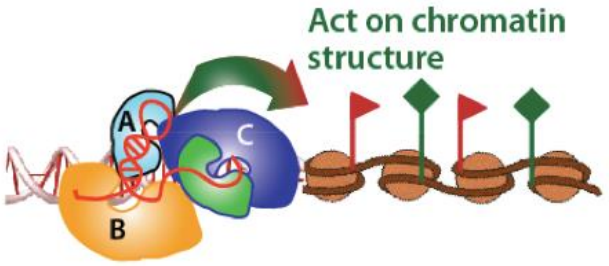
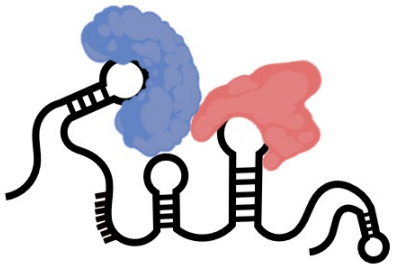
Epigenetic regulation lncRNAs



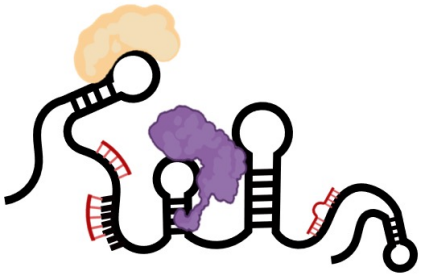
HOTTIP and HOTAIR lncRNAs

lncRNAs mechanisms of action



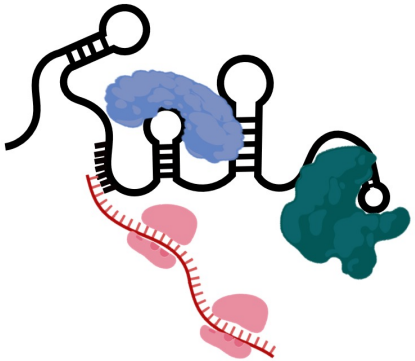
SCAFFOLD

lncRNAs act as central platforms to bring together in space and time multiple effectors and transiently assemble ribonucleoprotein complexes



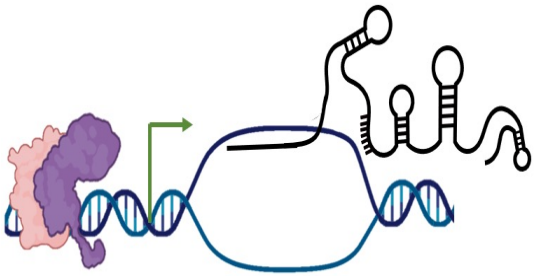
DECOY

lncRNAs can scavenge proteins and miRNAs, thereby limiting their availability and preventing them from binding their targets, even sequestering them within specific cellular subdomains



GUIDE

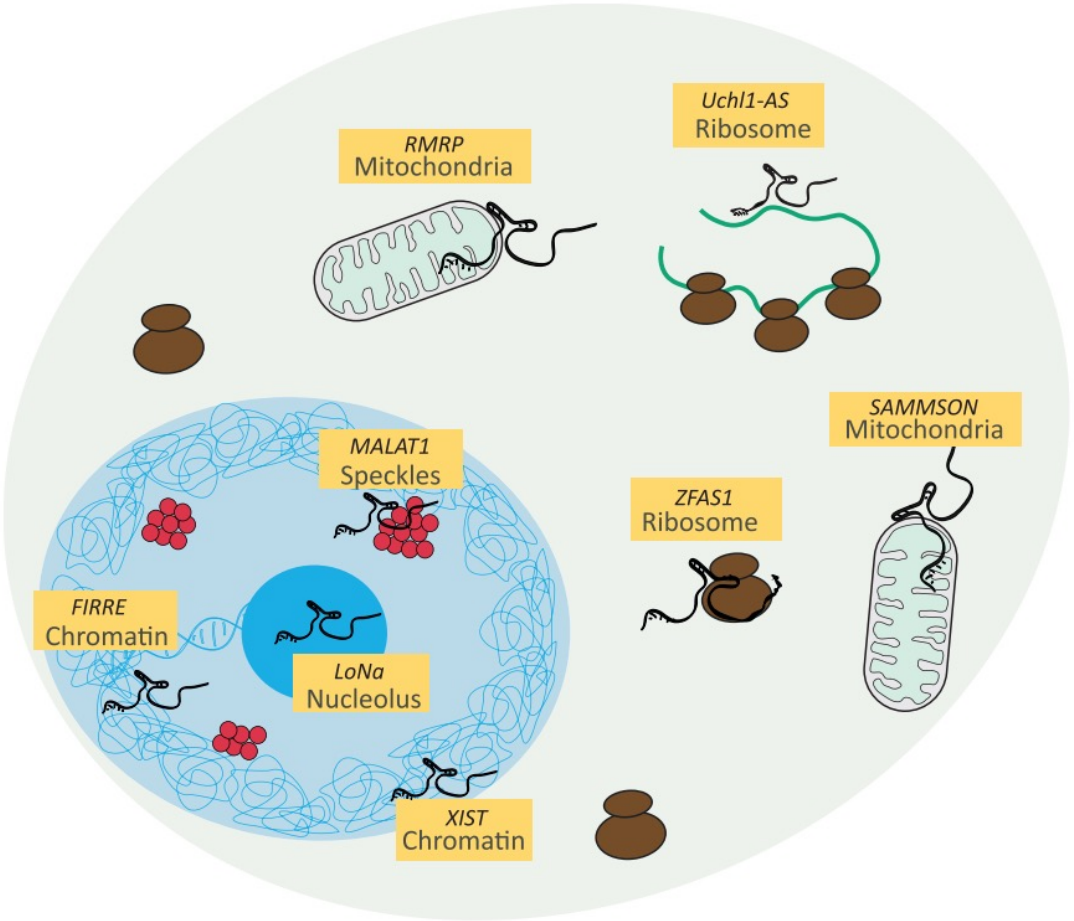
lncRNAs bind their partners and direct their localization to specific targets



SIGNAL

The act of a lncRNA transcription itself has a regulatory purpose

lncRNAs localisation



(Carlevaro-Fita J and Johnson R; 2019)

The range of molecular partners with which a lncRNA can interact (and, consequently, the functions it can assume) are associated with their unique subcellular localization

Nucleus:

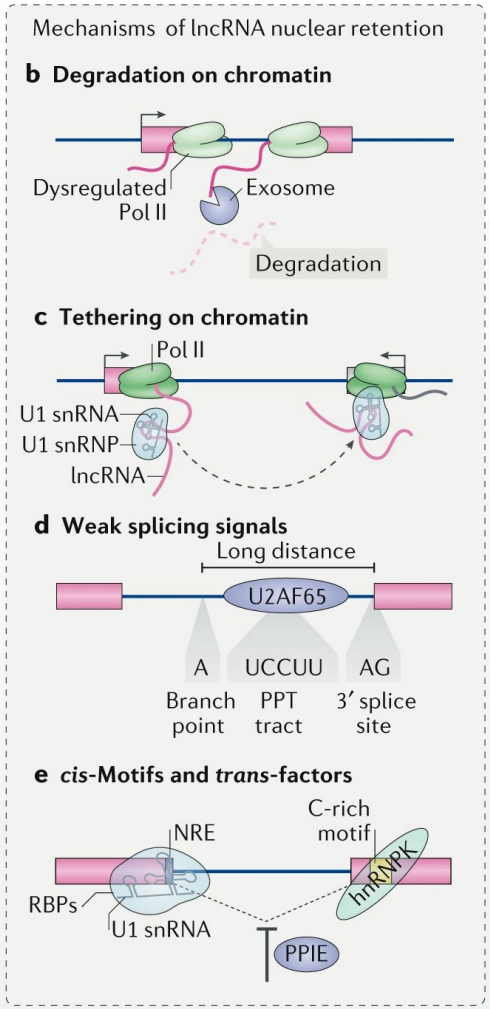
- Nucleoli
- Chromatin speckles
- Paraspeckles
- Nuclear membrane

Cytoplasm:

- Mitochondria
- Ribosomes
- Extracellular membranes
- Exosomes

But remember that subcellular localization of lncRNAs can be a dynamic process!

lncRNAs localisation



(Statello L, Guo CJ, Huarte M; 2021)

Degradation on chromatin:
A significant fraction of lncRNAs are transcribed by phosphorylation-dysregulated Pol II. These are weakly co-transcriptionally spliced and their transcription termination is independent of polyadenylation signals, leading to temporal accumulation of lncRNAs on chromatin, followed by their rapid degradation by the RNA exosome

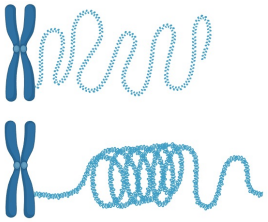
Tethering on chromatin:
Some chromatin-localized lncRNAs contain high levels of U1 small nuclear RNA binding sites, which recruit the U1 snRNP to transcriptionally engaged Pol II, resulting in the tethering of non-coding RNAs to chromatin

Weak splicing signals:
Overall, lncRNAs are spliced less efficiently than mRNAs. They have weaker internal splicing signals and longer distances between the 3′ splice site and the branch point, which correlate with augmented nuclear retention.

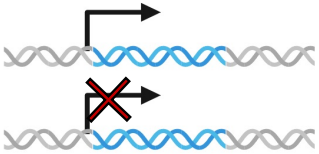
cis-Motifs and trans-factors:
lncRNAs often contain embedded sequence motifs that can recruit certain nuclear factors, which promote the nuclear localization and function of the lncRNA (trans). Repeat elements also likely have roles in driving lncRNA nuclear retention (cis).

Nuclear lncRNAs

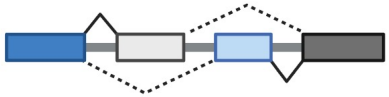
1. Chromatin organisation



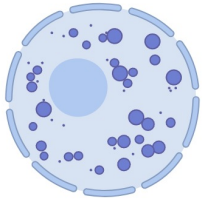
2. Transcription regulation



3. Post-transcription regulation

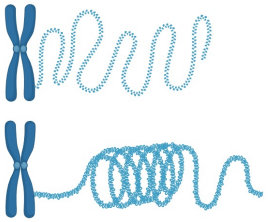


4. Nuclear structure



Nuclear lncRNAs

1. Chromatin organisation



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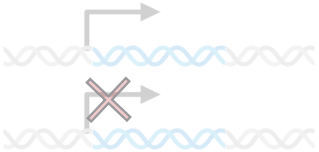


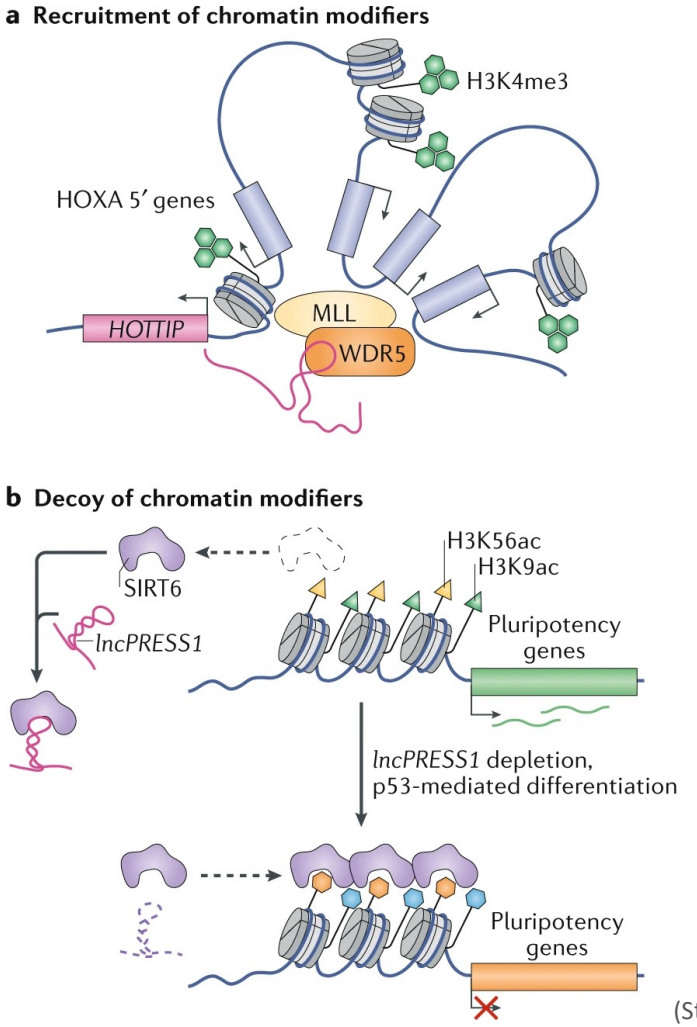
3. Post-transcription regulation

4. Nuclear structure

1

Chromatin organisation

cheRNAs: chromatin-enriched RNAs



Recruitment of chromatin-modulating proteins to chromatin:
Some nuclear lncRNAs can influence chromatin architecture by interacting with chromatin-modulating proteins (such as SWI/SNF or PRC), promoting their recruitment and/or association to chromatin. The interaction can be direct or mediated by Nonchromatin-modifying proteins.

Decoy of chromatin-associated proteins away from chromatin:
Nuclear lncRNAs can also influence gene expression by preventing the association of specific chromatin factors (such as histone deacetylase, methyl transferase, or chromatin- remodeling complexes) to specific gene loci.

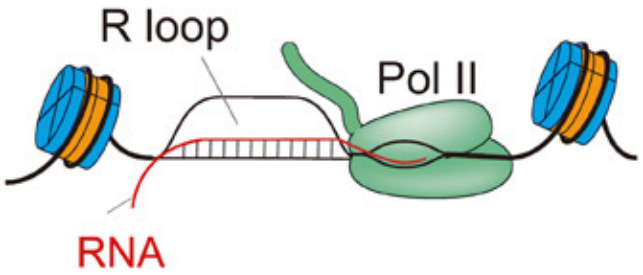
(Statello L, Guo CJ, Huarte M; 2021)

1

Chromatin organisation – RNA:DNA binding

lncRNAs can generate hybrid structures with DNA to influence chromatin accessibility

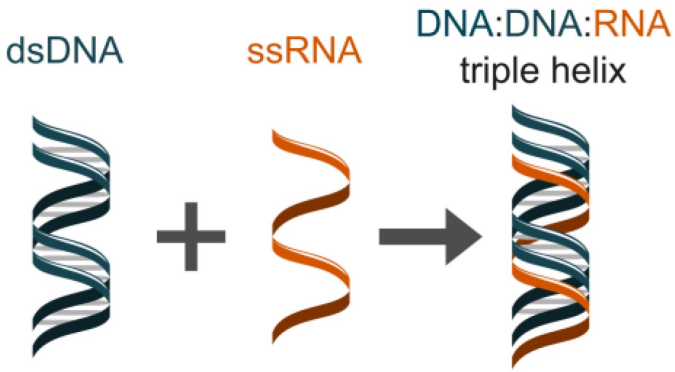
R-loop



(Wang K et al ; 2021)

An **heteroduplex** formed when nascent RNA invades dsDNA during transcription, resulting in a DNA-RNA hybrid and a displaced ssDNA.

DNA:DNA:RNA triple helix



(Warwick T , Brandes RP and Leisegang MS; 2023)

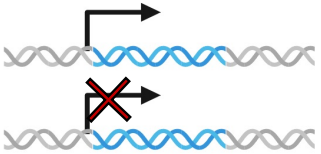
A **triplex** formed by the accommodation of a ssRNA in the major groove of dsDNA with sequence specificity.

These structures can be then recognised and bound by chromatin modifiers and transcription factors/repressors

Nuclear lncRNAs

1 Chromatin organisation

2 Transcription regulation

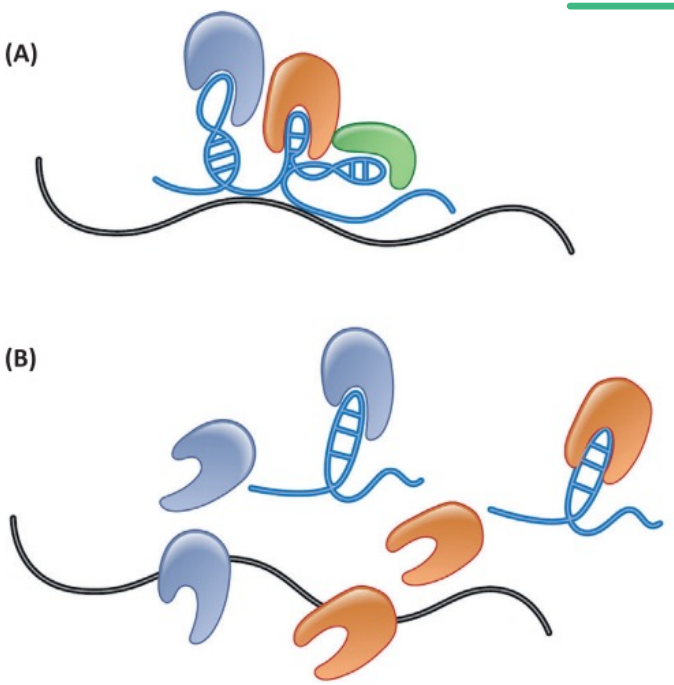


3 Post-transcription regulation

4 Nuclear structure

2

Transcription regulation



(Adapted from Sun Q, Hao Q, and Prasanth K; 2018)

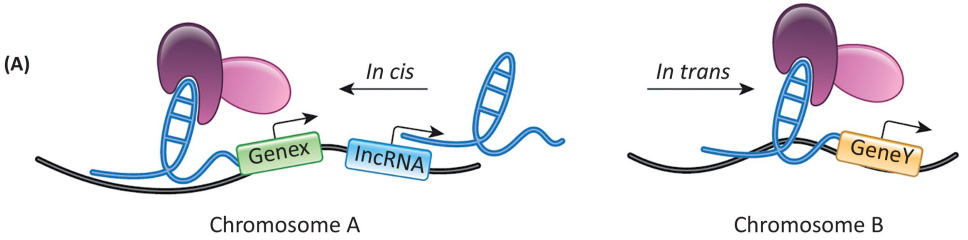
As in the case of chromatin-modulating proteins, also transcription factors or repressors can be guided by lncRNAs to the promoters of target genes (guide) or, alternatively, lncRNAs can prevent their association to specific gene loci (decoy).

Moreover, lncRNAs also regulate transcription by influencing TF activity. For example, by promoting or preventing the association between a TF and its co-activator or repressor.

2

Transcription regulation – cis/trans difference

cis-acting lncRNAs:
Which are positioned in proximity of their transcription sites and can occasionally spread their effect to long distances on the same chromosome

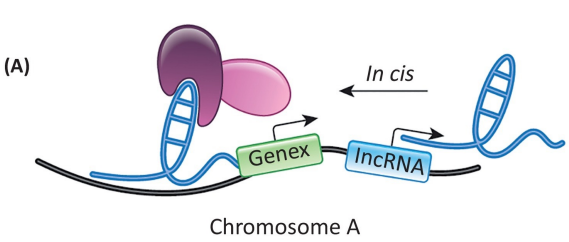


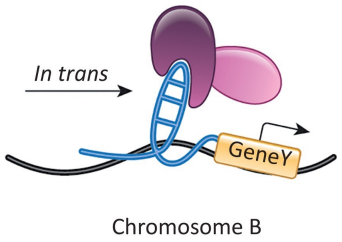
Trans-acting lncRNAs:
Which are relocated from their site of synthesis.

