigenetic modifiers, organizing nuclear substructures, co-regulating multiple genes in the same TAD, and mediating long-range chromatin interactions. LncRNAs are also involved in many different events, including development, immune responses, and diseases. Consequently, lncRNAs are expected to serve as novel biomarkers and therapeutic targets [49]. More details remain to be elucidated.

Although nuclear lncRNAs function in a wide variety of events, the fundamental property that shared among all RNAs and RNA binding proteins may exist. Identification of the property and elucidation of how it is regulated in the nucleus remain to be investigated. The mechanism by which each lncRNA is expressed, localized, or recruited to the specific sites in the genome may be another layer of gene regulation, in the context of the 3D genome architecture.

Conflict of interest statement

Nothing declared.

Acknowledgements

We apologize to authors whose work could not be cited due to space constraints. We would like to thank the members of the Saitoh laboratory (The Cancer Institute of JFCR) for helpful discussions. This work was supported by JSPS KAKENHI Grant Numbers JP17H05013 [to H.T.], JP19K23736 [to T.Y.], JP18H05531 [to N.S.], and JP18K19310 [to N.S.], and by grants from the Takeda Science Foundation [to N.S.], The Vehicle Racing Commemorative Foundation [to N.S.], and a Research Grant of the Princess Takamatsu Cancer Research Fund [to N.S.].

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Luger K, Mäder AW, Richmond RK, Sargent DF, Richmond TJ: **Crystal structure of the nucleosome core particle at 2.8Å resolution**. *Nature* 1997, **389**:251-260.

2. Dekker J, Marti-Renom MA, Mirny LA: **Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data**. *Nat Rev Genet* 2013, **14**:390-403.

3. Dekker J, Rippe K, Dekker M, Kleckner N: **Capturing chromosome conformation**. *Science* 2002, **295**:1306-1311.

4. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, Hu M, Liu JS, Ren B: **Topological domains in mammalian genomes identified by analysis of chromatin interactions**. *Nature* 2012, **485**:376-380.

5. Rao SSP, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, Sanborn AL, Machol I, Omer AD, Lander ES *et al.*: **A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping**. *Cell* 2014, **159**:1665-1680.

6. Wijchers PJ, Krijger PHL, Geeven G, Zhu Y, Denker A, Verstegen MJAM, Valdes-Quezada C, Vermeulen C, Janssen M, Teunissen H *et al.*: **Cause and consequence of tethering a SubTAD to different nuclear compartments**. *Mol Cell* 2016, **61**:461-473.

7. Nagano T, Lubling Y, Várnai C, Dudley C, Leung W, Baran Y, Mendelson Cohen N, Wingett S, Fraser P, Tanay A: **Cell-cycle dynamics of chromosomal organization at single-cell resolution**. *Nature* 2017, **547**:61-67.

8. Flyamer IM, Gassler J, Imakaev M, Brandão HB, Ulianov SV, Abdennur N, Razin SV, Mirny LA, Tachibana-Konwalski K: **Single-nucleus Hi-C reveals unique chromatin reorganization at oocyte-to-zygote transition**. *Nature* 2017, **544**:110-114.

9. Lupiáñez DG, Kraft K, Heinrich V, Krawitz P, Brancati F, Klopocki E, Horn D, Kayserili H, Opitz JM, Laxova R *et al.*: **Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions**. *Cell* 2015, **161**:1012-1025.

10. Hu S, Lv P, Yan Z, Wen B: **Disruption of nuclear speckles reduces chromatin interactions in active compartments**. *Epigenetics Chromatin* 2019, **12**:1-12.

11. Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO *et al.*: **Comprehensive mapping of long-range interactions reveals folding principles of the human genome**. *Science* 2009, **326**:289-293.

12. Bonner J, Widholm J: **Molecular complementarity between nuclear DNA and organ-specific chromosomal RNA**. *Proc Natl Acad Sci U S A* 1967, **57**:1379-1385.

13. Holoubek V, Deacon NJ, Buckle DW, Naora H: **A small chromatin-associated RNA homologous to repetitive DNA sequences**. *Eur J Biochem* 1983, **137**:249-256.

14. Huang RC, Bonner J: **Histone-bound RNA, a component of native nucleohistone**. *Proc Natl Acad Sci U S A* 1965, **54**:960-967.

15. Schubert T, Pusch MC, Diermeier S, Benes V, Kremmer E, Imhof A, Längst G: **Df31 protein and snoRNAs maintain accessible higher-order structures of chromatin**. *Mol Cell* 2012, **48**:434-444.

16. Zhao Y, Li H, Fang S, Kang Y, Wu W, Hao Y, Li Z, Bu D, Sun N, Zhang MQ *et al.*: **NONCODE 2016: an informative and valuable data source of long non-coding RNAs**. *Nucleic Acids Res* 2016, **44**:D203-8.

