Role of Long non-coding RNAs in Epigenetic Regulation

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OBJECTIVES

- Describe lncRNAs as functional regulatory molecules
- Remark their importance in biological processes
- Review their main characteristics, functions and molecular mechanisms
- Review epigenetic mechanisms where lncRNA play an important role
- Exemplify their importance in development with Xist, a lncRNA that drives the X chromosome inactivation in Mammals.

METHODOLOGY

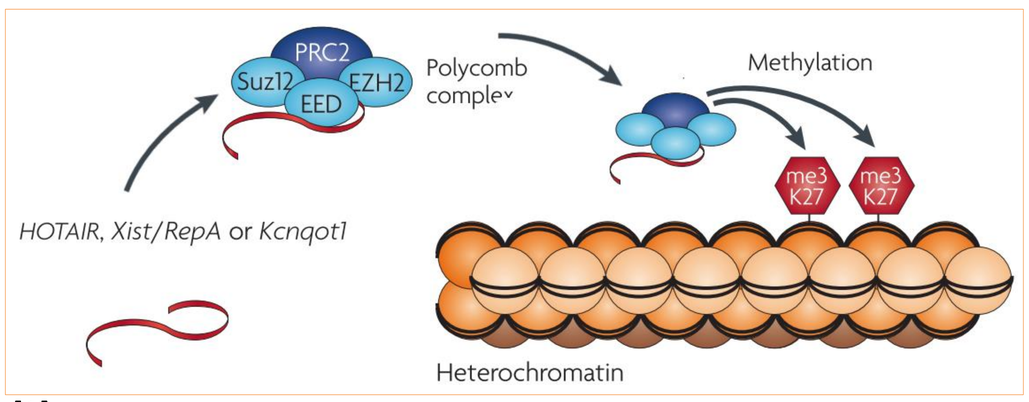
- Bibliographic research with scientific tools like Web of Science and PubMed to obtain review articles published in the past 5 years.
- Use of reference books and Ph.D. Theses in Epigenetics to get a knowledge background in the field.
- Attendance to "V Jornada de Cromàtina i Epigenètica – SCB" about non-coding RNAs and Heterochromatin.

INTRODUCTION TO LONG NON-CODING RNAS AS REGULATORY ELEMENTS

- lncRNAs are a novel regulatory RNA molecule more than 200 bp long and without a coding ORF. Their nuclear localization make them suitable to regulate gene expression during development.
- lncRNAs are classified following their genomic position. Enhancer RNAs (eRNAs) and upstream antisense RNAs (uaRNAs) are located near regulatory regions and represent up to the 80% of known lncRNAs. Antisense transcripts and Long intergenic non-coding RNA (LincRNAs) represent the other 20%. However, the last groups as act as independently regulatory molecules and have crucial roles in epigenetics.
- Although their low conservation rate, their tissue specificity and their protein interaction prove their biological importance in several development processes.
- Current experimental techniques used for their identification are SAGE, CAGE, RNAseq and Chip-seq technologies. All of them detect expressed coding and non-coding regions.

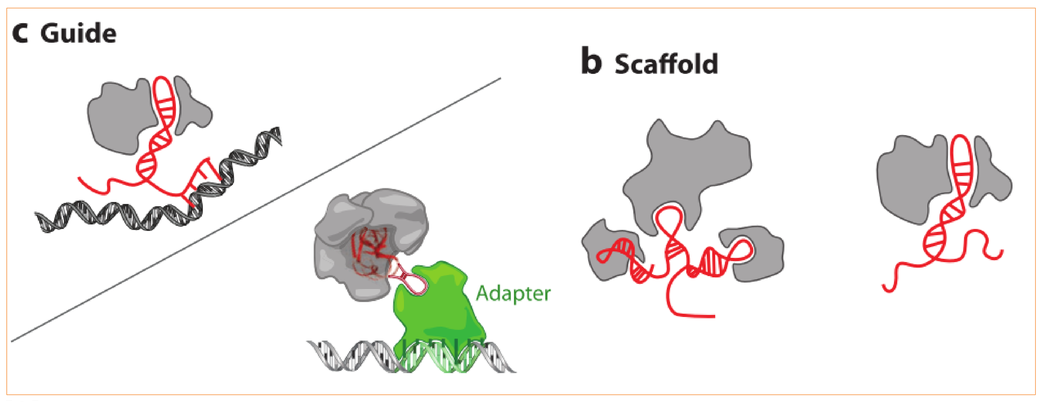
FUNCTIONS, MECHANISMS AND EPIGENETIC EFFECTORS

The main **function of lncRNA** in regulation is to control expression of defined genes. For their role in development, they modulate chromatin structure recruiting epigenetic modification proteins to defined sites of the genome. Examples of this function are HOTAIR, Xist and Kcnqot1. HOTAIR is involved in limb formation and cancer meanwhile Kcnqot1 is involved with the imprinting of its loci.