2

Transcription regulation – cis/trans difference

cis-acting lncRNAs:
Which are positioned in proximity of their transcription sites and can occasionally spread their effect to long distances on the same chromosome





Trans-acting lncRNAs:
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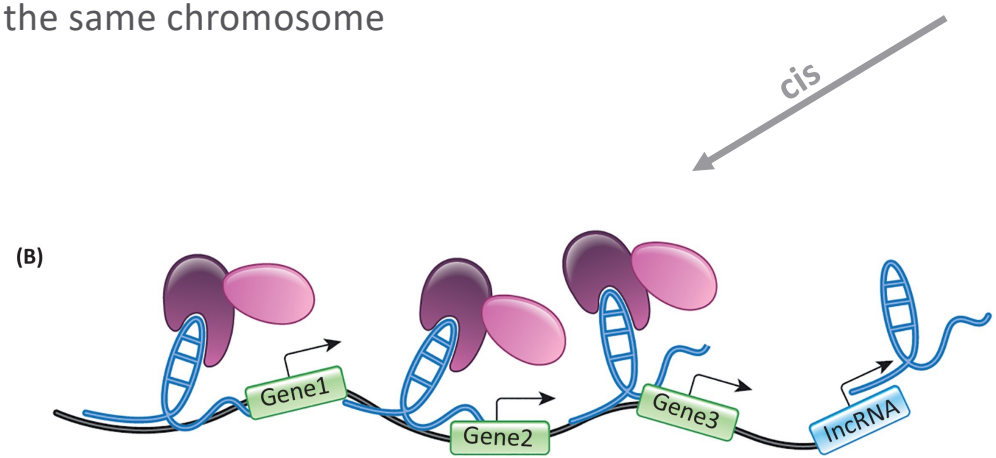
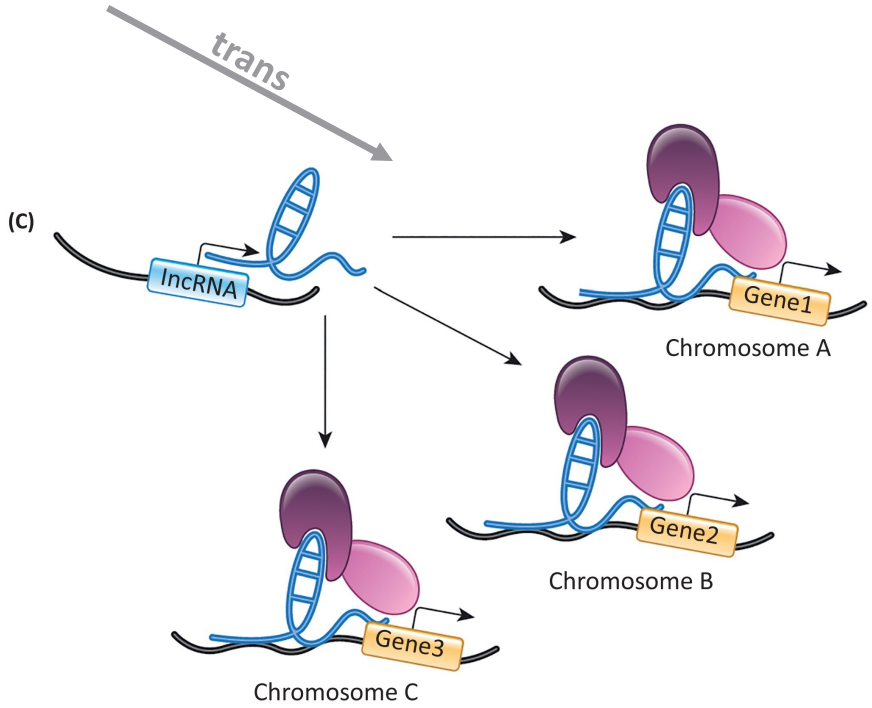




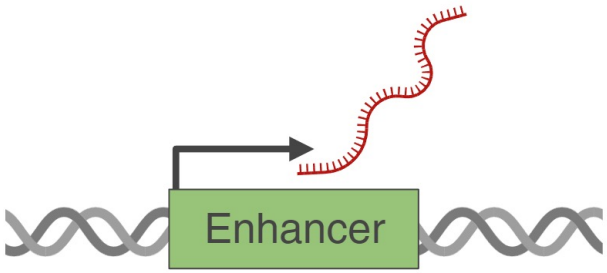
In several instances, lncRNAs modulate the expression of multiple genes (both in cis or in trans)

(Adapted from Sun Q, Hao Q, and Prasanth K; 2018)

2

Transcription regulation - eRNAs

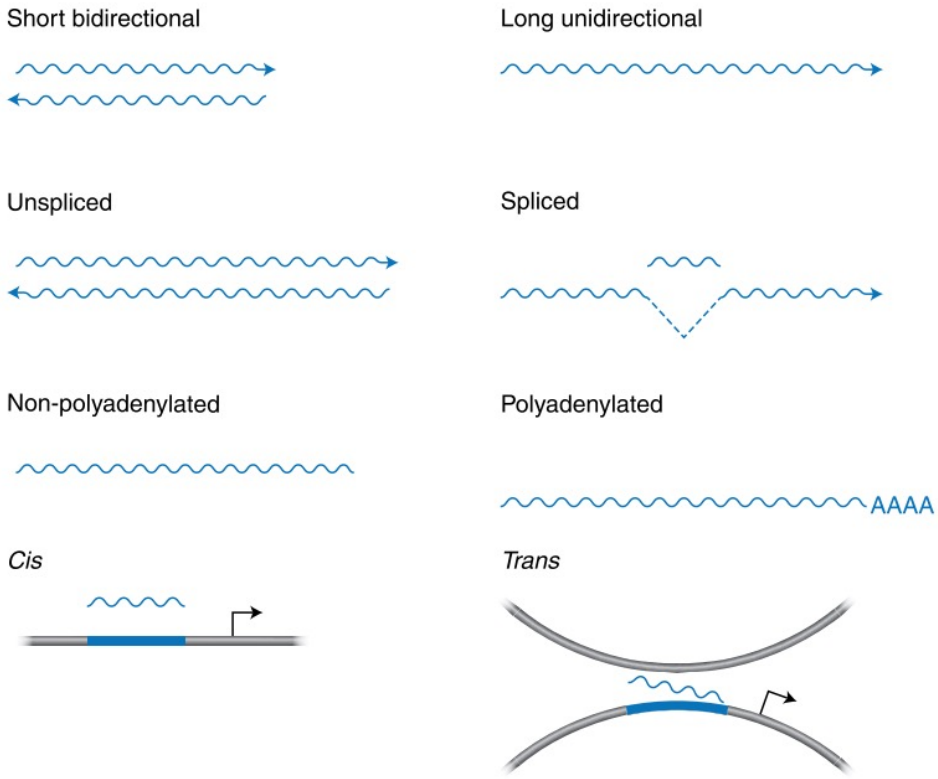
lncRNAs can be transcribed from enhancer regions



eRNAs expression has become a hallmark of active enhancers, however it remains to be resolved whether enhancer transcription, eRNAs themselves, or both, are important for enhancer activity.

eRNAs
enhancer RNAs
(2D-eRNA)

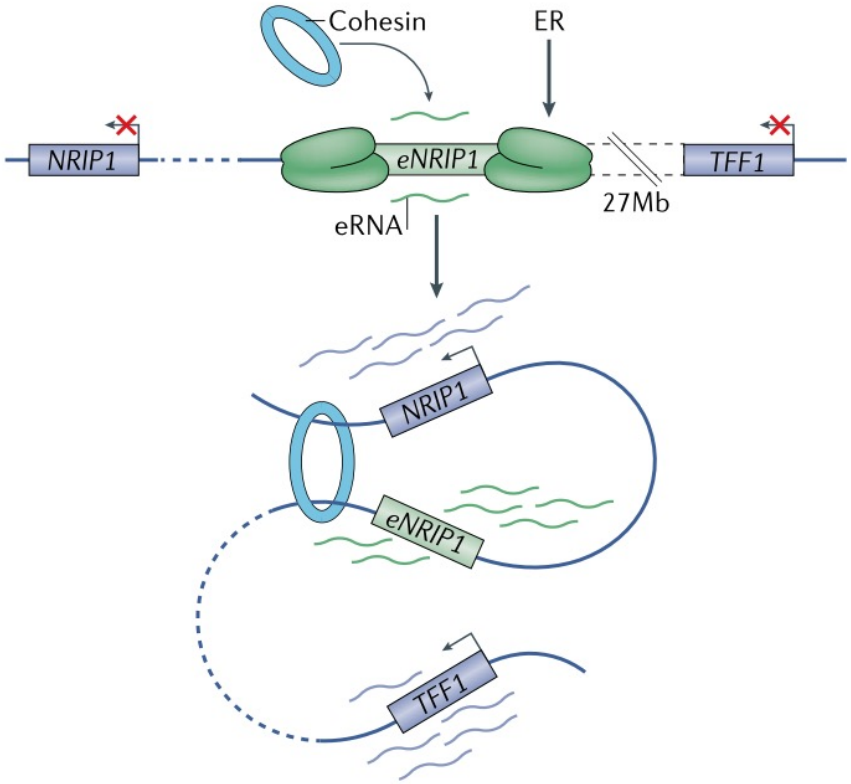
elncRNAs
enhancer-associated lncRNAs
(1D-eRNA)



(Sartorelli V and Lauberth SM; 2020)

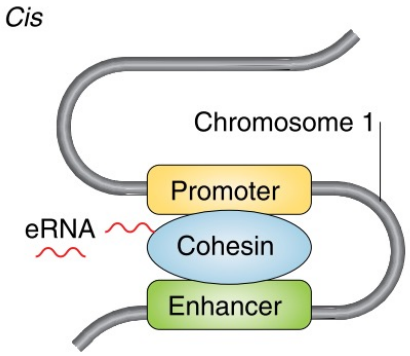
2

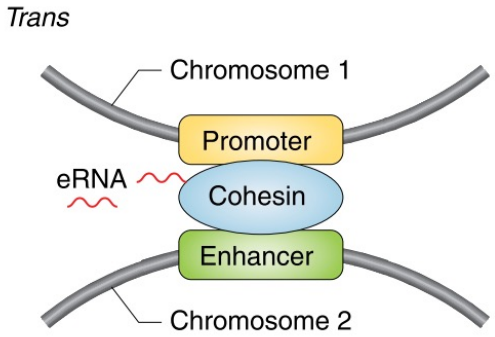
Transcription regulation - eRNAs



(Statello L, Guo CJ, Huarte M; 2021)

An important feature of some eRNAs and elncRNAs is their ability to regulate gene expression by directly promoting specific enhancer–promoter looping through the recruitment of looping factors.



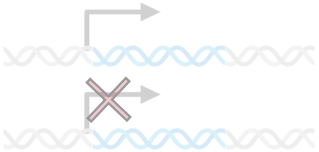


(Sartorelli V and Lauberth SM; 2020)

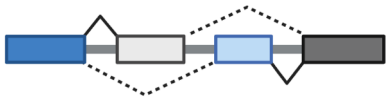
Nuclear lncRNAs

1 Chromatin organisation

2 Transcription regulation



3 Post-transcription regulation



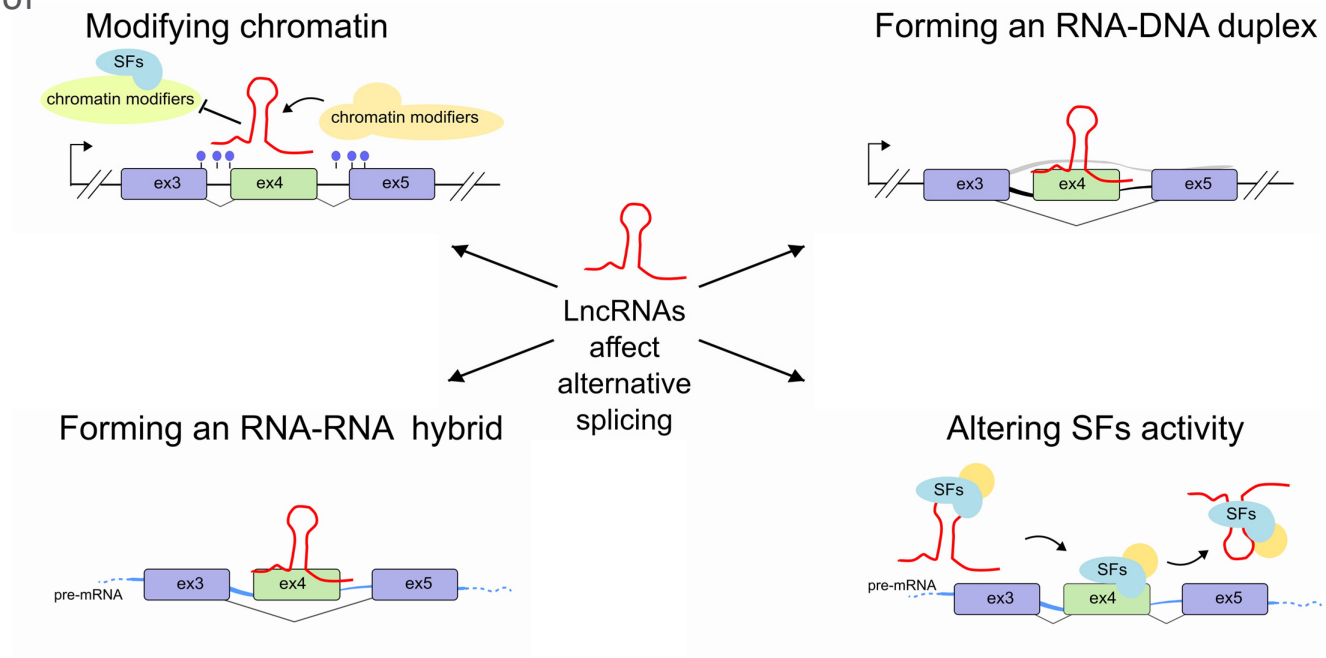
4 Nuclear structure

3

Post-transcription regulation - splicing

Modifying chromatin structure is likely to play an important role in modulating the effects of transcription on AS

R-loops promote transcriptional pausing, which coincides with SF recruitment and AS



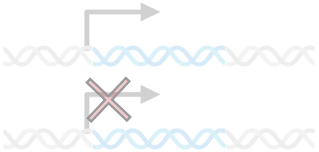
Hybridizing with the pre-mRNA molecule could prevent the binding of the spliceosome, resulting in exon skipping

lncRNAs could promote SF recruitment or decoy into specific subnuclear compartments

Nuclear lncRNAs

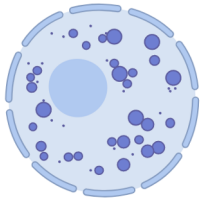
1 Chromatin organisation

2 Transcription regulation



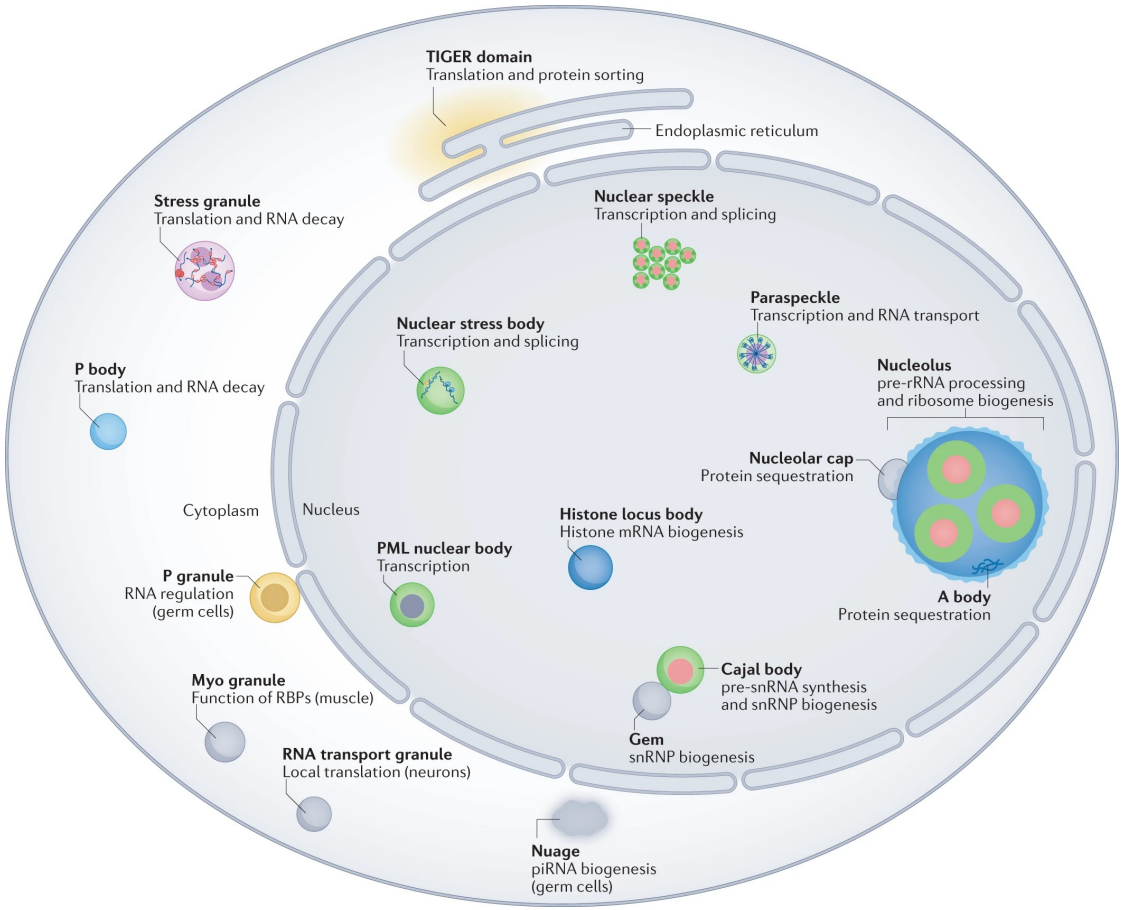
3 Post-transcription regulation

4 Nuclear structure



4

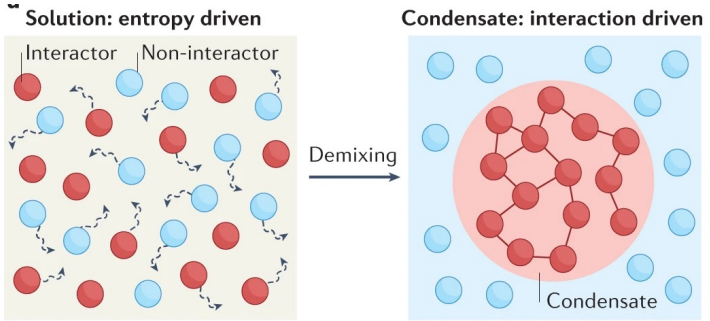
Nuclear structure - Membraneless Organelles (MLOs)



(Hirose T, Ninomiya K, Nakagawa S and Yamazaki T; 2023)

Membraneless Organelles (MLOs):
Biomolecular condensates, which contribute to intracellular compartmentalization of biological functions, by undergoing phase separation.

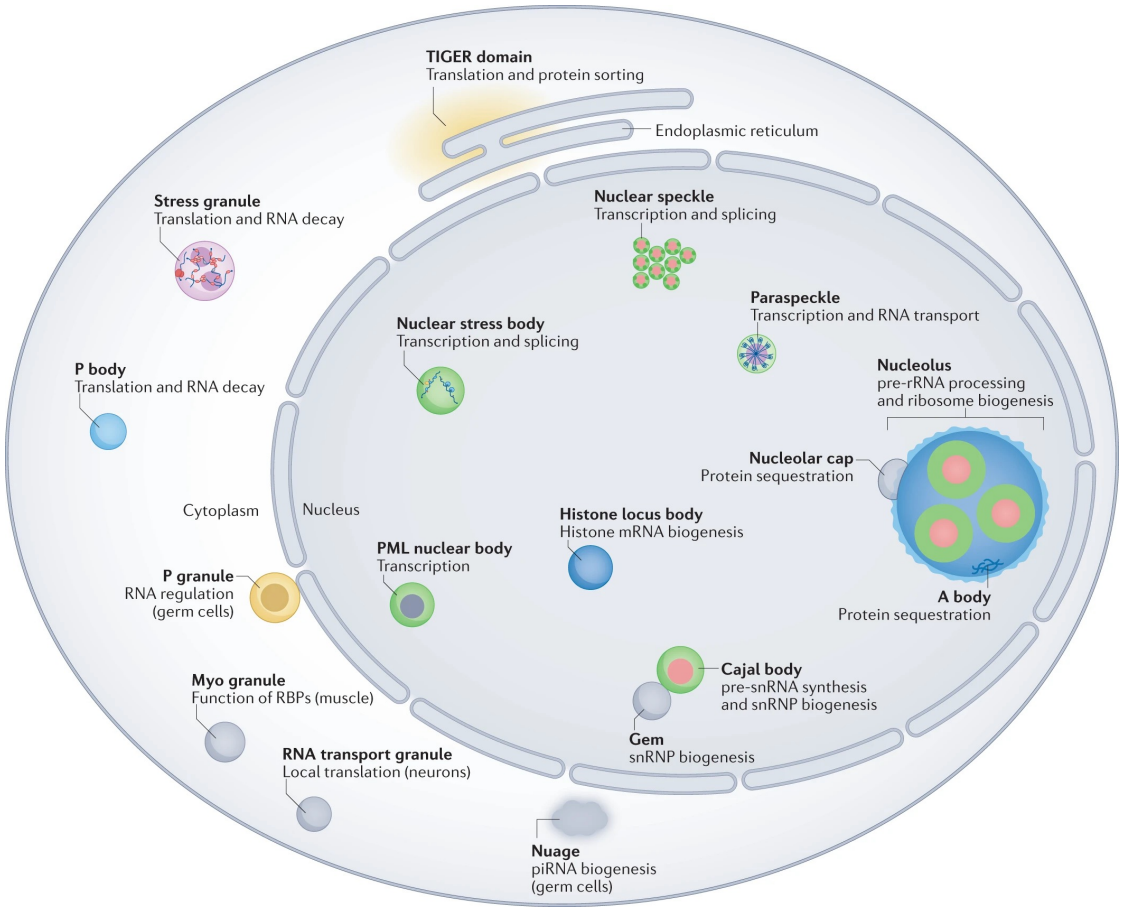
Liquid-liquid phase separation (LLPS) :
The spontaneous separation of a uniform solution into distinct liquid phases, driven by molecular interactions. It occurs when interactions among specific subsets of molecules (proteins and nucleic acids) surpass the tendency of the system to remain disordered in solution due to entropy.