17. Lyon MF: **Gene action in the X-chromosome of the mouse (*Mus musculus* L.)**. *Nature* 1961, **190**:372-373.

18. Boumil RM, Lee JT: **Forty years of decoding the silence in X-chromosome inactivation**. *Hum Mol Genet* 2001, **10**:2225-2232.

19. Brown CJ, Hendrich BD, Rupert JL, Lafrenière RG, Xing Y, Lawrence J, Willard HF: **The human XIST gene: analysis of a 17 kb inactive X-specific RNA that contains conserved repeats and is highly localized within the nucleus**. *Cell* 1992, **71**:527-542.

20. Brown SD: **XIST and the mapping of the X chromosome inactivation centre**. *Bioessays* 1991, **13**:607-612.

21. Borsani G, Tonlorenzi R, Simmler MC, Dandolo L, Arnaud D, Capra V, Grompe M, Pizzuti A, Muzny D, Lawrence C *et al.*: **Characterization of a murine gene expressed from the inactive X chromosome**. *Nature* 1991, **351**:325-329.

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

23. Penny GD, Kay GF, Sheardown SA, Rastan S, Brockdorff N: **Requirement for Xist in X chromosome inactivation**. *Nature* 1996, **379**:131-137.

24. Marahrens Y, Panning B, Dausman J, Strauss W, Jaenisch R: **Xist-deficient mice are defective in dosage compensation but not spermatogenesis**. *Genes Dev* 1997, **11**:156-166.

25. Lee JT, Jaenisch R: **Long-range cis effects of ectopic X-inactivation centres on a mouse autosome**. *Nature* 1997, **386**:275-279.

26. Lee JT, Strauss WM, Dausman JA, Jaenisch R: **A 450 kb transgene displays properties of the mammalian X-inactivation center**. *Cell* 1996, **86**:83-94.

27. Sun S, Del Rosario BC, Szanto A, Ogawa Y, Jeon Y, Lee JT: **Jpx RNA activates Xist by evicting CTCF**. *Cell* 2013, **153**:1537-1551.

28. •• Colognori D, Sunwoo H, Kriz AJ, Wang C-Y, Lee JT: **Xist deletional analysis reveals an interdependency between Xist RNA and polycomb complexes for spreading along the inactive X**. *Mol Cell* 2019, **74**:101-117.e10

This paper showed that the *Xist* RNA and the Polycomb complexes are mutually required for both to be spread along the Xi. Perturbing either *Xist* or Polycomb spreading impaired *de novo* Xi silencing.

29. Bousard A, Raposo AC, Żylicz JJ, Picard C, Pires VB, Qi Y, Gil C, Syx L, Chang HY, Heard E *et al.*: **The role of Xist-mediated Polycomb recruitment in the initiation of X-chromosome inactivation**. *EMBO Rep* 2019, **20**:e48019.

30. Pintacuda G, Wei G, Roustan C, Kirmizitas BA, Solcan N, Cerase A, Castello A, Mohammed S, Moindrot B, Nesterova TB *et al.*: **hnRNPK recruits PCGF3/5-PRC1 to the Xist RNA B-repeat to establish polycomb-mediated chromosomal silencing**. *Mol Cell* 2017, **68**:955-969.e10.

31. Almeida M, Pintacuda G, Masui O, Koseki Y, Gdula M, Cerase A, Brown D, Mould A, Innocent C, Nakayama M *et al.*: **PCGF3/5-PRC1 initiates Polycomb recruitment in X chromosome inactivation**. *Science* 2017, **356**:1081-1084.

32. Zhao J, Sun BK, Erwin JA, Song J-J, Lee JT: **Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome**. *Science* 2008, **322**:750-756.

33. McHugh CA, Chen C-K, Chow A, Surka CF, Tran C, McDonel P, Pandya-Jones A, Blanco M, Burghard C, Moradian A *et al.*: **The Xist lncRNA interacts directly with SHARP to silence transcription through HDAC3**. *Nature* 2015, **521**:232-236.

34. Minajigi A, Froberg J, Wei C, Sunwoo H, Kesner B, Colognori D, Lessing D, Payer B, Boukhali M, Haas W *et al.*: **Chromosomes. A comprehensive Xist interactome reveals cohesin repulsion and an RNA-directed chromosome conformation**. *Science* 2015, **349**.

35. Giorgetti L, Lajoie BR, Carter AC, Attia M, Zhan Y, Xu J, Chen CJ, Kaplan N, Chang HY, Heard E *et al.*: **Structural organization of the inactive X chromosome in the mouse**. *Nature* 2016, **535**:575-579.

36. Nagano T, Mitchell JA, Sanz LA, Pauler FM, Ferguson-Smith AC, Feil R, Fraser P: **The Air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin**. *Science* 2008, **322**:1717-1720.