[1]

Molecular mechanisms of lncRNAs can be classified by the way their sequence domains interact with effector proteins. For epigenetic modifications, the two most important molecular mechanisms reported are **Guiding** effector proteins through the genome to their target sites and working as **Scaffold** molecule for large protein complexes.



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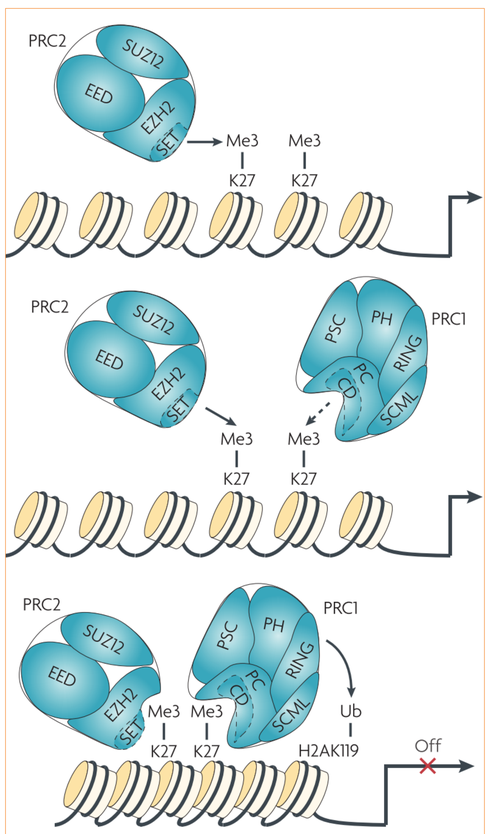
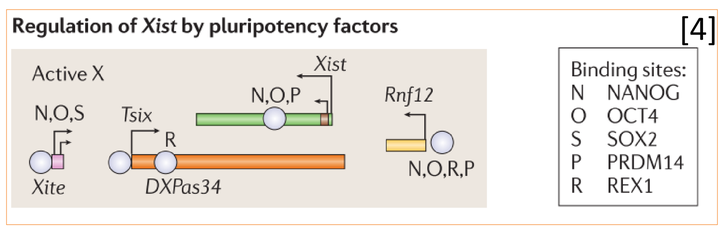


[3]

lncRNAs recruit epigenetic modifiers which are mostly Polycomb Group proteins. They are involved with gene silencing and development. They are known to maintain the celular memory of decissions performed during embryogenesis. Their mechanism is based on two protein complexes, PRC1 and PRC2. First, PRC2 is recruited by lncRNAs and generate H3K27me3 marks in defined sites. This epigenetic marks are detected by PRC1 which establishes a long lasting repression.

X CHROMOSOME INACTIVATION

- Xist is a long non-coding RNA located in the X inactivation center (XIC), a region of the X chromosome which is sufficient and necessary to trigger the X chromosome inactivation (XCI) in mammals.
- This same locus also contains other coding and non-coding genes that participate in the regulation of Xist. This development event generates a compacted X chromosome known as Barr Body which is almost silenced.