(Mehta S and Zhang J; 2022)

4

Nuclear structure - Membraneless Organelles (MLOs)



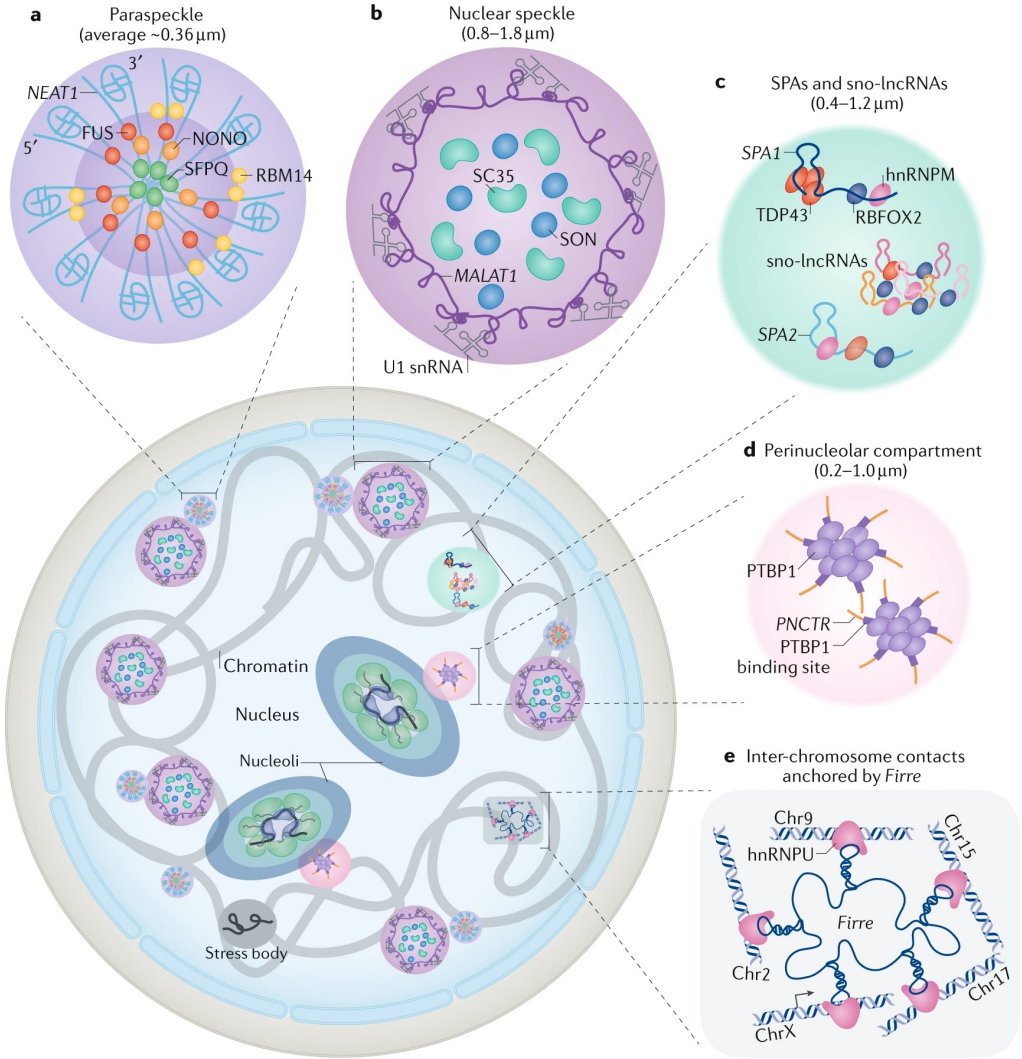
(Hirose T, Ninomiya K, Nakagawa S and Yamazaki T; 2023)

The functions of Membraneless Organelles include macromolecule biogenesis, gene expression, DNA repair and chromatin organization.

- **Nucleolus:** ensures the correct ribosome biogenesis, which sequentially involves pre-rRNA transcription, modification and processing, and ribosome assembly.
- **Cajal body:** has roles in the biogenesis of snRNPs, which eventually form part of the spliceosome.
- **Nuclear speckle:** can act as reservoirs of RNA processing factors (for splicing, transcription…) or as gene expression hubs, promoting transcription and co-transcriptional processes.
- **Paraspeckle:** sequesters mRNAs with stem loops in the 3’ UTR, suppressing their gene expression, and specific RBPs, deregulating their transcriptional control of target genes.

4

Nuclear structure - Membraneless Organelles (MLOs)

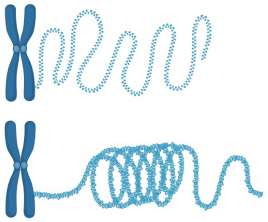


lncRNAs are crucial for the form, composition and function of phase-separated RNA–protein condensates and have a central role in organizing the genome and gene expression by the formation of spatial compartments and transcriptional condensates

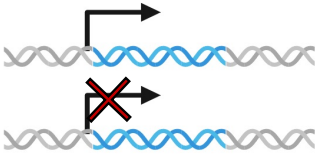
(Statello L, Guo CJ, Huarte M; 2021
See also: Mattick JS et al; 2023)

Nuclear lncRNAs

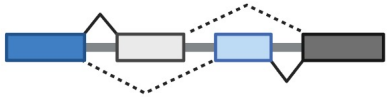
1. Chromatin organisation



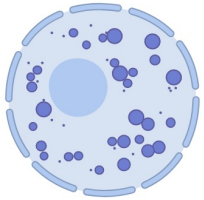
2. Transcription regulation



3. Post-transcription regulation

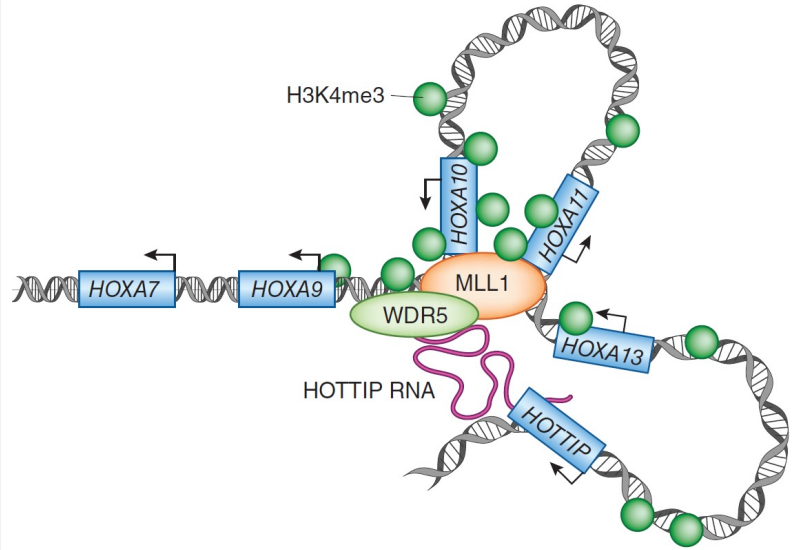


4. Nuclear structure



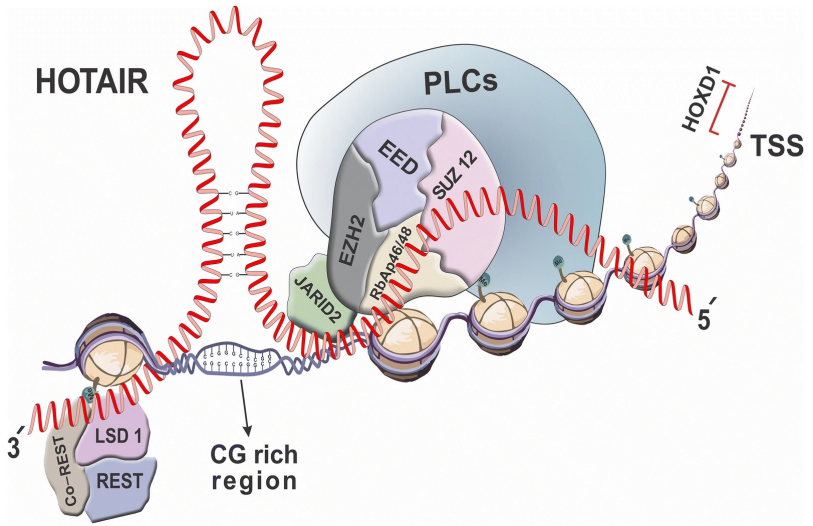
Nuclear lncRNAs - Examples

cis-acting lncRNA



HOTTIP

trans-acting lncRNA



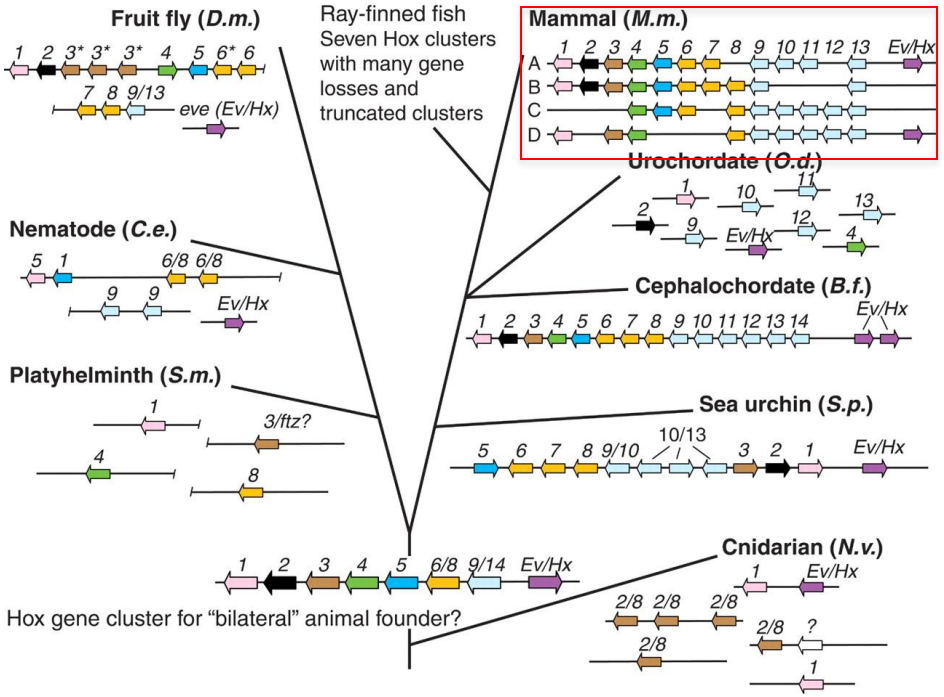
HOTAIR

HOX loci

•In mammals, **39 HOX transcription factors** clustered on four chromosomal loci, termed HOXA through HOXD, are essential for specifying the positional identities of cells.

•The temporal and spatial pattern of HOX gene expression is often correlated to their genomic location within each loci, a property termed **colinearity**.

•Transcription of many ncRNAs has been observed in fly, mouse, and human *HOX loci*



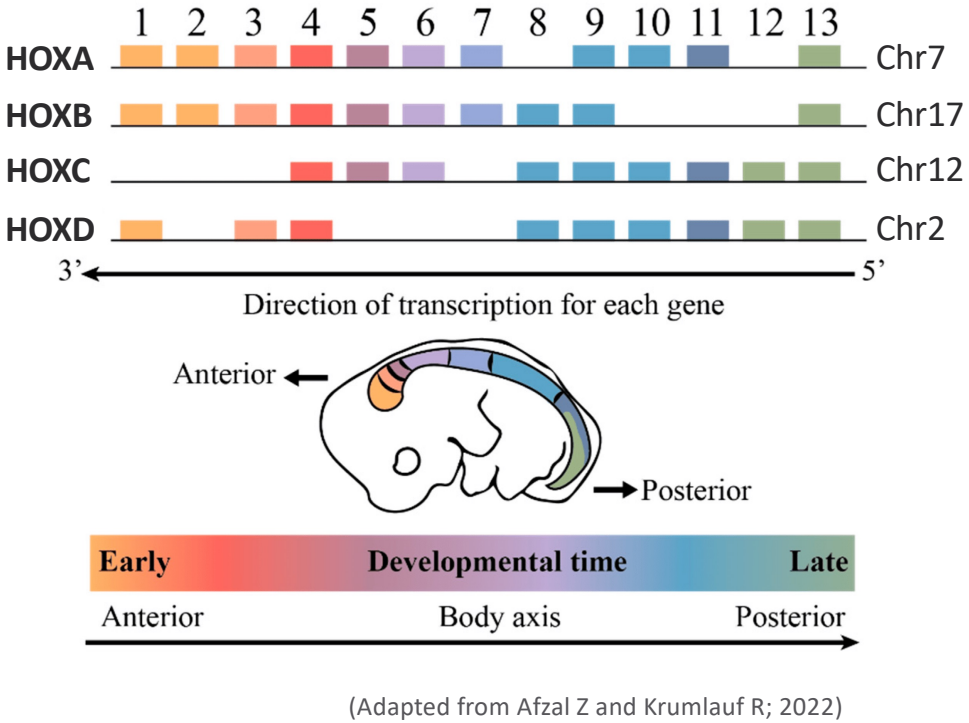
HOTTIP – HOX loci

The **homeotic (Hox) genes** are the genes responsible for correct spatial body development (providing the antero-posterior and dorso-ventral information)

Highly conserved in vertebrates, they are located contiguously in clusters (in mammals 4 clusters and 39 Hox genes), encode transcription factors and share three basic mechanisms of regulation of expression:

- **Spatial collinearity:** the position of a Hox gene 3’ to 5’ within a Hox cluster corresponds to its expression in the animal along the A-P axis.
- **Posterior prevalence:** Hox genes that are positioned more 5’ in the cluster will have a dominant phenotype to those more 3’.
- **Temporal collinearity:** Hox genes are expressed temporally in an order corresponding to their positions from 3’ to 5’ within each cluster.

Transcription of many **ncRNAs** has been observed in Hox loci



(Adapted from Afzal Z and Krumlauf R; 2022)

Polycomb and MLL/Trithorax Complexes

- The ON and OFF states of ***Hox*** and other key developmental genes are maintained by the **MLL/Trithorax (Trx)** and **polycomb group (PcG)** proteins, which mediate trimethylation of histone H3 lysine 4 (H3K4me3) to activate genes or lysine 27 (H3K27me3) to repress genes.

H3K4me3

Trithorax-group Proteins

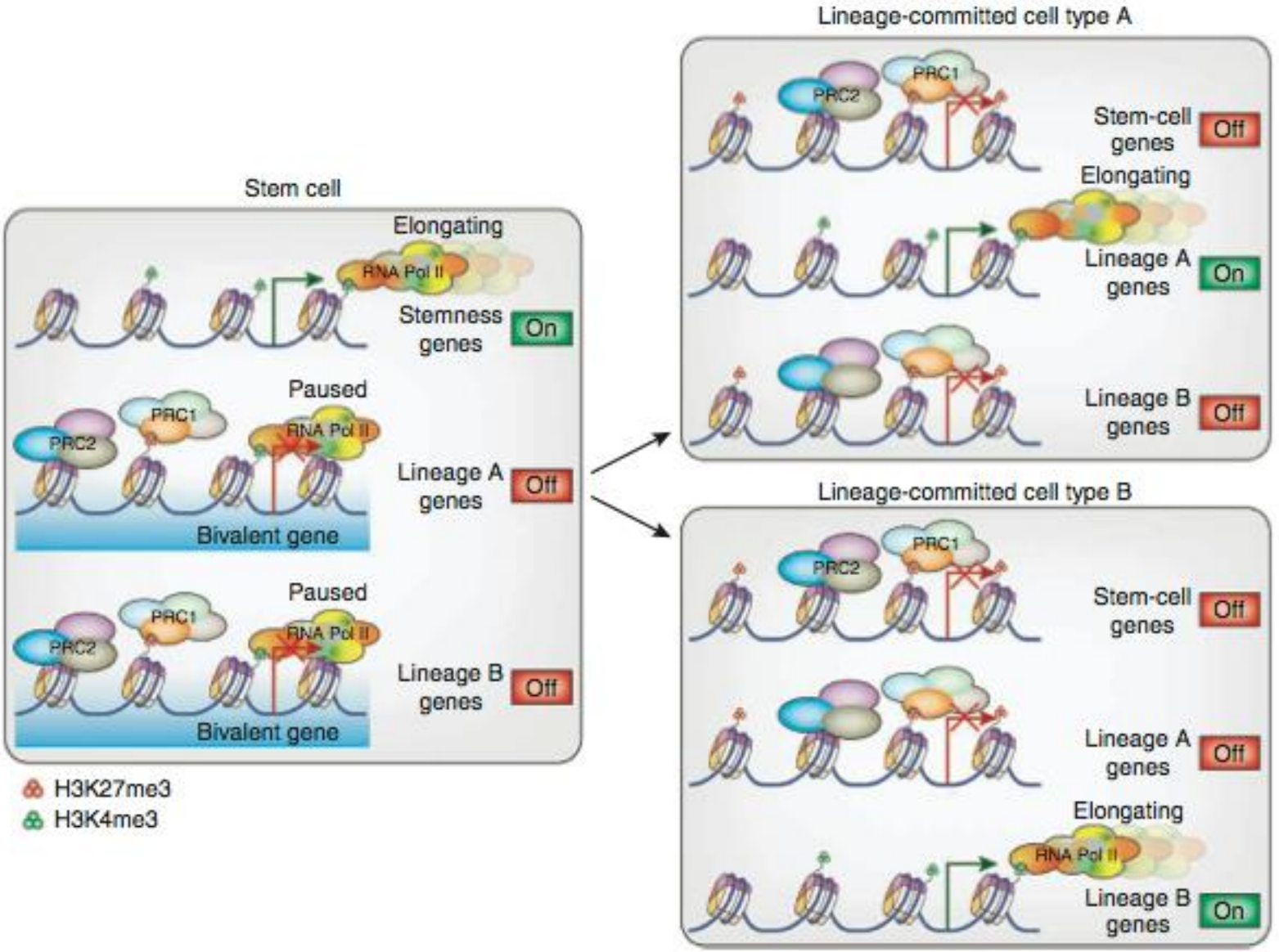
- Maintains an active state
- Counteracts the action of PcG proteins

H3K27me3

Polycomb-group Proteins

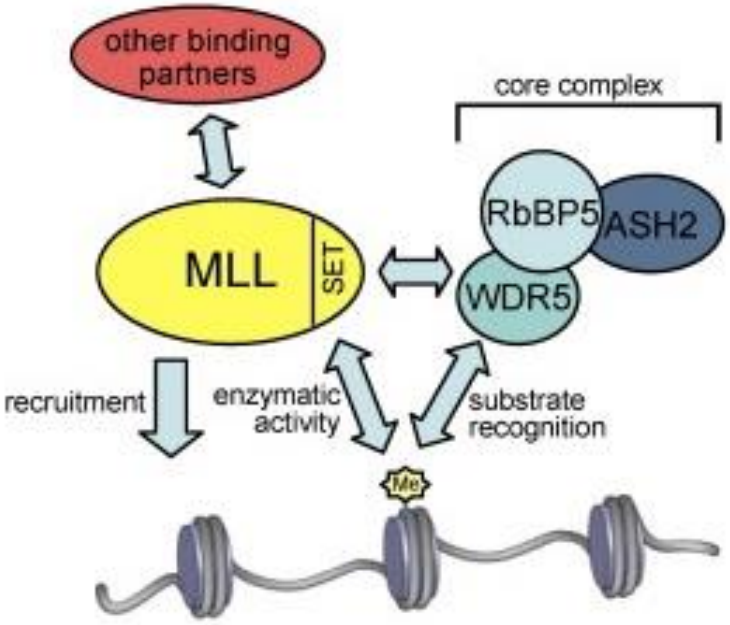
- Maintains a silenced state
- Prevents chromatin remodelling

Role of MLL and PRC complexes in stem-cell fate



Writing the H3K4 Methylation Mark

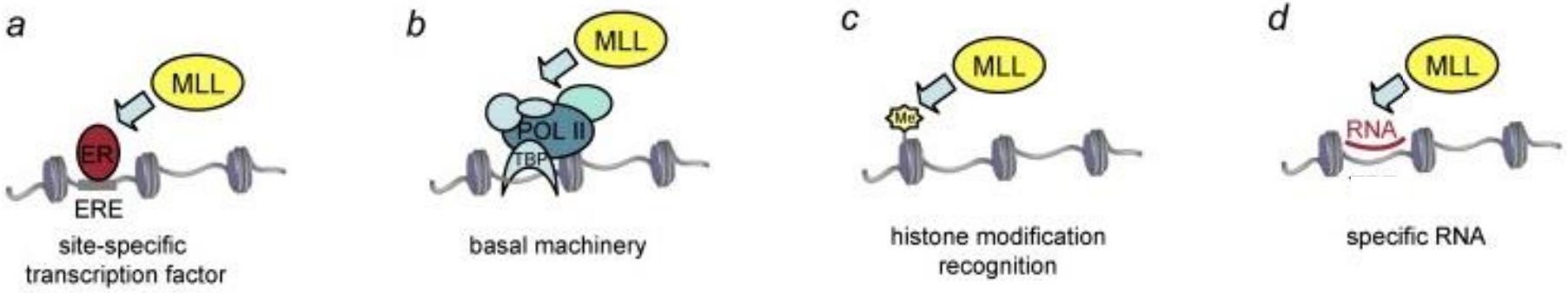
- TrxC (MLL in mammals) methylates H3K4 and recruits HAT and remodeling complexes
- Acetylated H3K9 prevents methylation, and prevents HP1 binding



MLL-family HMTs associate with the core complex containing **RbBP5**, **WDR5**, and **ASH2**. The core complex cooperates with the catalytic SET domain to methylate H3K4, whereas other regions of the MLL protein are involved in association with other protein partners and in recruitment of the MLL complex to the target genes. **WDR5** plays a role in substrate recognition and presentation, with preferential, but not exclusive, binding to the H3K4me2 substrate.