37. Latos PA, Pauler FM, Koerner MV, Şenergin HB, Hudson QJ, Stocsits RR, Allhoff W, Stricker SH, Klement RM, Warczok KE *et al.*: **Airn transcriptional overlap, but not its lncRNA products, induces imprinted Igf2r silencing**. *Science* 2012, **338**:1469-1472.

38. Santoro F, Mayer D, Klement RM, Warczok KE, Stukalov A, Barlow DP, Pauler FM: **Imprinted Igf2r silencing depends on continuous Airn lncRNA expression and is not restricted to a developmental window**. *Development* 2013, **140**:1184-1195.

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

40. Hacisuleyman E, Goff LA, Trapnell C, Williams A, Henao-Mejia J, Sun L, McClanahan P, Hendrickson DG, Sauvageau M, Kelley DR *et al.*: **Topological organization of multichromosomal regions by the long intergenic noncoding RNA Firre**. *Nat Struct Mol Biol* 2014, **21**:198-206.

41. • Lewandowski JP, Lee JC, Hwang T, Sunwoo H, Goldstein JM, Groff AF, Chang NP, Mallard W, Williams A, Henao-Meija J *et al.*: **The Firre locus produces a trans-acting RNA molecule that functions in hematopoiesis**. *Nat Commun* 2019, **10**:5137

Lewandowski *et al.* reported the physiological role of *Firre* in mouse hematopoiesis. They showed that the deletion of the *Firre* locus did not change the expression of neighboring genes in the X chromosome, but rather changed those on autosomes, supporting the idea that *Firre* is a trans-acting lncRNA.

42. Yang F, Deng X, Ma W, Berletch JB, Rabaia N, Wei G, Moore JM, Filippova GN, Xu J, Liu Y *et al.*: **The lncRNA Firre anchors the inactive X chromosome to the nucleolus by binding CTCF and maintains H3K27me3 methylation**. *Genome Biol* 2015, **16**:52.

43. •• Fanucchi S, Fok ET, Dalla E, Shibayama Y, Börner K, Chang EY, Stoychev S, Imakaev M, Grimm D, Wang KC *et al.*: **Immune genes are primed for robust transcription by proximal long noncoding RNAs located in nuclear compartments**. *Nat Genet* 2019, **51**:138-150

Fanucchi *et al.* showed that the *UMLILO* lncRNA mediates the 3D genome architecture that brings H3K4me3, which makes TNF (tumor necrosis factor)-responsive genes primed for robust transcription in the establishment of trained immunity.

44. Wang KC, Yang YW, Liu B, Sanyal A, Corces-Zimmerman R, Chen Y, Lajoie BR, Protacio A, Flynn RA, Gupta RA *et al.*: **A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression**. *Nature* 2011, **472**:120-124.

45. Yang YW, Flynn RA, Chen Y, Qu K, Wan B, Wang KC, Lei M, Chang HY: **Essential role of lncRNA binding for WDR5 maintenance of active chromatin and embryonic stem cell pluripotency**. *eLife* 2014, **3**:e02046.

46. • Abdalla MOA, Yamamoto T, Maehara K, Nogami J, Ohkawa Y, Miura H, Poonperm R, Hiratani I, Nakayama H, Nakao M *et al.*: **The Eleanor ncRNAs activate the topological domain of the ESR1 locus to balance against apoptosis**. *Nat Commun* 2019, **10**:3778

This paper showed that the *ELEANOR* lncRNAs delineate the active TAD in the recurrent breast cancer model cells. *ELEANORS* mediate the long-range chromatin interactions between the genes for cell proliferation (*ESR1*) and apoptosis (FOXO3). Disruption of this balance by *ELEANOR* inhibition with an estrogen-like compound interrupted the chromatin interactions and led to cell death. This may explain the paradoxical estrogen therapy for recurrent breast cancer patients, at least partly.

47. Yamamoto T, Sakamoto C, Tachiwana H, Kumabe M, Matsui T, Yamashita T, Shinagawa M, Ochiai K, Saitoh N, Nakao M: **Endocrine therapy-resistant breast cancer model cells are inhibited by soybean glyceollin I through Eleanor non-coding RNA**. *Sci Rep* 2018, **8**:15202.

48. • Tomita S, Abdalla MOA, Fujiwara S, Matsumori H, Maehara K, Ohkawa Y, Iwase H, Saitoh N, Nakao M: **A cluster of noncoding RNAs activates the ESR1 locus during breast cancer adaptation**. *Nat Commun* 2015, **6** 6966-15

This paper reports the first demonstration that a cluster of lncRNAs, *ELEANORS*, is produced from a large chromatin domain including the *ESR1* locus, and forms RNA clouds in the ER-positive endocrine-therapy resistant breast cancer cell model. *ELEANORS* activate the *ESR1* gene, which is required for the adaptation of the cancer cells to the therapeutic environment.

49. Slack FJ, Chinnaiyan AM: **The role of non-coding RNAs in oncology**. *Cell* 2019, **179**:1033-1055.