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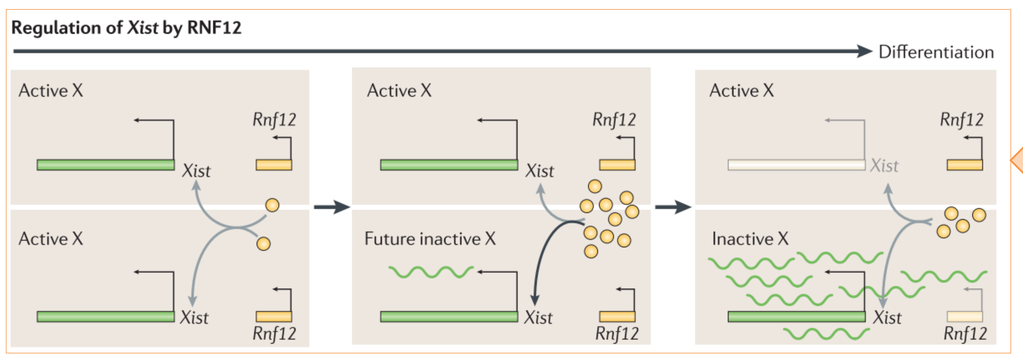
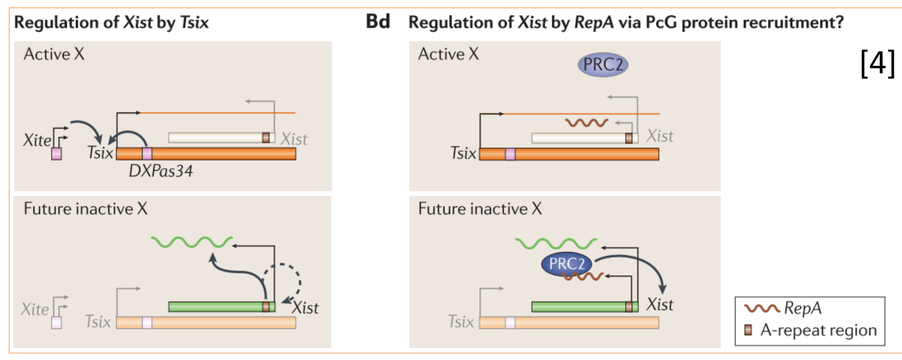
NANOG, OCT4, SOX2 and other **pluripotency factors** inhibit in trans the expression of Xist in multiple point of its regulation. This inhibition cause a reactivation of the Barr Bodies in pluripotent cells.



[4]

Although **Rnf12** is located in the X inactivation center (XIC), it regulates Xist expression also in trans. Rnf12 increase Xist transcription and then is repressed by the lncRNA in a feedback loop. It is thought to participate in the monoallelic expression of Xist.



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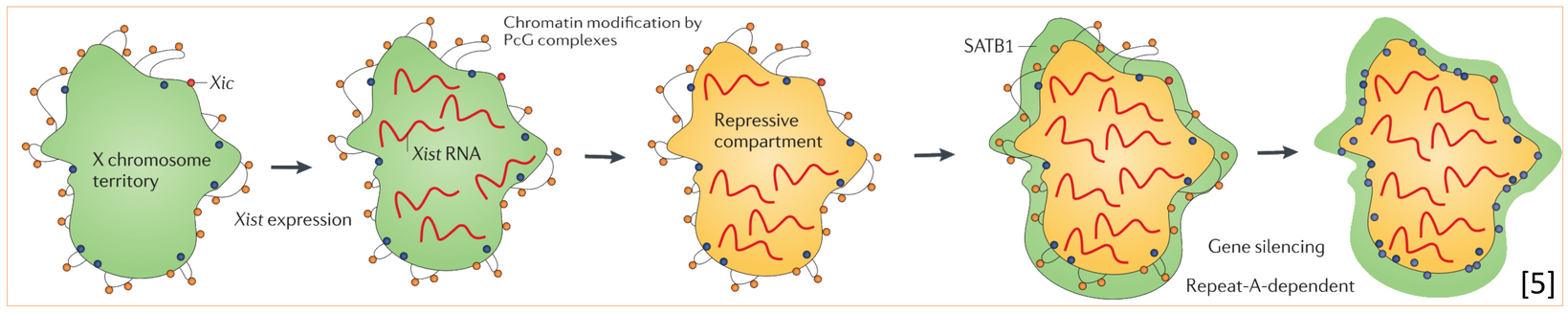
Tsix is an antisense non coding transcript located in Xist locus. Its expression is regulated by DxPas34 and Xite, also non coding elements. Tsix acts as decoy for PRC2 complexes that try to bind Xist. The equilibrium established by these two elements permits a precise decision wether a chromosome may be or not silenced.

The **steps of X chromosome Inactivation (XCI)** consist in a first expression of Xist in the center of the chromosome 3D region. Xist recruit PcG proteins and silence the central and non-coding region. STAB1 is later recruited by repA, a sequence domain inside Xist, and silence the outlying and active genes.



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CONCLUSIONS

- lncRNAs are emerging regulatory elements for genetic networks in eukaryotes that raise the complexity of already existing gene regulation.
- There are not enough tools to understand their functions and mechanisms. The introduction of a modular code for lncRNA seems a promising concept that can help to predict them and their functions by computational methods.
- X chromosome inactivation is a key example to understand how a lncRNA is capable to recruit epigenetic modifiers and silence a whole chromosome in a crucial development process.

REFERENCES

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