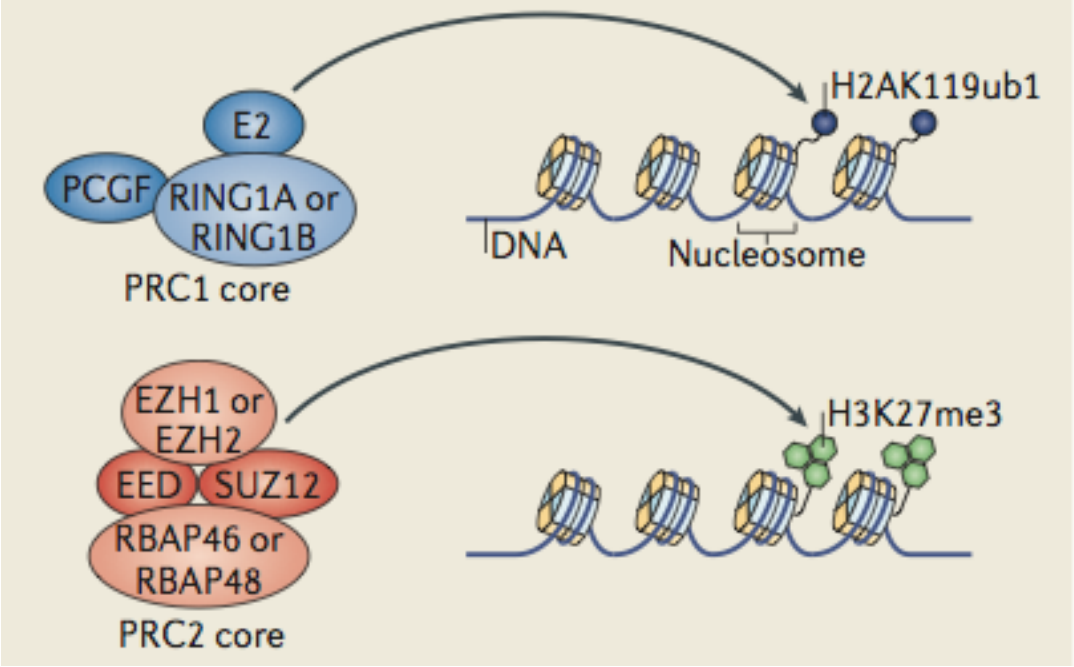
Trithorax Complex (MLL) recruitment to target genes

Mechanisms of H3K4 methyltransferase recruitment to the target genes. Although precise mechanisms of recruitment remain to be determined, the existing literature suggests that H3K4 methyltransferases are recruited to and/or stabilized on chromatin by a combination of mechanisms involving **association with site-specific transcription factors** (a), **basal machine**ry (b), **histone modification recognition** (c), and **specific RNAs** (d).



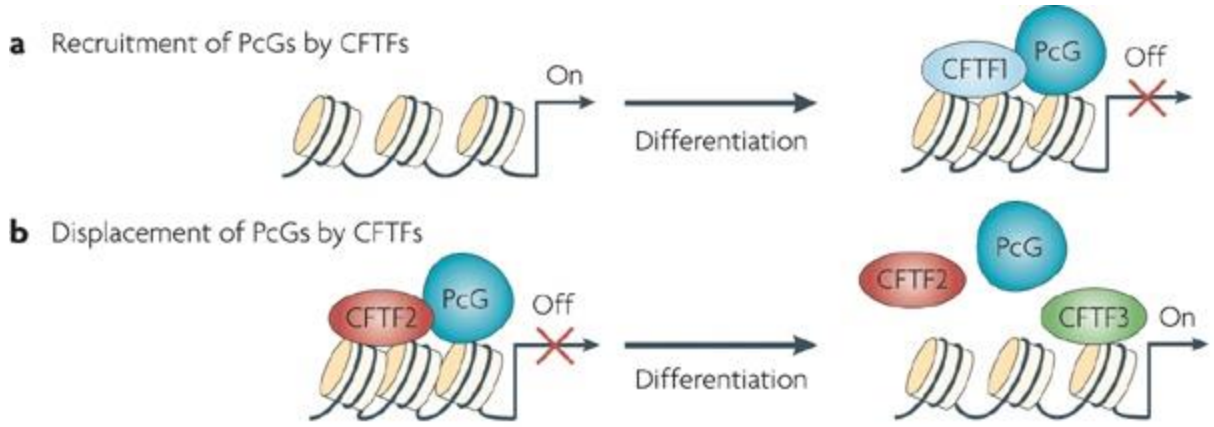
Core PRCs and their chromatin-modifying activities

•**Polycomb repressive complexes** (PRCs) repress transcription by a mechanism that involves the modification of chromatin.



PcG recruitment to target genes

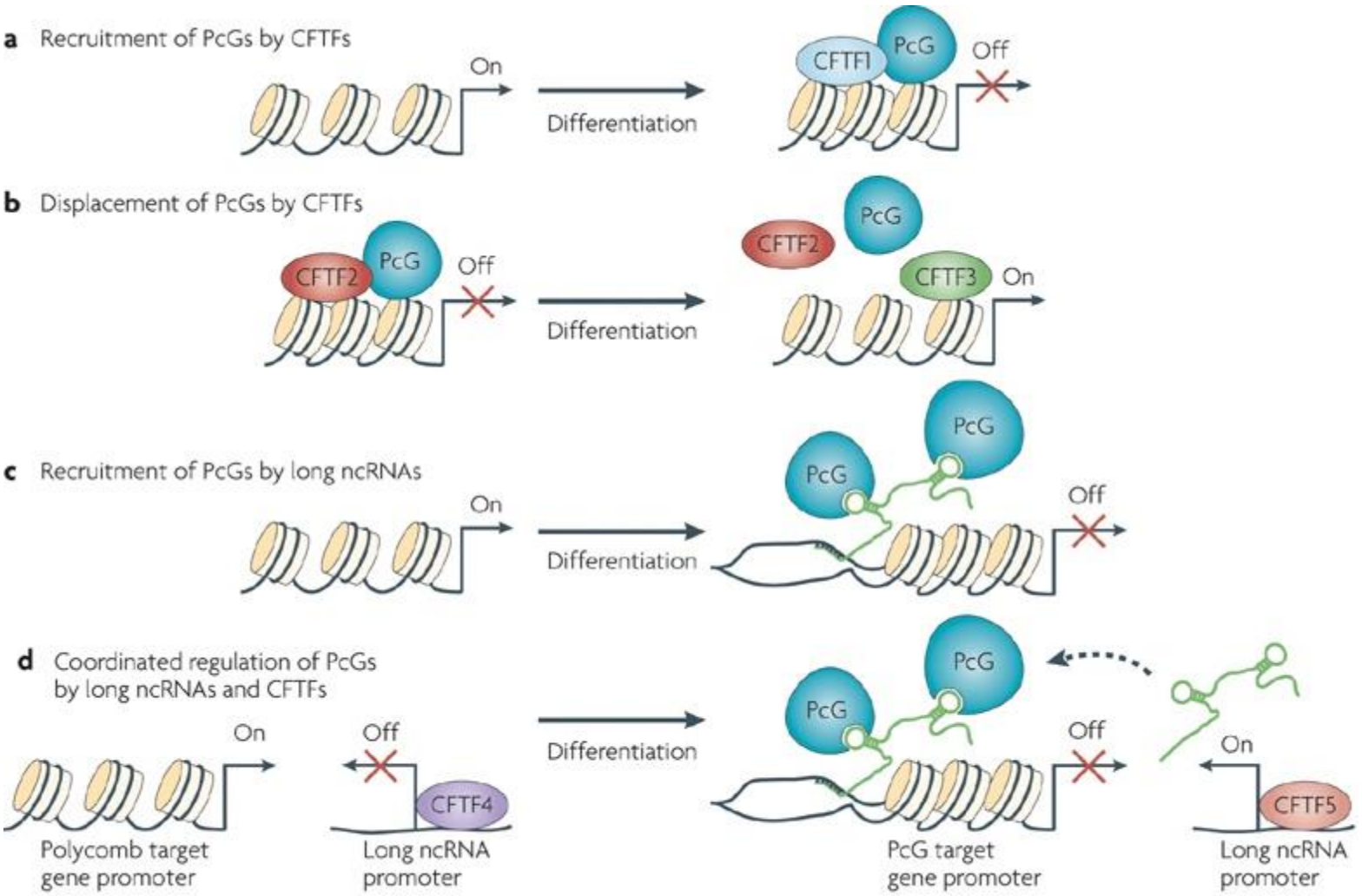
•PcG proteins do not have the ability to bind specific DNA motifs



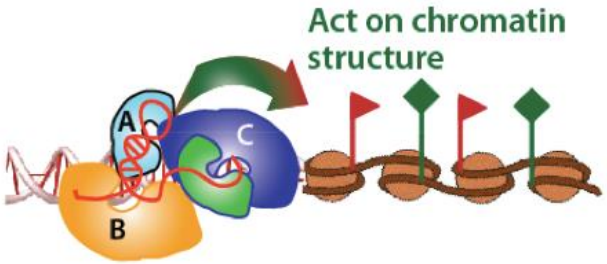
•<u>cell fate transcription factors</u> **CFTFs** may regulate PRC binding to specific promoters

PcG recruitment to target genes

•Long non coding RNAs may also regulate PRC binding to specific promoters



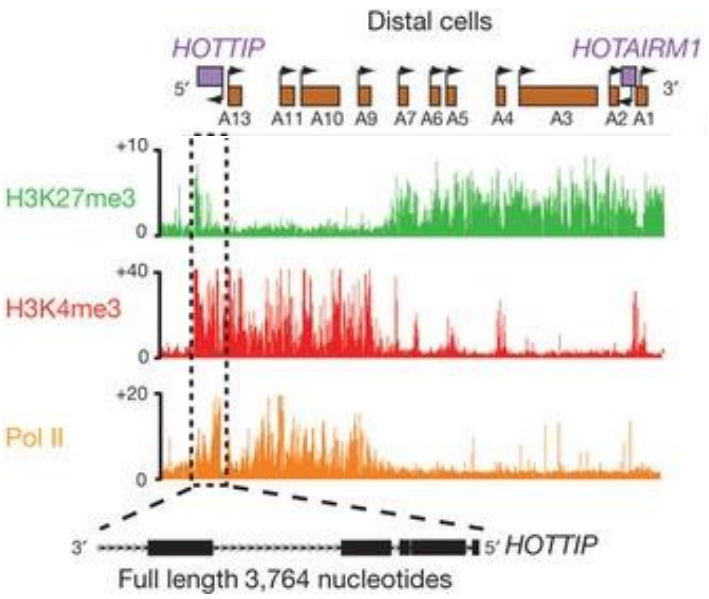
Epigenetic regulation by cis-acting lncRNAs

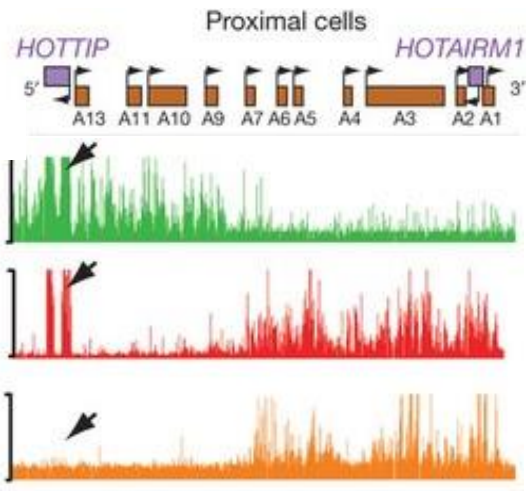


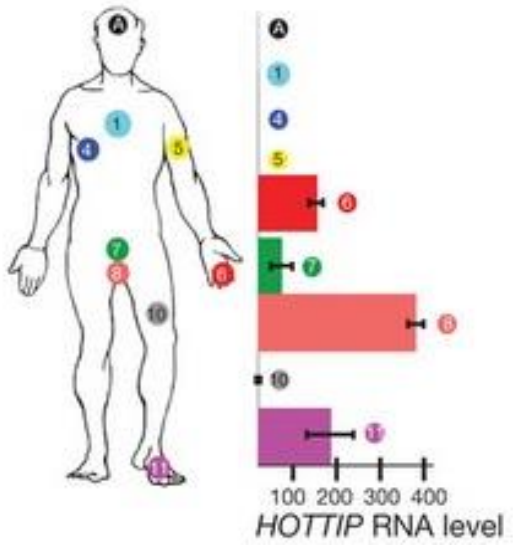
HOTTIP lncRNA

HOTTIP is a lncRNA transcribed in distal anatomic sites

The cis-acting lncRNA **HOXA distal transcript antisense RNA** (**HOTTIP**), which is produced from the 5' end of the human HOXA locus upstream of HOXA13, was identified in human primary fibroblasts. In anatomically distal cells (for example, foreskin and foot fibroblasts), HOTTIP expression correlates with gene activation (proximal HOXA genes) while anatomically proximal cells (for example, lung fibroblasts) have the diametrically opposite pattern.



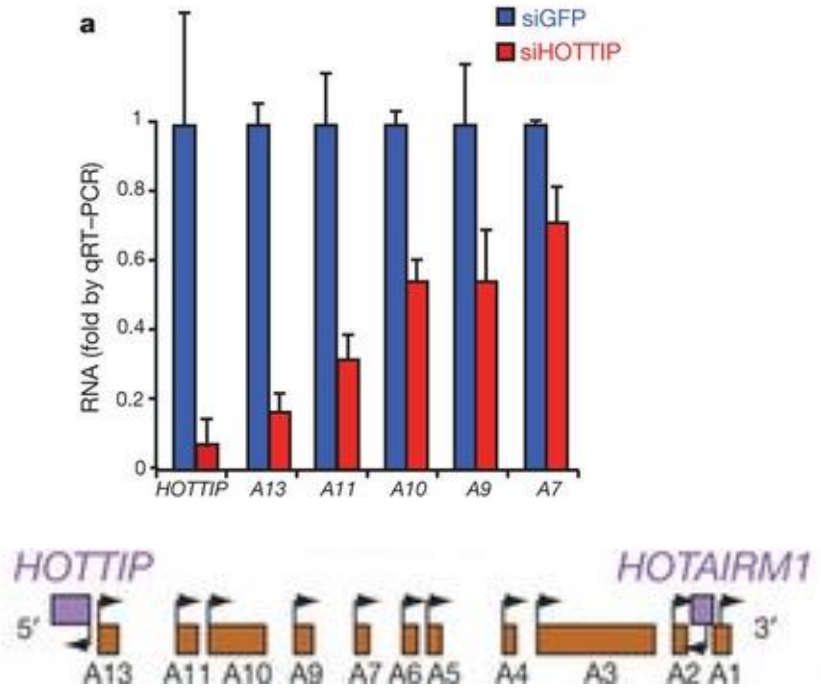




Wang et al., 2011. Nature

HOTTIP is required for coordinate activation of 5′ *HOXA* genes

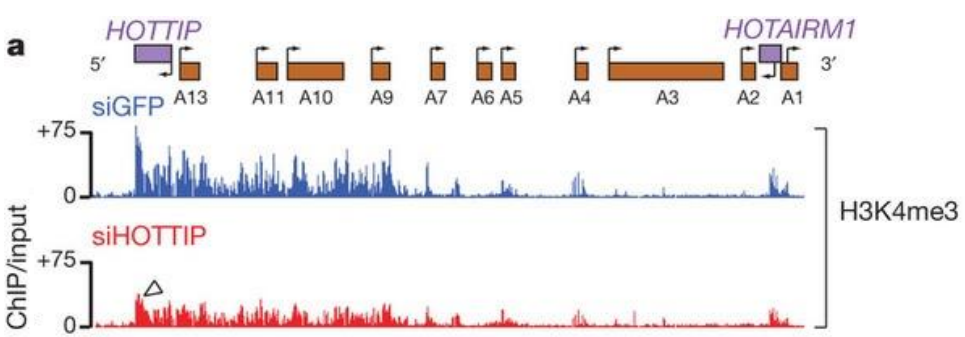
Knock down of HOTTIP RNA in fibroblasts from a distal anatomic site (foreskin), abrogated expression of distal HOXA genes across 40 kilobases with a trend dependent on the distance to HOTTIP. The strongest blockade was observed for HOXA13 and HOXA11, with progressively less severe effects on HOXA10, HOXA9 and HOXA7. HOXD gene expression was not affect.

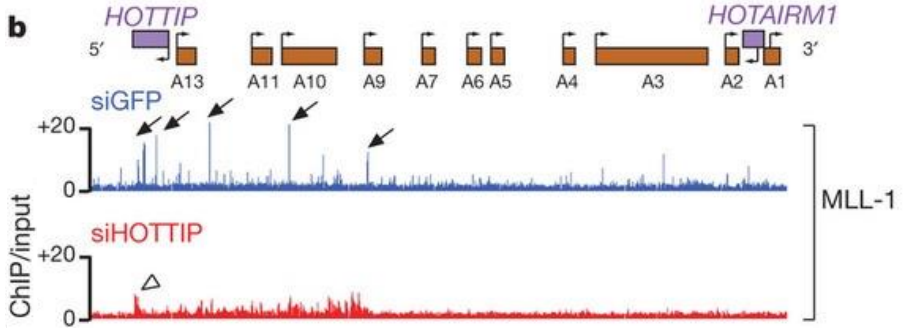


Wang et al., 2011. Nature

HOTTIP RNA is required for the active chromatin state of 5′ *HOXA* cluster

HOTTIP RNA knockdown led to broad loss of H3K4me3 across the *HOXA* locus, most prominently over 5′ *HOXA* and *HOTTIP* gene itself. MLL1 is recruited to promoters of *HOX* genes to maintain their activation states. Strikingly, *HOTTIP* RNA knockdown abrogated the peaks of MLL1 occupancy near TSS, resulting in diffuse and less intense binding of MLL1 across *HOXA* cluster, most prominently over the 5′ *HOXA* domain.

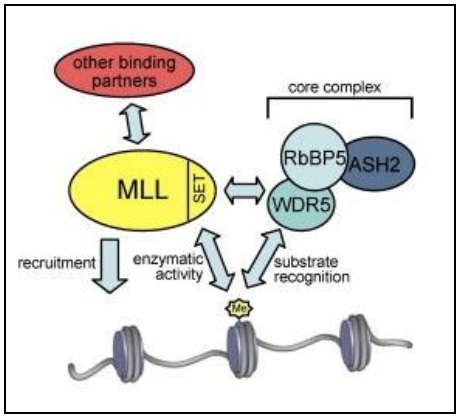


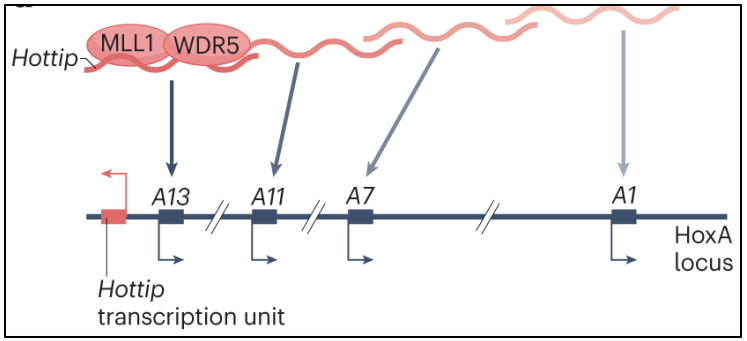


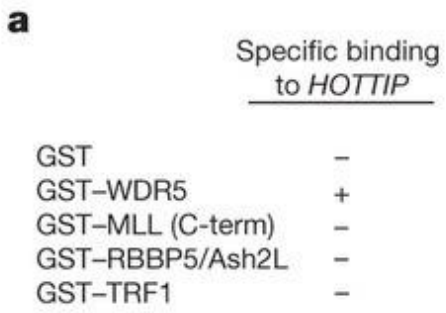
Wang et al., 2011. Nature

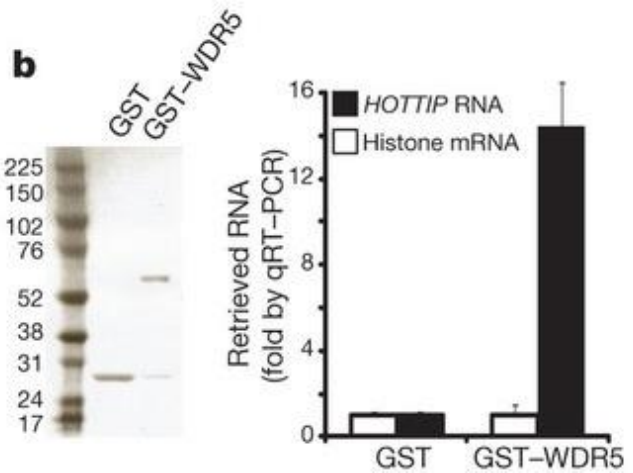
HOTTIP RNA interacts with the MLL component WDR5

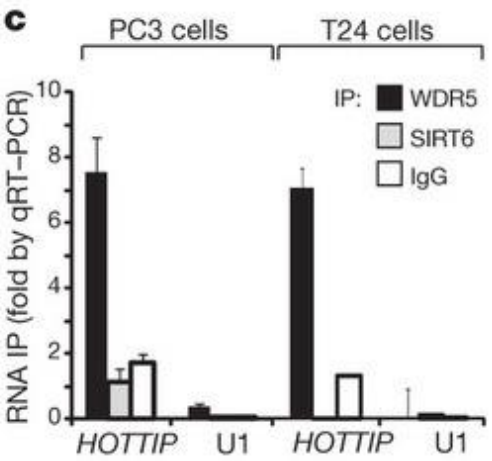
Purified, *in-vitro*-transcribed, full-length *HOTTIP* RNA bound specifically to recombinant glutathione-*S*-transferase-conjugated WDR5 (GST–WDR5), but not to GST, RBBP5, ASH2L, or the telomeric protein TRF1 (a, b). Immunoprecipitation of endogenous WDR5 from two different cell lines each specifically retrieved endogenous *HOTTIP* RNA (c), indicating that WDR5 and *HOTTIP* RNA interact in living cells.







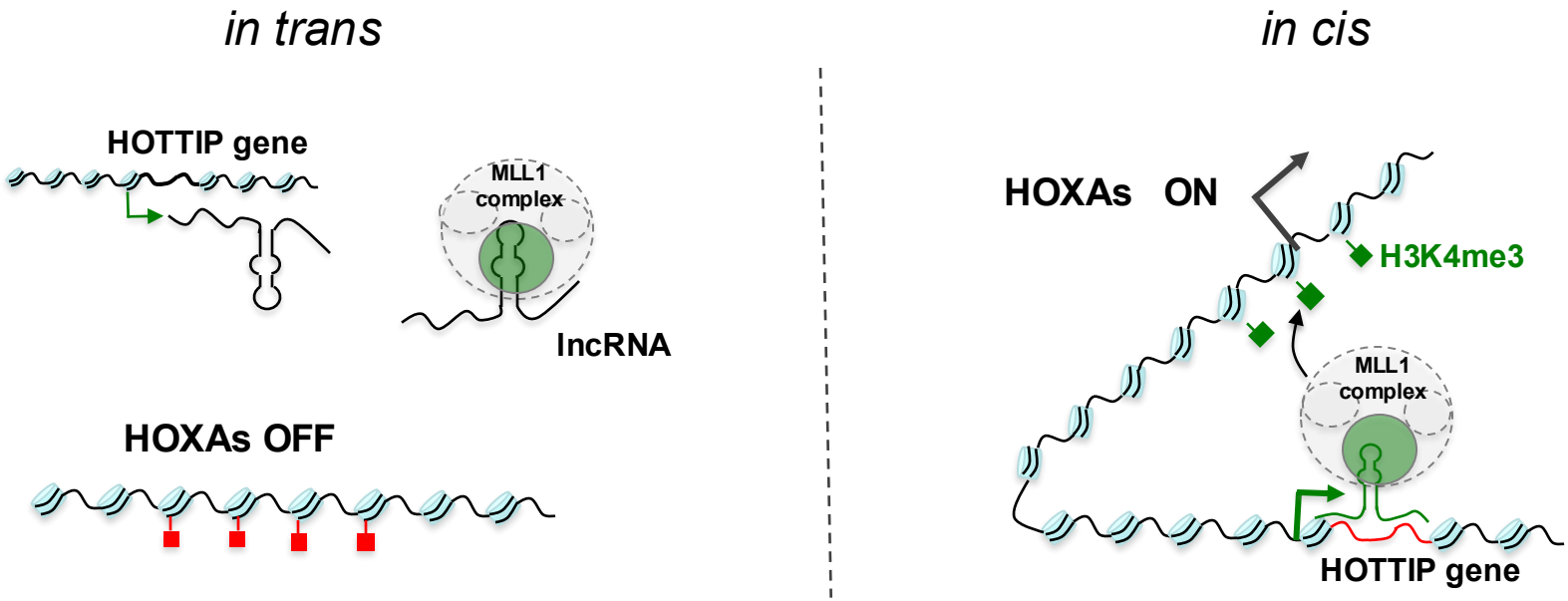




Wang et al., 2011. Nature

HOTTIP RNA does not function in trans

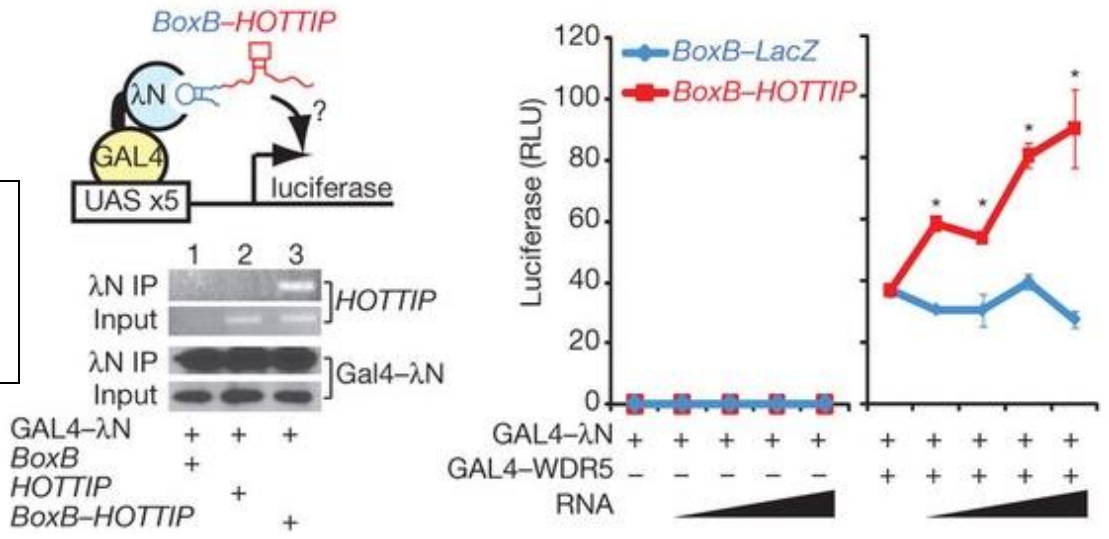
Ectopic expression of *HOTTIP* RNA by retroviral transduction of lung fibroblasts, which do not express *HOTTIP*, failed to activate expression of distal *HOXA* genes, and did not change H3K4me3 and H3K27me3 patterns (data not shown). Ectopically expressed *HOTTIP* RNA, being transcribed from retroviral insertion sites scattered randomly in the genome, may not be able to find 5′*HOXA* genes. In contrast, endogenous *HOTTIP* RNA is directly positioned near the 5′ *HOXA* genes by chromosomal looping, allowing interaction and control



HOTTIP RNA recruitment potentiates transcription

To test cis-acting targeting mechanism, they engineered an allele of HOTTIP RNA that can be artificially recruited to a reporter gene. Addition of five copies of the BoxB RNA element to HOTTIP RNA allows the fusion transcript to be recruited to the lN RNA binding domain fused to a GAL4 DNA-binding domain. Recruitment of HOTTIP RNA to a silent GAL4 promoter is not sufficient to initiate transcription <u>but can significantly boost transcription if the promoter is also bound by WDR5.</u>

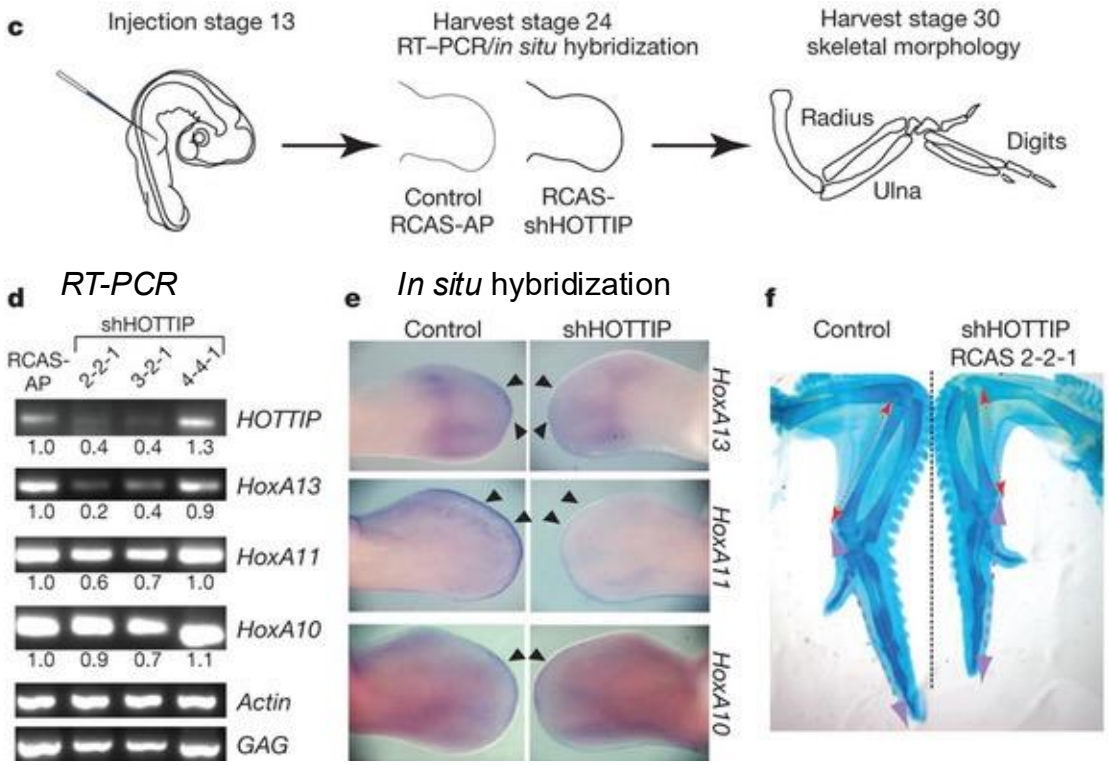
IP of GAL4- λN specifically retrieves *BoxB–HOTTIP* RNA



Wang et al., 2011. Nature

HOTTIP RNA controls activation of distal *Hox* genes *in vivo*.

The function of *HOTTIP* RNA *in vivo* was analyse by RNAi in the developing chick limb bud. Limbs depleted of *HOTTIP* RNA showed notable shortening and bending of distal bony elements. This phenotype resembled some of the defects in mice lacking *HoxA11* and *HoxA13*.

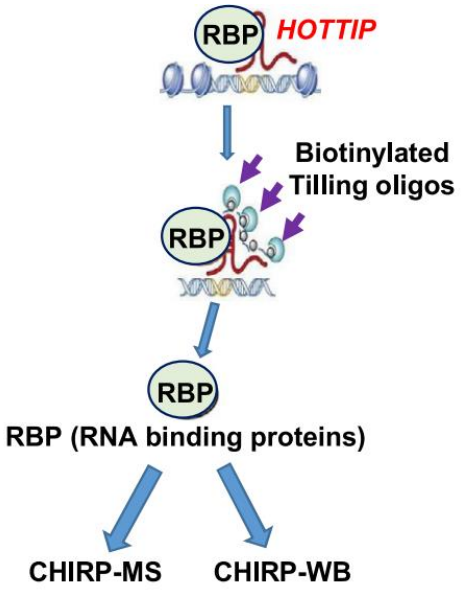


Wang et al., 2011. Nature

Molecular Cell Article 2022

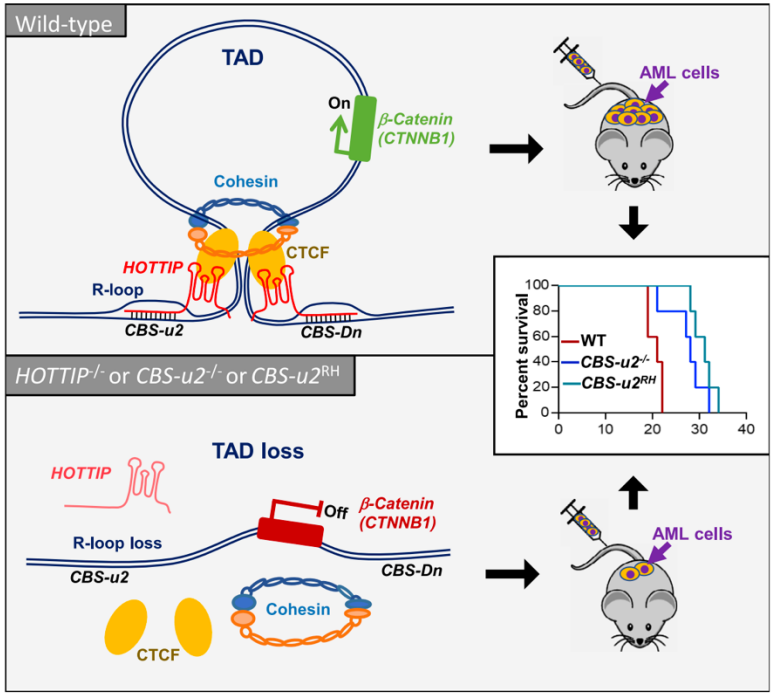
HOTTIP-dependent R-loop formation regulates CTCF boundary activity and TAD integrity in leukemia

Huacheng Luo,[1,12] Ganqian Zhu,[2,12] Melanie A. Eshelman,[1,12] Tsz Kan Fung,[3,11] Qian Lai,[1,4] Fei Wang,[5] Bernd B. Zeisig,[3,11] Julia Lesperance,[1] Xiaoyan Ma,[1,5] Shi Chen,[2] Nicholas Cesari,[1] Christopher Cogle,[6] Baoan Chen,[5] Bing Xu,[4] Feng-Chun Yang,[7,8] Chi Wai Eric So,[3,11,*] Yi Qiu,[9,10,*] Mingjiang Xu,[2,7,*] and Suming Huang[1,10,13,*]



LC-MS/MS identification of the *HOTTIP* protein complexes in AML cells (partial list of proteins):

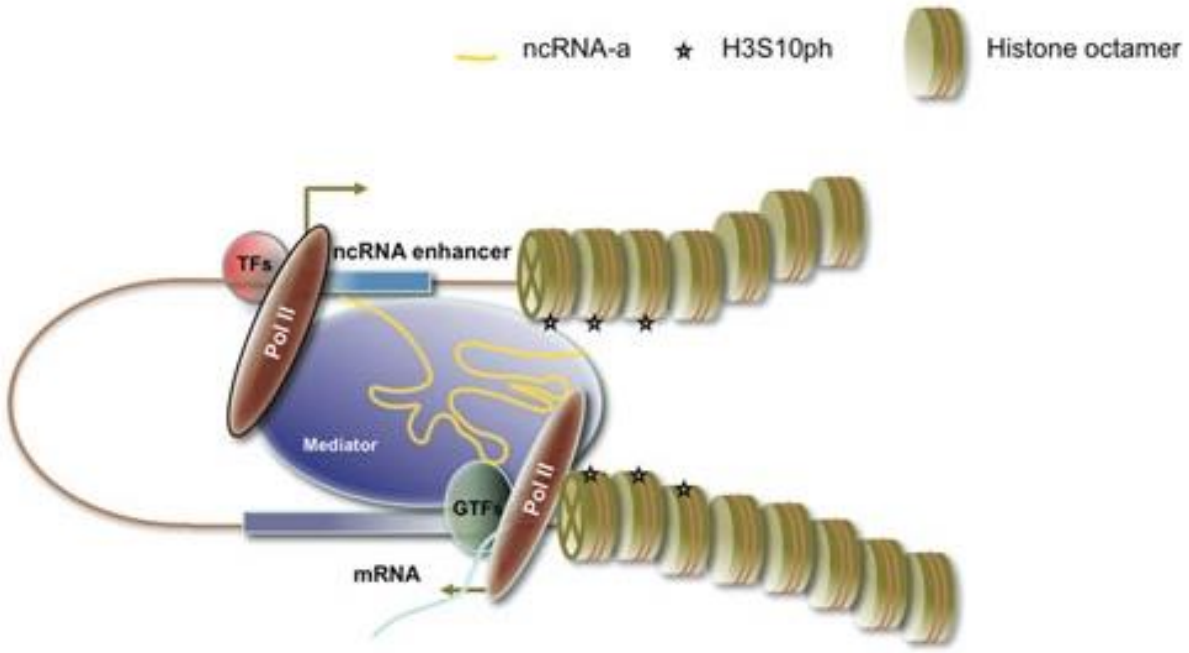
Protein ID	Size (kDa)	Uni No. Peptide	Function
SMC3	141	13	CTCF/cohesin
CTCF	130	7	
RAD21	71	7	
SMC1A	143	5	
LMNB1	66	12	Nuclear matrix
NPM1	33	6	
SATB1	86	1	
LMNB2	70	1	
HNRNPA2B1	37	8	RBPs
HNRNPL	64	6	
ASH2L	69	3	MLL1/DOT1L
WDR5	37	2	
KMT2A	320	1	
RBBP5	59	1	
DOT1L	185	1	
RPA1	68	3	Sensor/regulator of R-loops
DHX9	141	9	
PARP1	113	6	
SRSF1	28	4	
SUPT16H	120	3	
MCM3	91	5	
RPB3 (ROLR2C)	31	5	Transcription
LMO1	18	2	Hematopoietic TFs
RUNX1	49	1	
STAT5A	91	1	



CTCF-binding sites (CBSs)

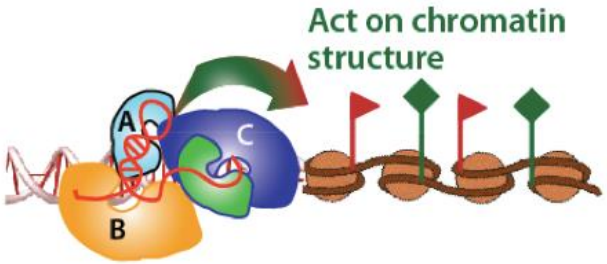
Long Noncoding RNAs with Enhancer-like Function

Many lncRNA with an enhancer-like function (eRNAs) similar to HOTTIP have been identified in human cell lines. Depletion of these lncRNAs led to decreased expression of their neighboring protein-coding genes. These RNA molecules help proteins in the cell to create a loop of DNA in order to open up genes for transcription. This eRNAs can attach to Mediator at multiple locations within the Mediator protein complex and Mediator itself can interact with the enhancer element site on DNA that encodes these activating ncRNAs.



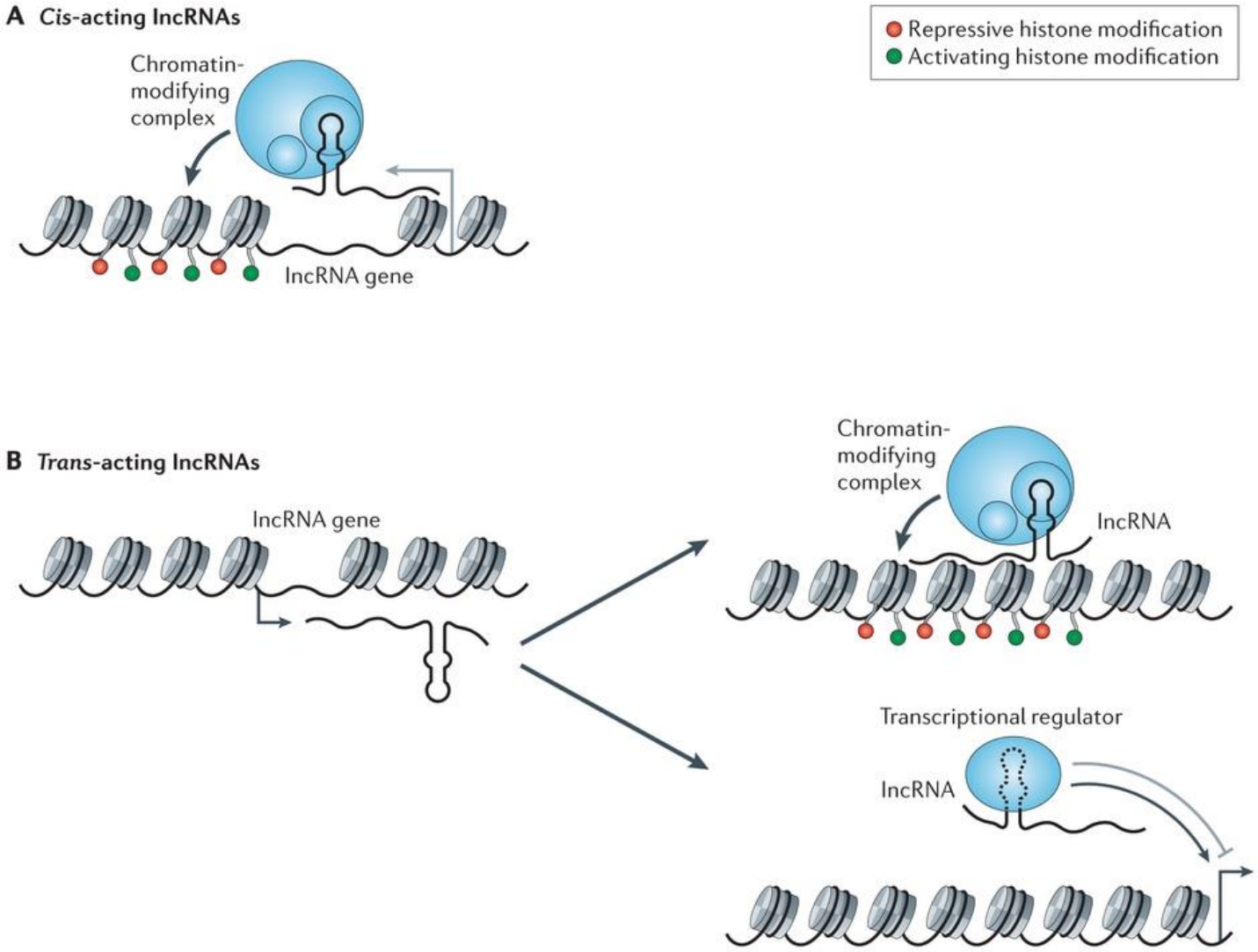
Ørom et al., 2010 Cell; Lai et al., 2013. Nature

Epigenetic regulation by trans-acting lncRNAs



HOTAIR lncRNA

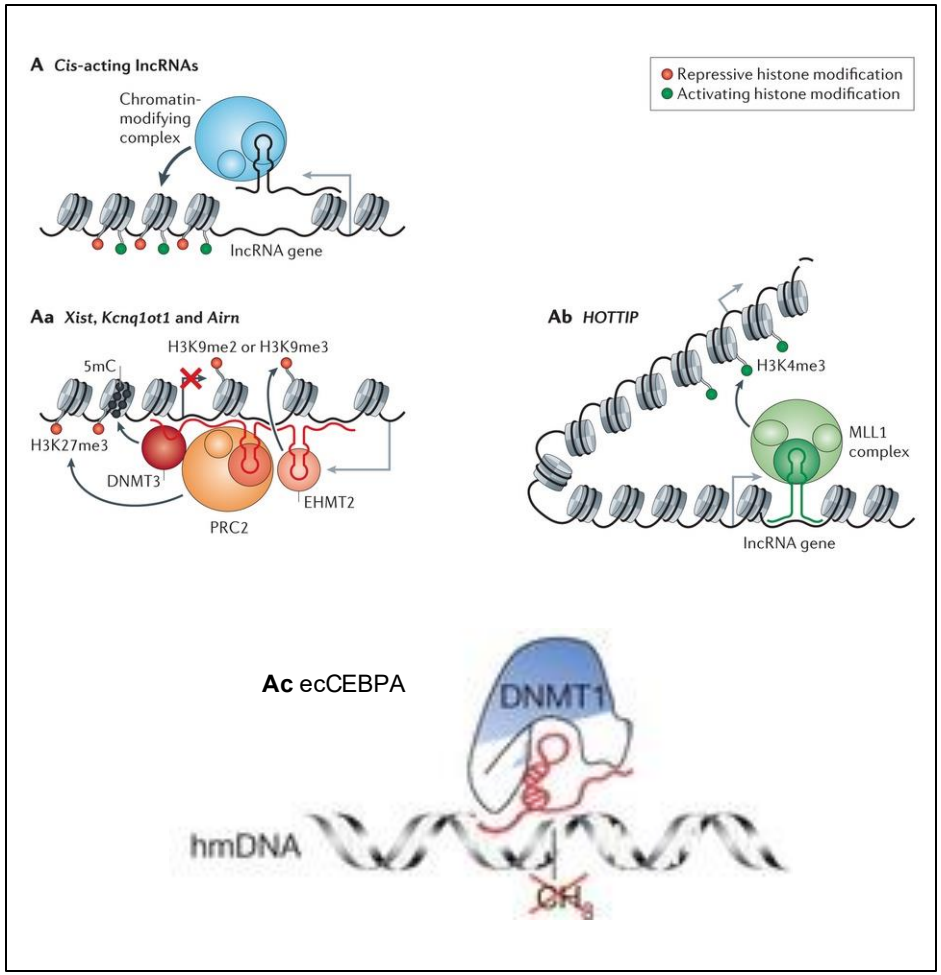
Trans-acting lncRNAs

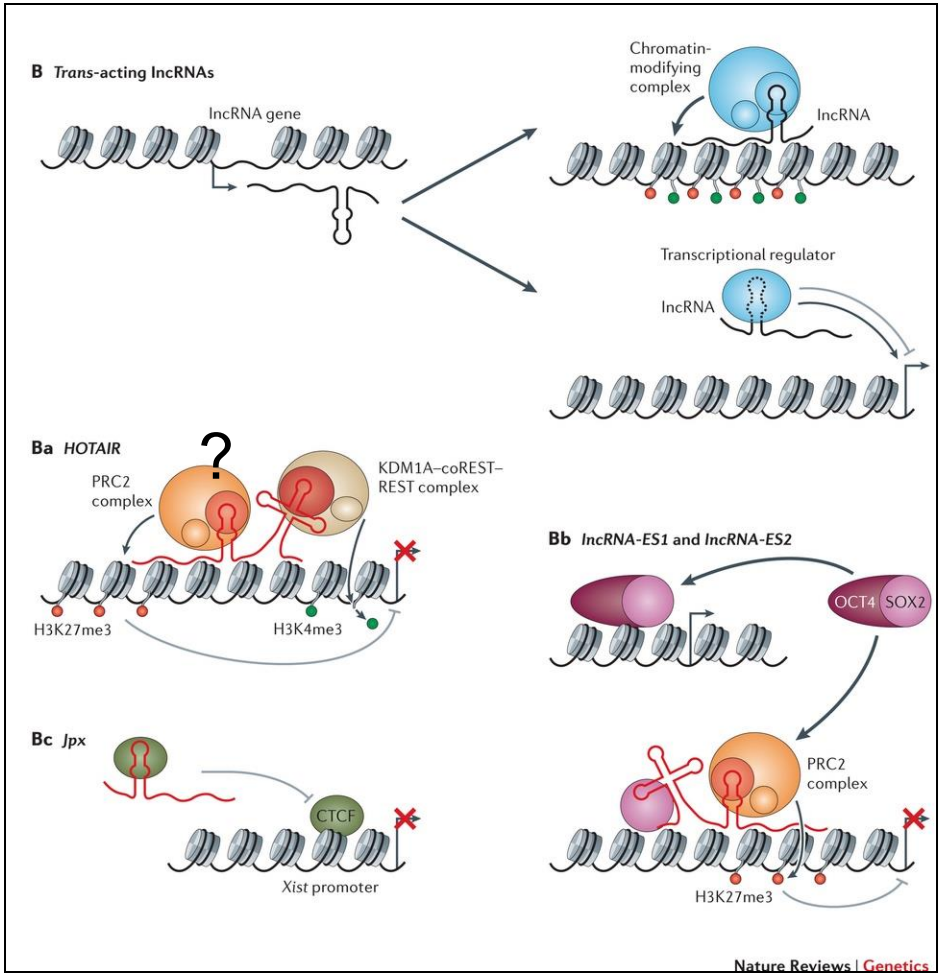


Models of nuclear lncRNA function

Cis-acting

Trans-acting





Polycomb and MLL/Trithorax Complexes

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Polycomb-group Proteins

- Maintains a silenced state
- Prevents chromatin remodelling

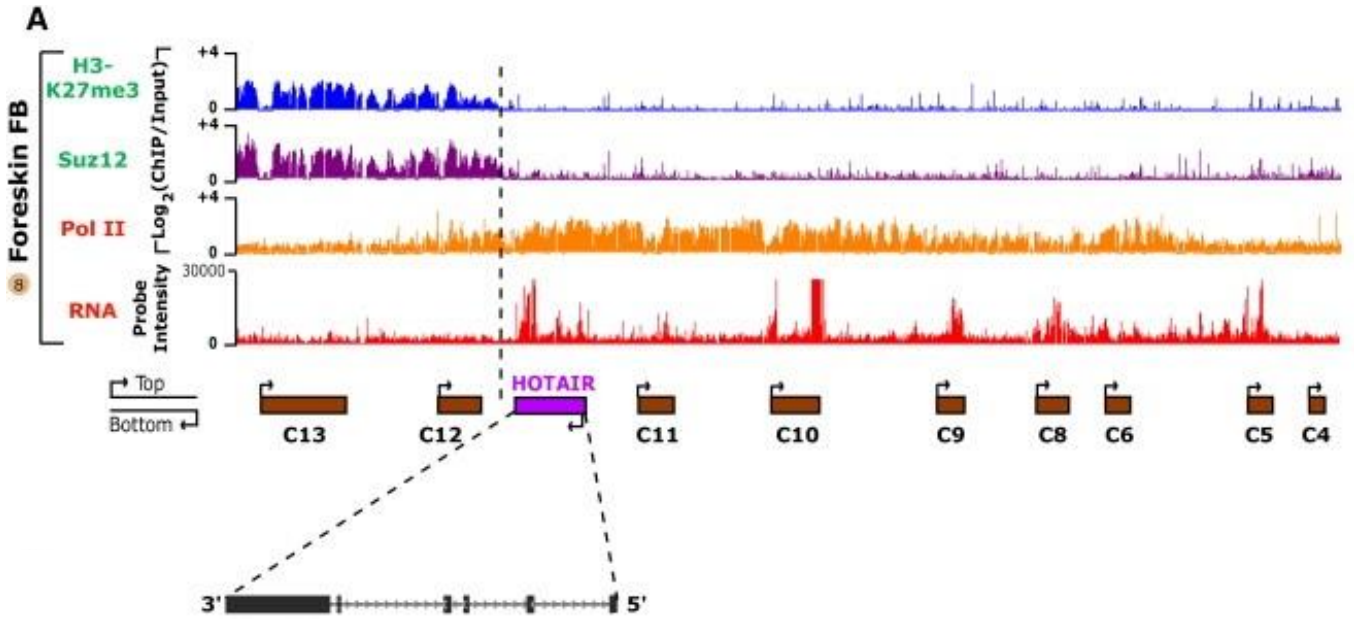
Trithorax-group Proteins

- Maintains an active state
- Counteracts the action of PcG proteins

HOTAIR: A ncRNA that Regulates Chromatin Silencing In *trans*

•ncRNAs produced from the HOX loci were identified by tiling array; one is transcribed in an antisense manner with respect to the canonical **HOXC** genes and was named **HOTAIR** (HOX Antisense Intergenic RNA).

•HOTAIR is preferentially expressed in posterior and distal sites



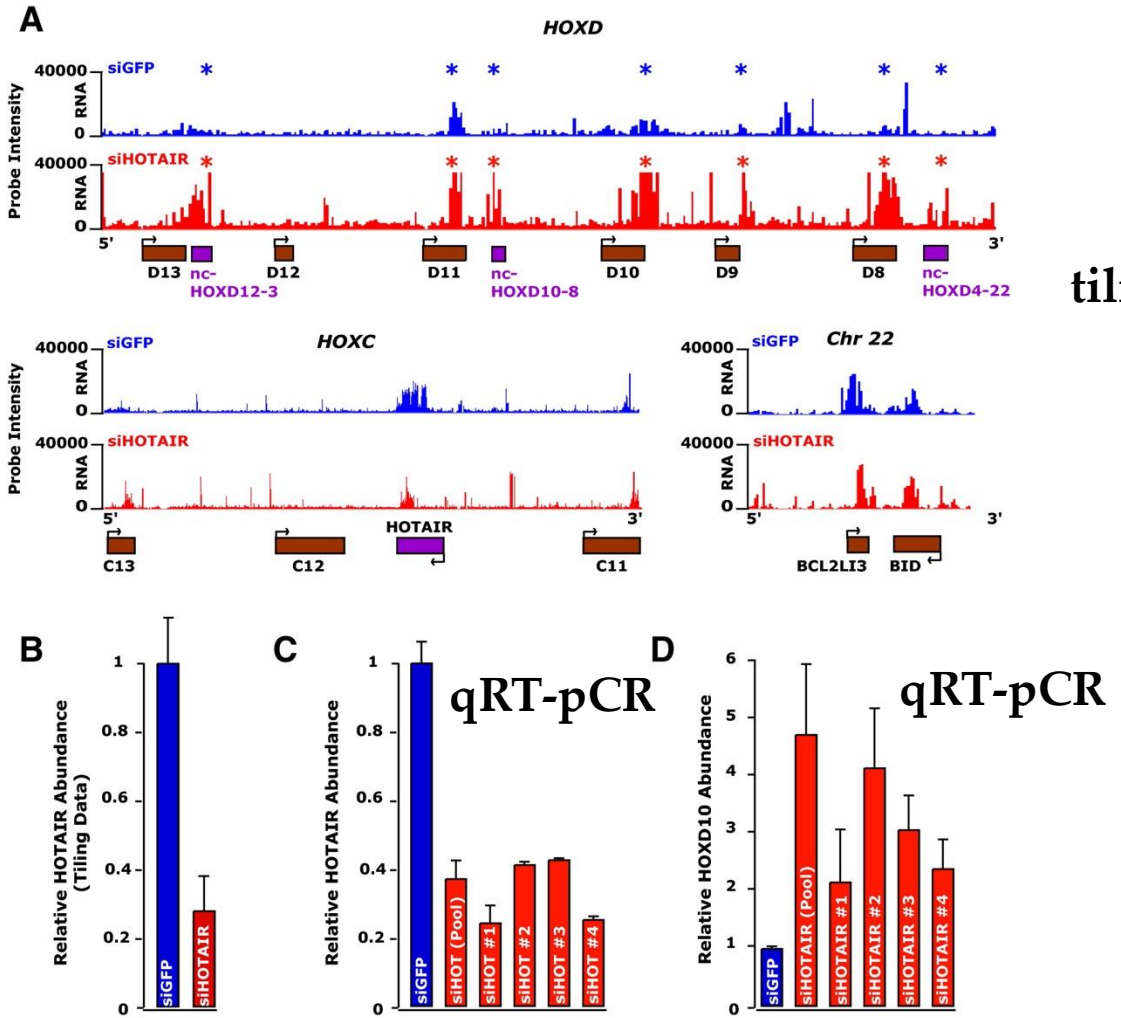
Rinn et al., 2007. Cell

HOTAIR ncRNA Enhances PRC2 Activity at the *HOXD Locus*

RNAi against HOTAIR in primary fibroblast led to dramatic transcriptional activation of the HOXD locus on chromosome 2 spanning over 40 kb, including HOXD8, HOXD9, HOXD10, HOXD11, and multiple ncRNAs

siGFP

siHOTAIR

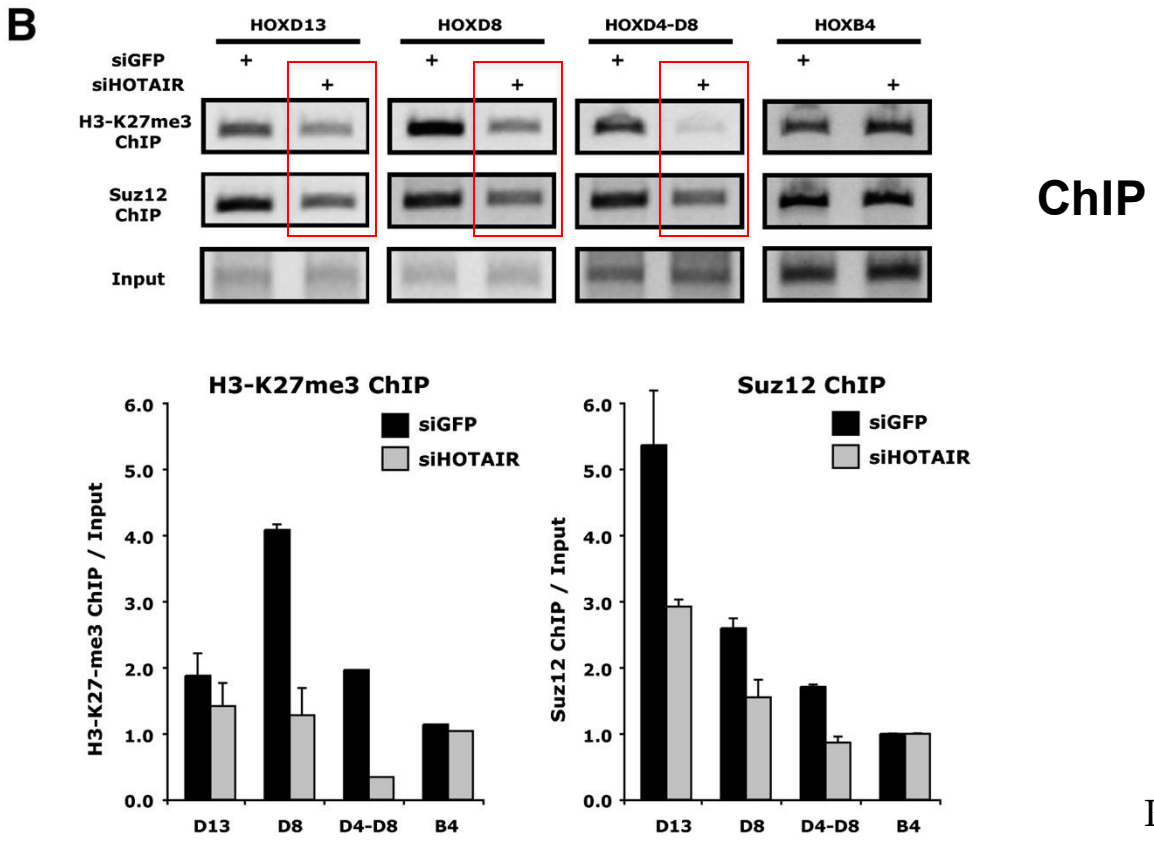


tiling array

Rinn et al., 2007. Cell

HOTAIR Enhances PRC2 Activity at the HOXD Locus

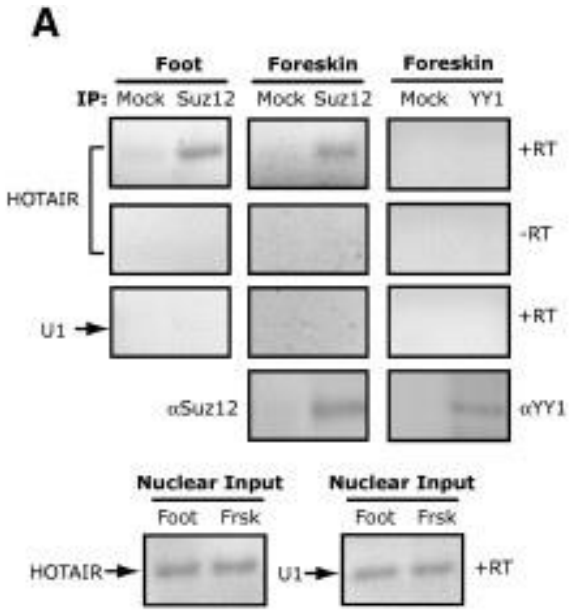
•HOTAIR is Required for **H3K27 Trimethylation** and **Suz12 (PRC2 component) occupancy** of the *HOXD Locus*



Rinn et al., 2007. Cell

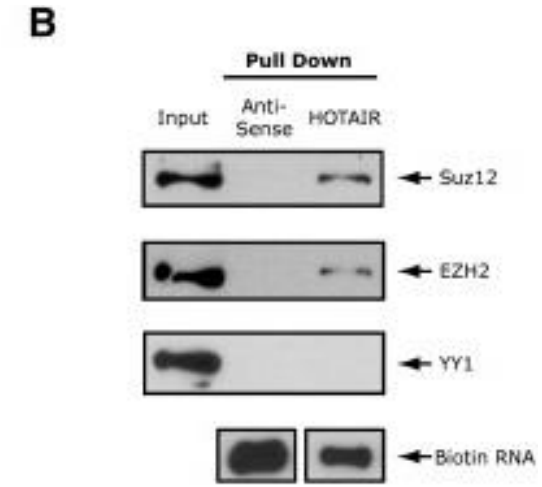
HOTAIR ncRNA Binds PRC2

Immunoprecipitation



•Immunoprecipitation of Suz12 retrieves endogenous HOTAIR

Exogenous RNA Pull Down



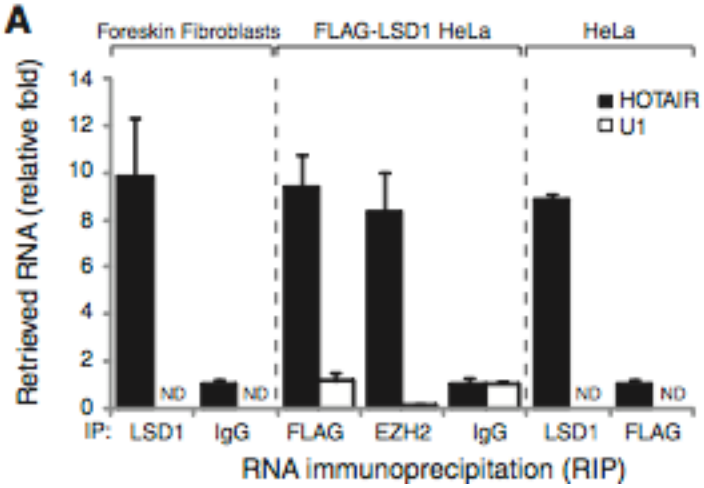
•In vitro-transcribed HOTAIR retrieves PRC2 subunits

Rinn et al., 2007. Cell

HOTAIR ncRNA Binds LSD1 KDM

The genomic regions flanking HOXD are also bound by **CoREST/REST** repressor complexes, which contain **LSD1** (KDM1/BHC110) that mediates enzymatic demethylation of H3K4me2 and that is required for proper repression of Hox genes.

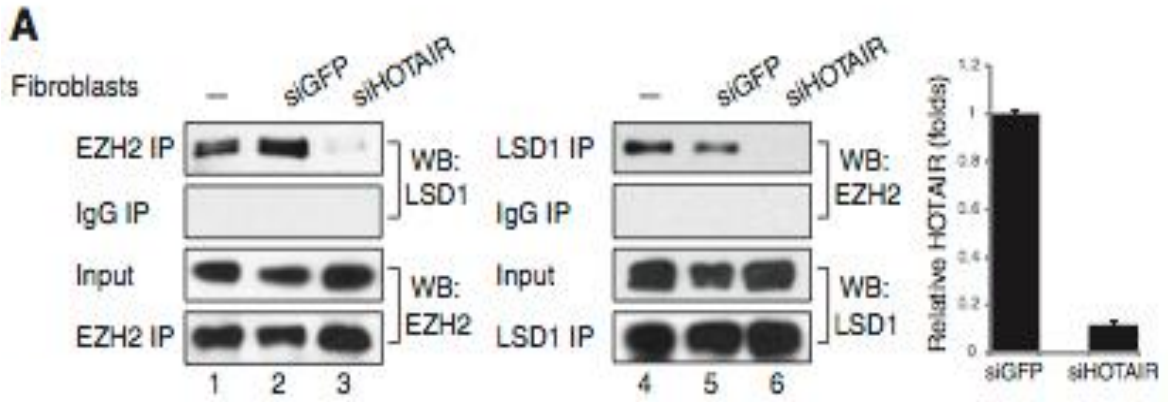
Immunoprecipitation of either endogenous LSD1 or FLAG-tagged LSD1 from primary foreskin fibroblasts or HeLa cells specifically retrieved endogenous HOTAIR RNA with enrichment comparable with that of EZH2 IP, the positive control



Tsai et al., 2010. Sience

HOTAIR is necessary for interaction between EZH2 and LSD1

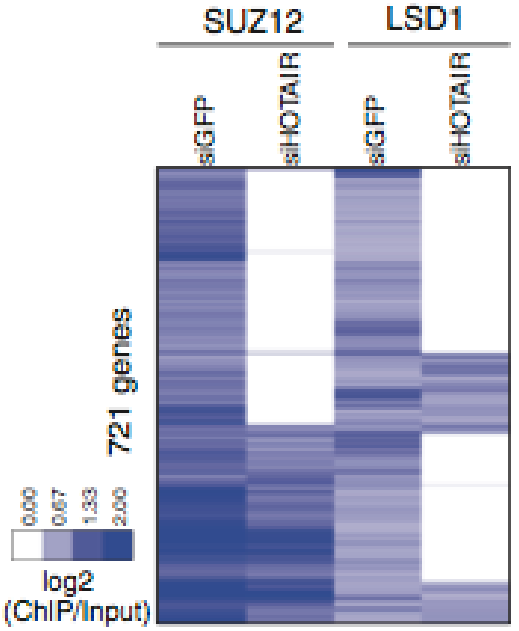
In foreskin fibroblasts, **EZH2** interacts with **LSD1** (lanes 1 and 4). Knockdown of HOTAIR (lanes 3 and 6), but not GFP (lanes 2 and 5), abolishes this interaction.

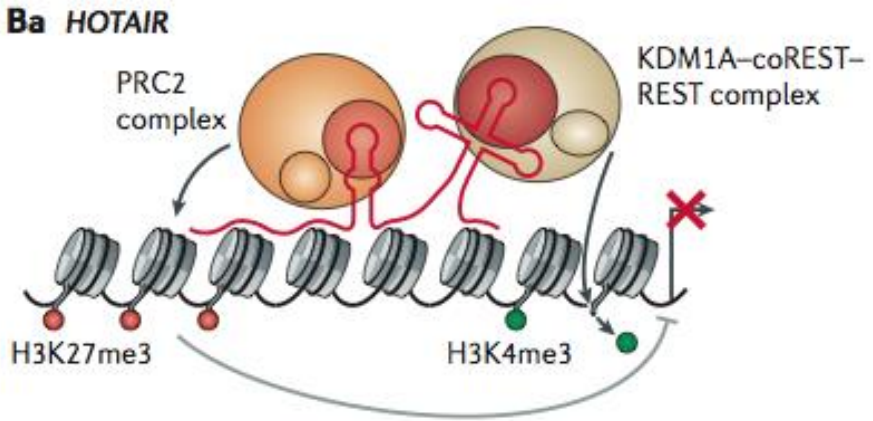


Tsai et al., 2010. Sience

HOTAIR coordinates localization of PRC2 and LSD1 genome-wide

Heat map of **SUZ12** and **LSD1** co-occupied genes (721 genes). Each column is an experiment; each row is a gene. HOTAIR knockdown led to concordant loss of SUZ12 and LSD1 occupancy. Chromatin occupancy is indicated in blue per the scale bar.

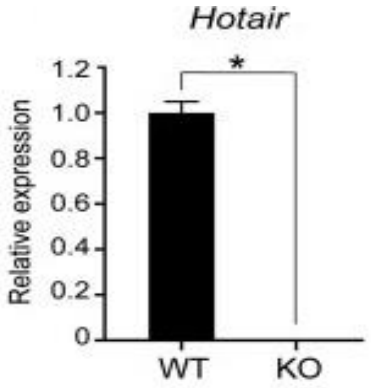


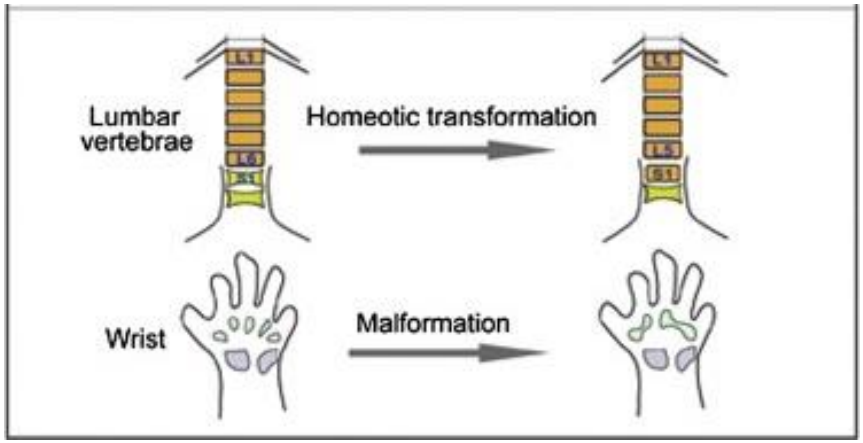


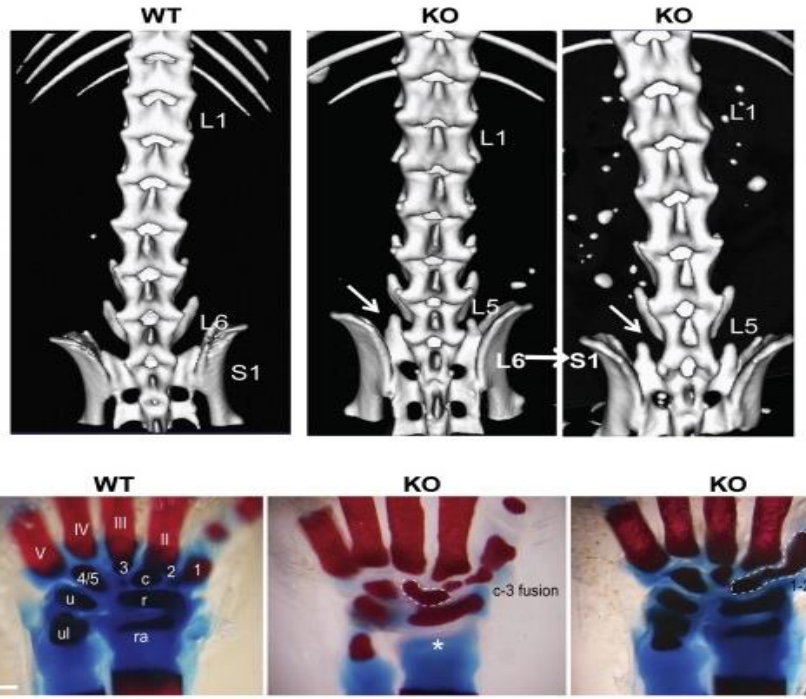
Tsai et al., 2010. Sience

Hotair KO Causes Homeotic Transformations

Targeted deletion of mouse Hotair lncRNA leads to derepression of hundreds of genes, resulting in homeotic transformation of the spine and malformation of metacarpal-carpal bones. RNA sequencing and conditional inactivation reveal an ongoing requirement of Hotair to repress HoxD genes.



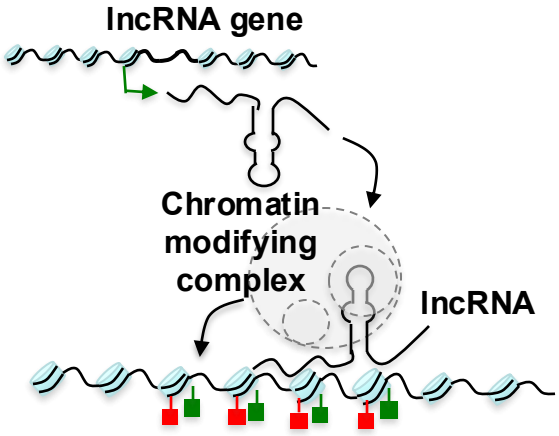


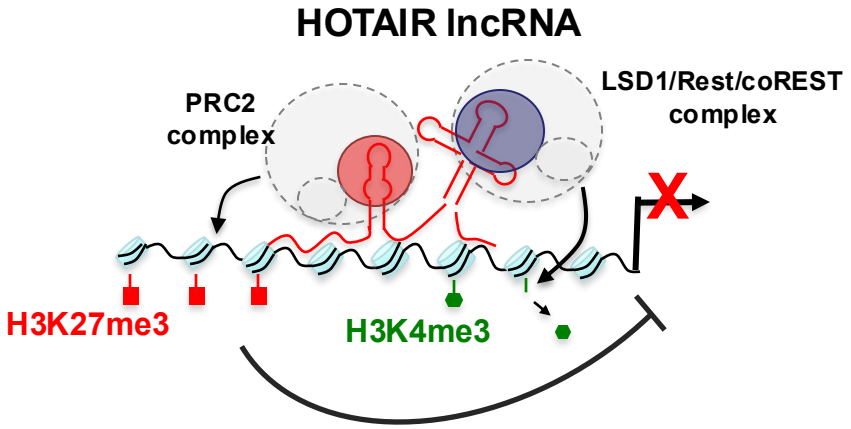


Li et al., 2013. Cell Reports

Epigenetic regulation by lncRNAs

These lncRNAs may act as scaffolds to bring two or more proteins into a complex or spatial proximity and may also act as guides to recruit proteins, such as chromatin modification enzymes, to DNA; this may occur through RNA-DNA interactions or through RNA interaction with a DNA-binding protein.

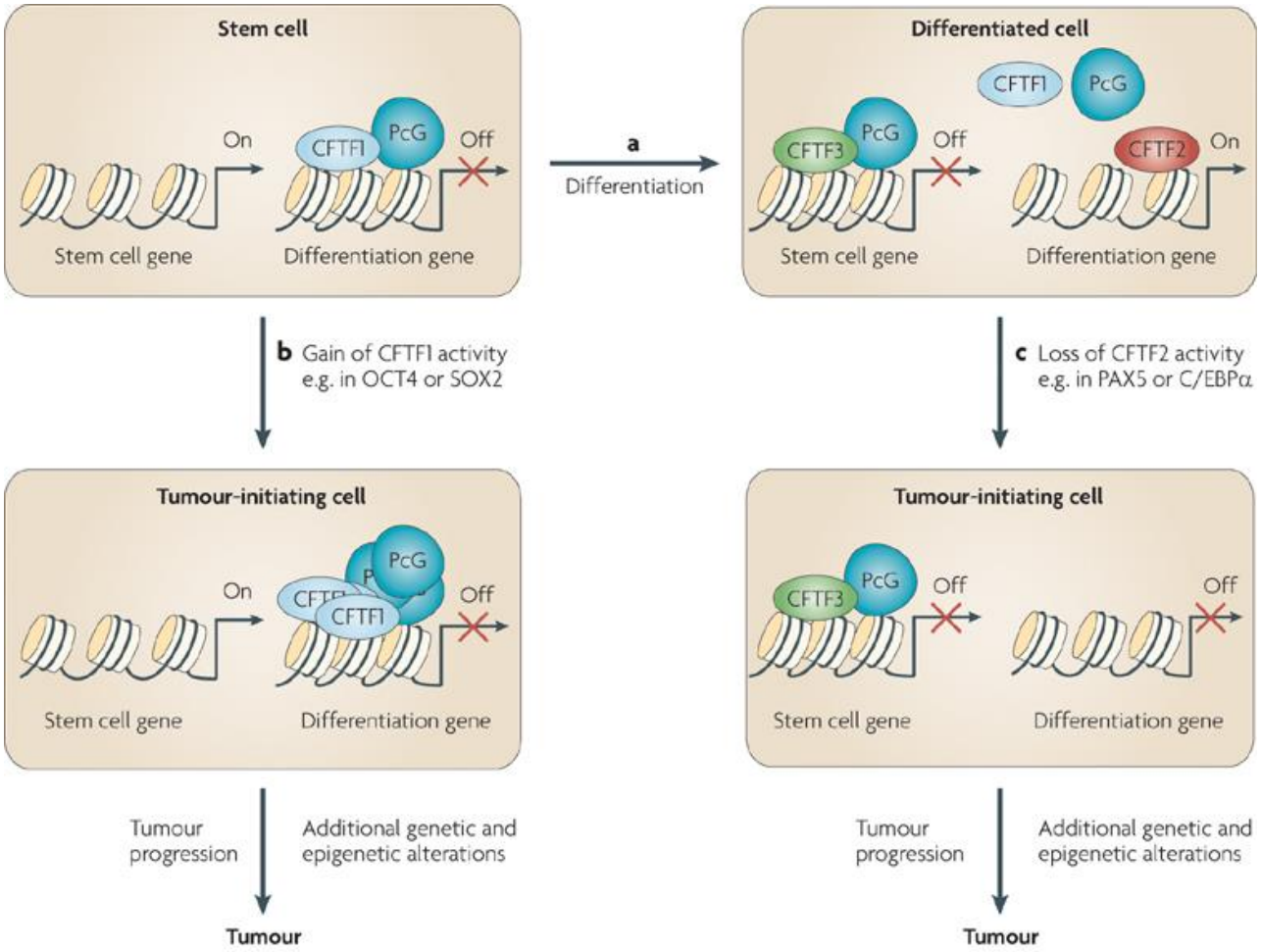




HOTAIR and cancer

PcGs and cancer

•Aberrant PcGs recruitment by the loss or gain of function of two putative CFTFs (cell fate transcription factors) may lead to the formation of a tumour-initiating cell



PcGs and cancer

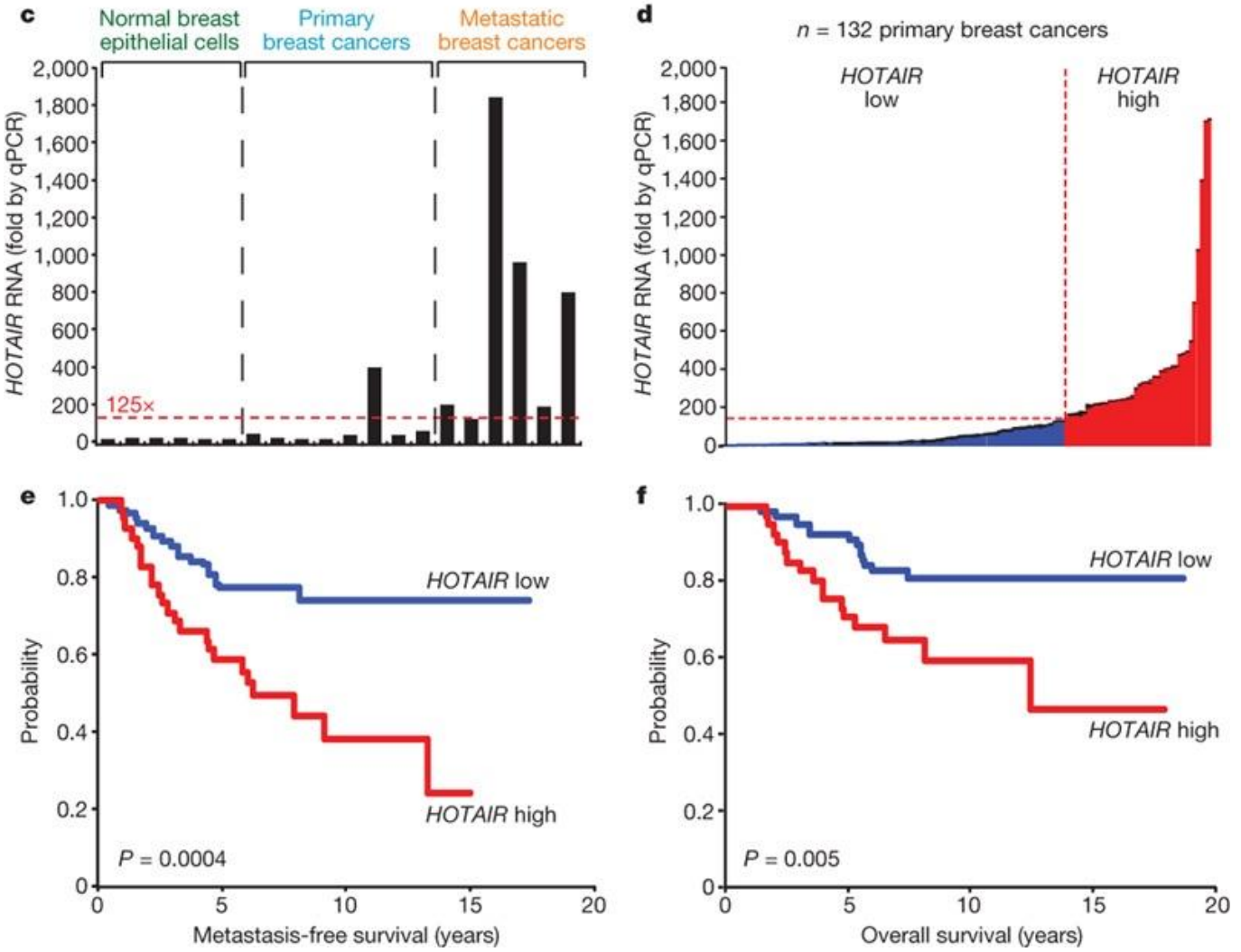
•PcG target genes accumulate DNA methylation on their promoters in cancer.

•The PcG proteins (e.g. EZH2 and CBX7) can physically associate with **DNA methyltransferases** suggesting a mechanism whereby the PcG proteins directly contribute to the altered DNA methylation profiles that are observed in multiple cancer types.

•PcG proteins and DNMTs cooperate to aberrantly silence pro-differentiation and anti-proliferative genes, which leads to the accumulation of a population of cells unable to respond to differentiation signals. The consequent block of differentiation may allow these tumour-initiating cells to linger and accumulate the additional epigenetic and/or genetic alterations necessary to develop into a tumour.

•Indeed several PcG proteins are highly expressed in cancer.

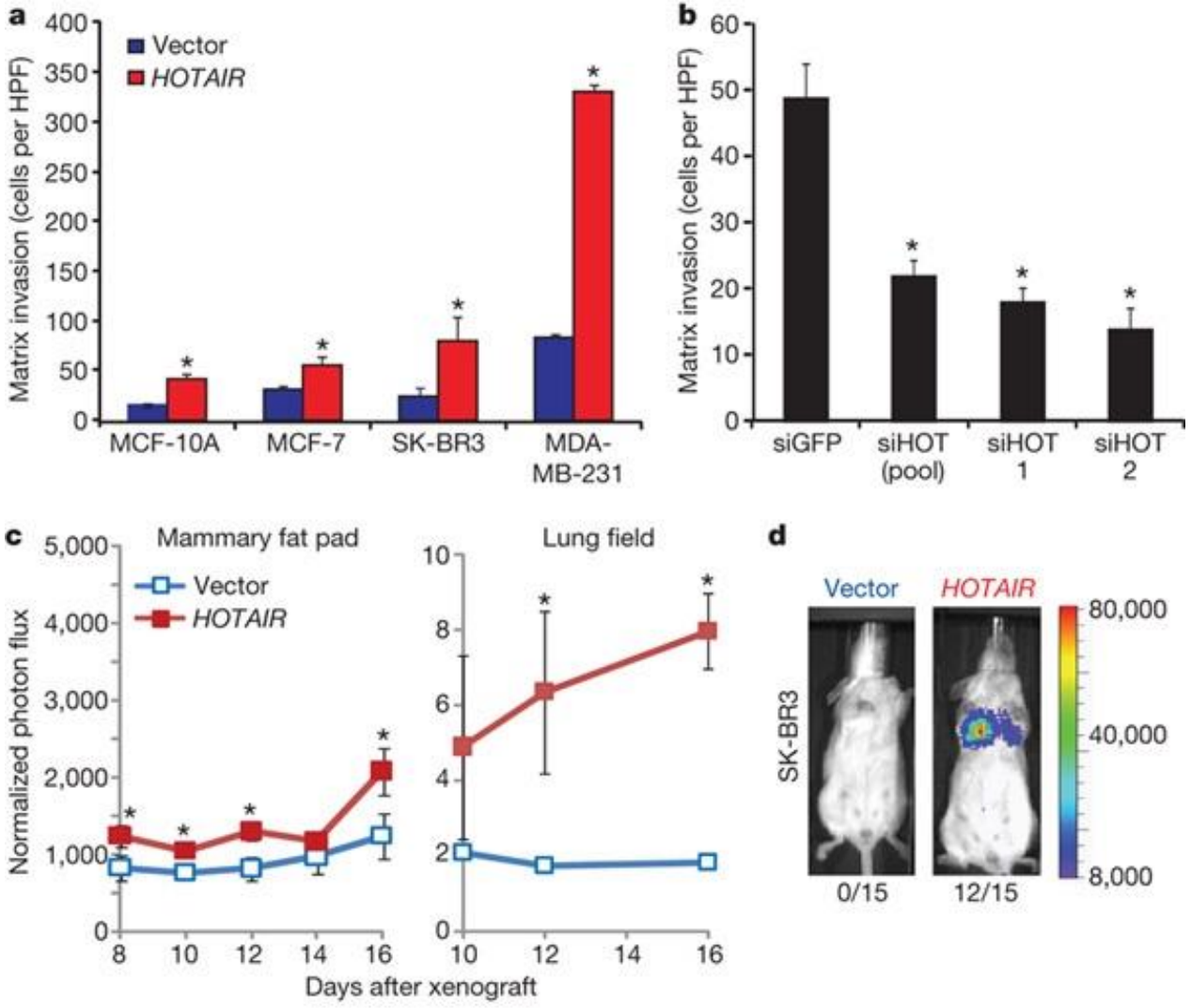
HOTAIR is up-regulated in breast carcinoma and have prognostic value for metastasis and survival.



1/3 of breast tumours has125-fold overexpression of HOTAIR

Gupta et al., 2010. Nature

HOTAIR promotes invasion of breast carcinoma cells

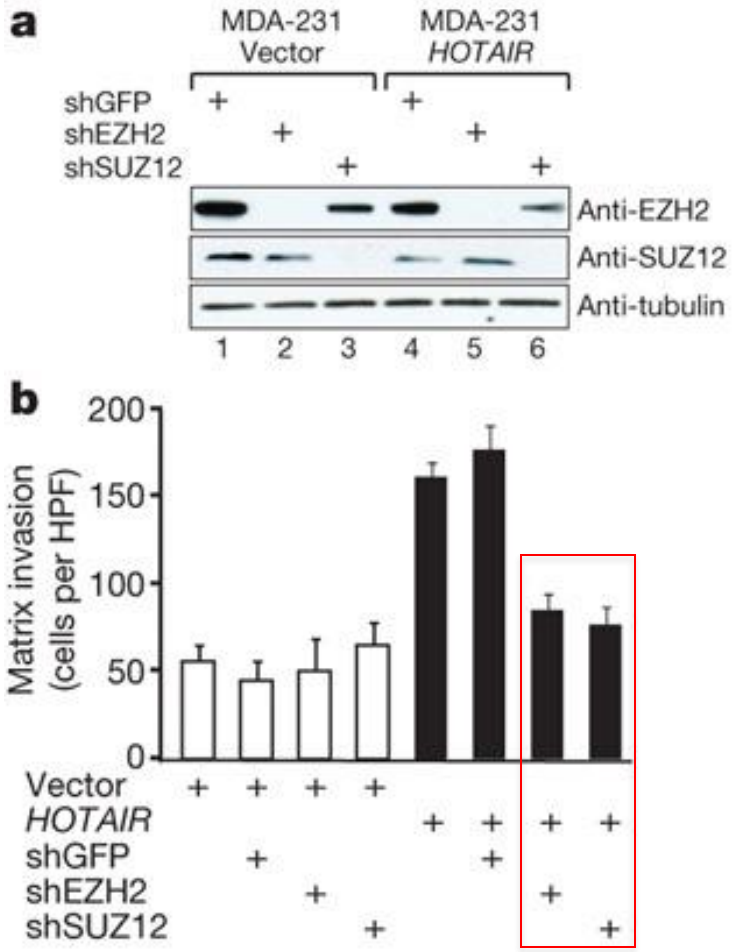


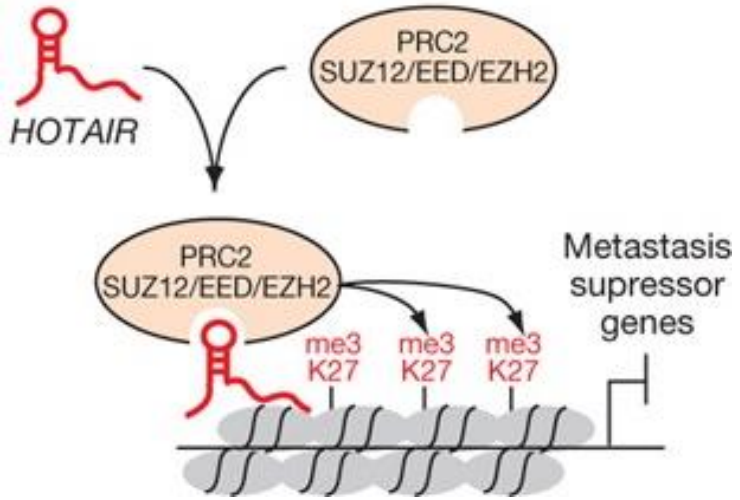
In vitro

In vivo

Gupta et al., 2010. Nature

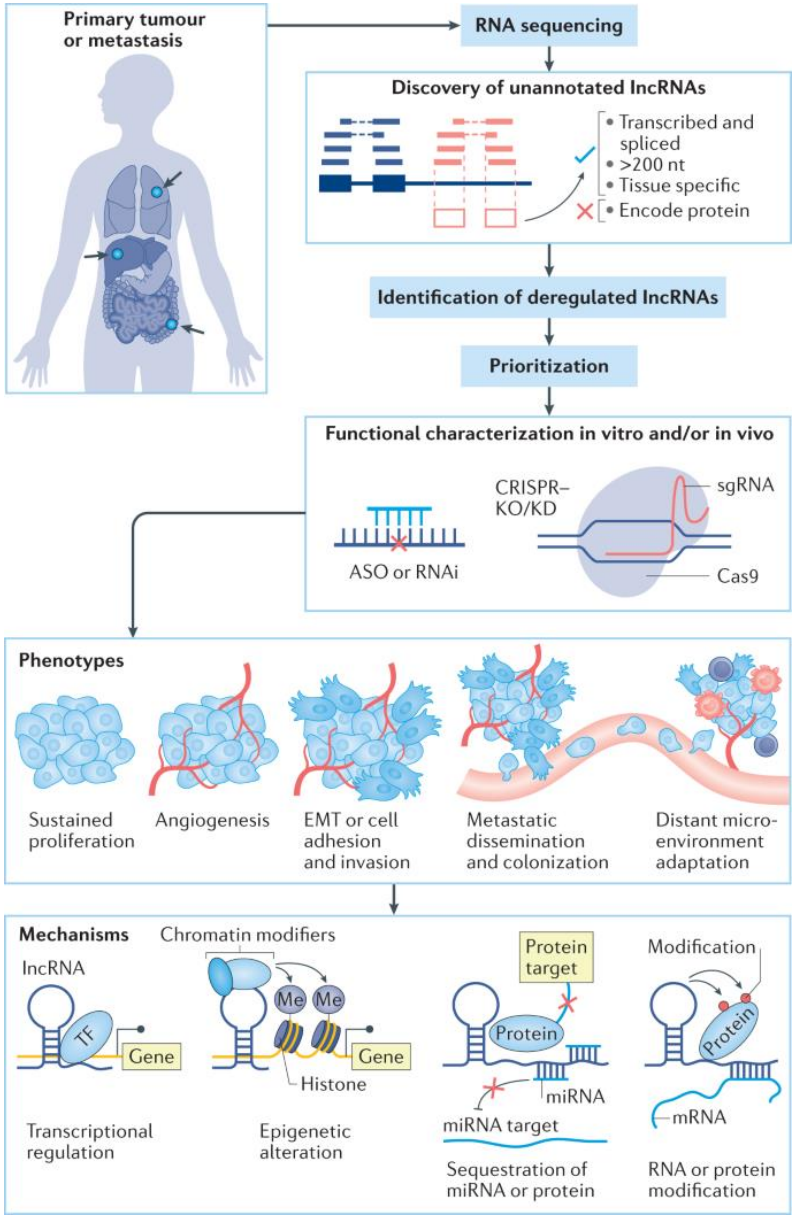
HOTAIR requires PRC2 for function





Gupta et al., 2010. Nature

lncRNAs in cancer



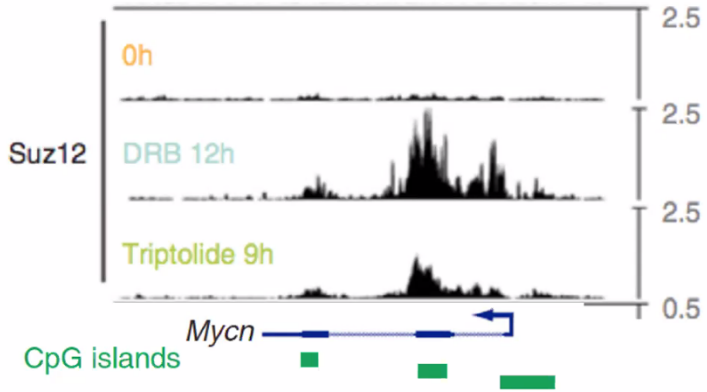
Nature Reviews Cancer (*Nat Rev Cancer*) | ISSN 1474-1768 (online)

2013-2019 Re-evaluating the foundations of lncRNA–Polycomb function

Gene activity regulates PRC2 recruitment

- **PRC2 recruitment or H3K27me3 is blocked by:**
  - Insertion of active promoter/enhancer (Jermann, 2014)
  - dCas9-VP64 (Hosogane, 2016)

- **PRC2 recruitment or H3K27me3 is induced by:**
  - Pol II inhibition (Riising, 2014)
  - Premature termination (Kaneko, 2014)
  - TSS deletion (Hosogane, 2016)



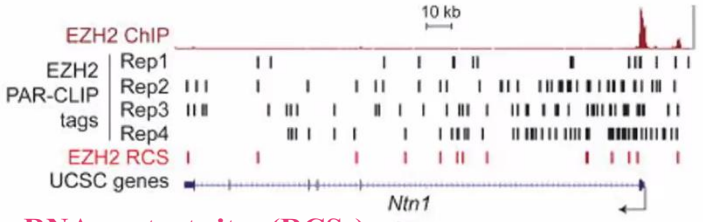
Riising et al., 2014

- **Suggests PRC2 "senses" gene activity and is only recruited *after* gene repression to maintain the repressed state.**
- **Could PRC2 sense nascent RNA?**

DRB acts as an inhibitor of transcriptional elongation by inhibiting Serine 2 phosphorylation (Ser2-P) of RNA Polymerase II (RNAPII), whereas Triptolide inhibits the ATPase activity of the XPB helicase subunit of TFIIH and induces proteasomal degradation of RNAPII

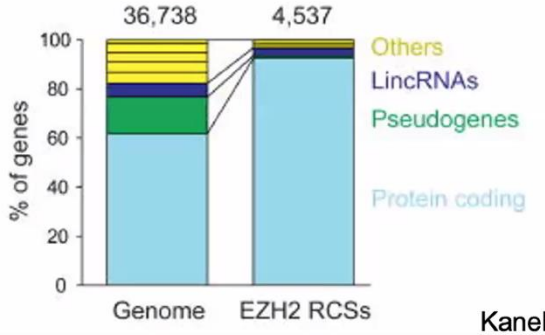
PRC2 preferentially interacts with pre-mRNAs

PAR-CLIP



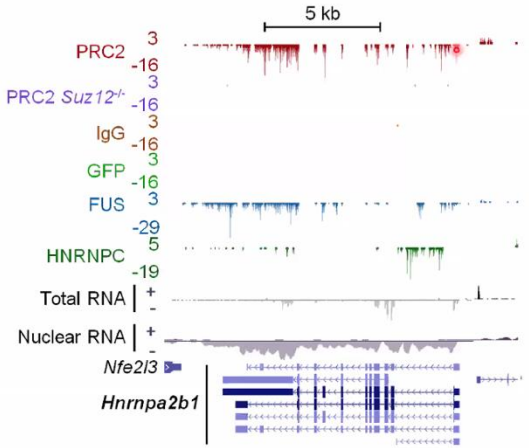
RNA contact sites (RCSs)

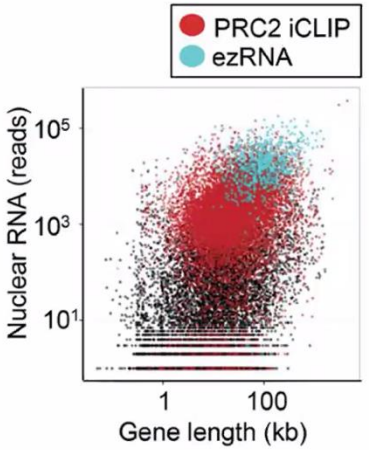
EZH2-binding RNAs ("ezRNAs")



Kaneko et al., 2013

iCLIP

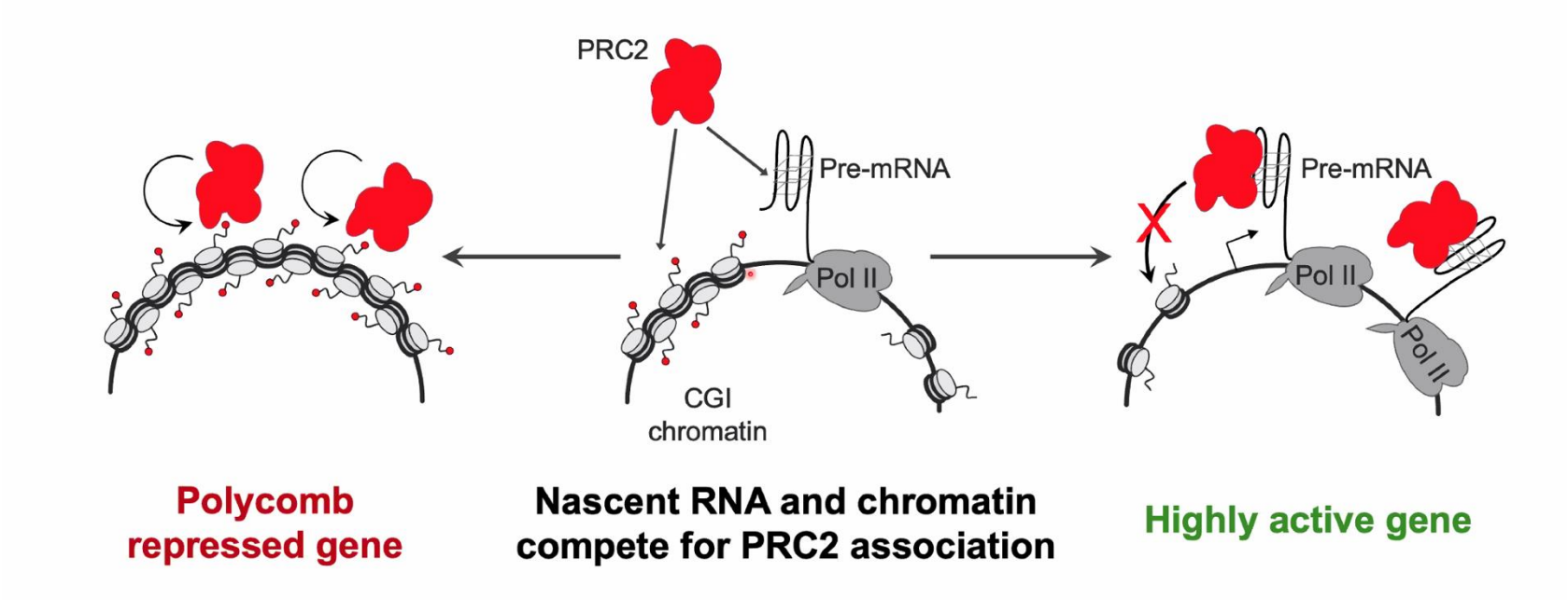




Beltran et al., 2016

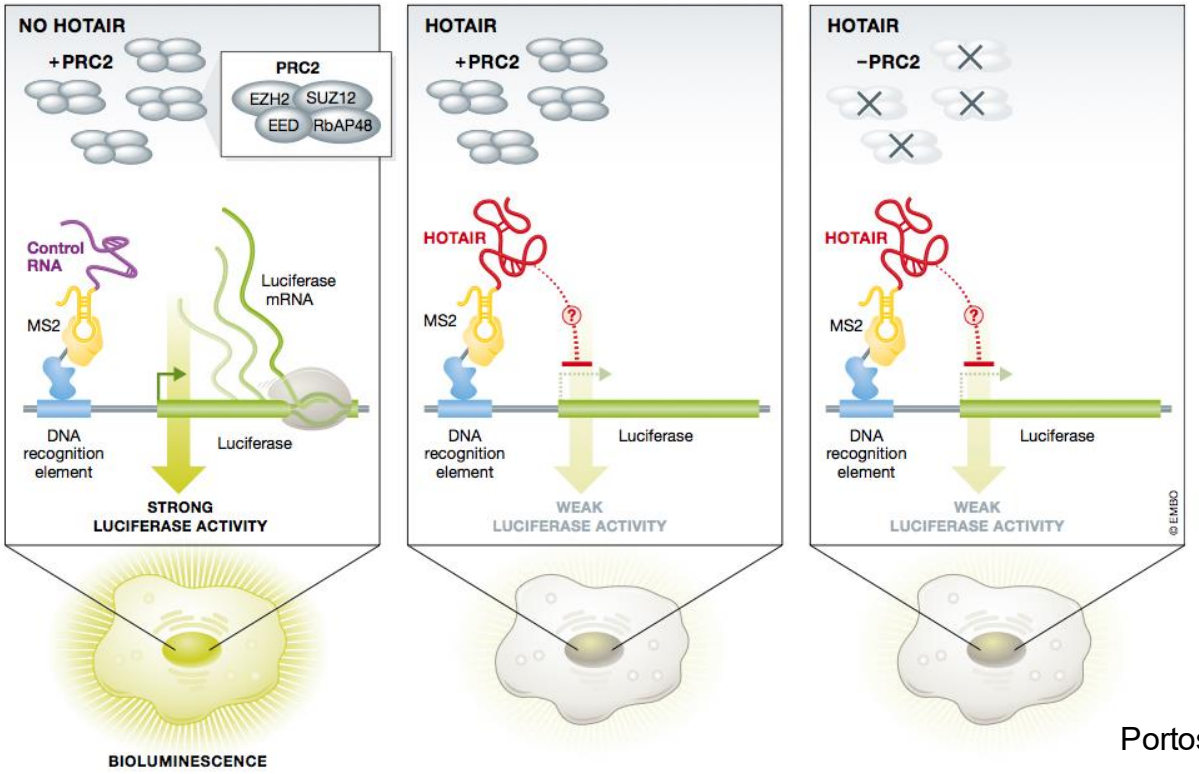
RNA prevents PRC2 recruitment to chromatin at active genes and inhibits its catalytic activity

Nascent RNA antagonises PRC2 association with active genes



PRC2 is not required for HOTAIR-mediated silencing of gene expression

The MS2 coat protein is fused with the Gal4-DNA binding domain to recognize an engineered UAS/Gal4-binding site upstream of a luciferase reporter gene. In this system, a specific RNA (HOTAIR or control RNAs) is fused to an MS2 hairpin which binds tightly to the MS2 coat protein and is tethered upstream of the reporter gene allowing for direct measurements of their impact on gene expression through measurement of luciferase levels. Recruitment of control RNAs had no impact on luciferase expression (left panel), whereas recruitment of the HOTAIR lncRNA leads to silencing of the luciferase reporter (middle panel). Notably, HOTAIR can silence transcription of the reporter gene even in cells lacking the PRC2 components (right panel).



Portoso et al., 2017 EMBO J