

LINE-1 regulates cortical development by acting as long non-coding RNA

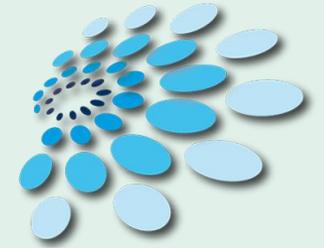
Damiano Mangoni, Stefano Gustincich et.al

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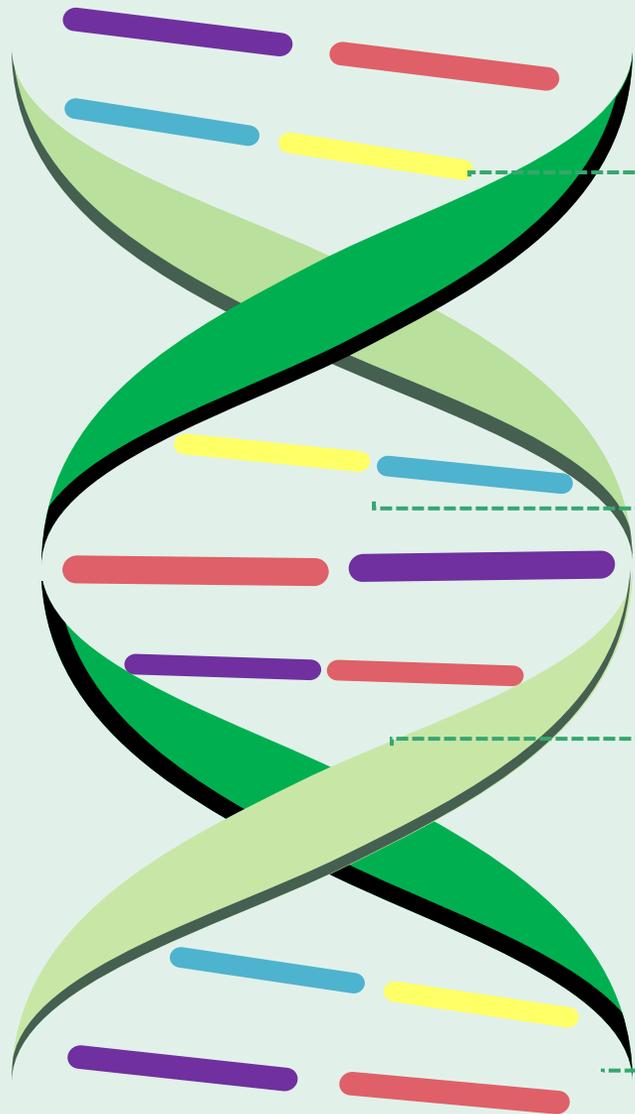


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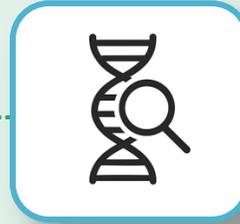


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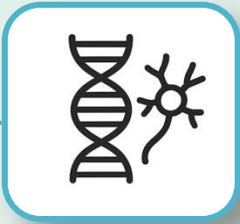




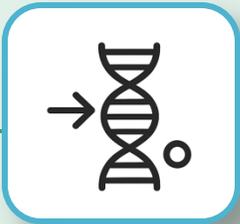
INTRODUCTION



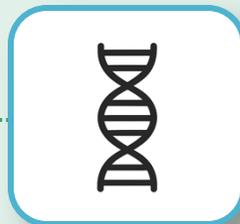
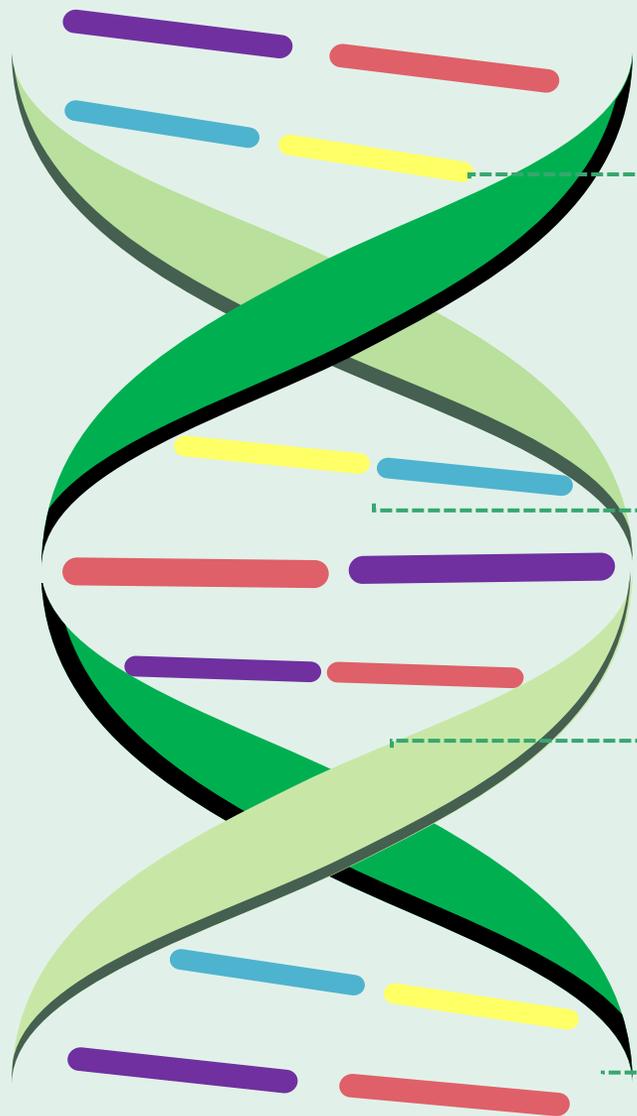
EXPERIMENT



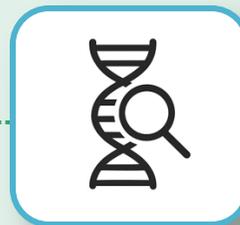
CONCLUSIONS



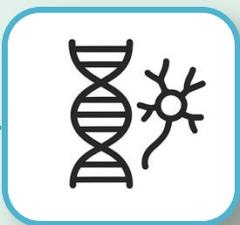
FUTURE OUTLOOK



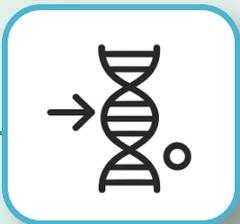
INTRODUCTION



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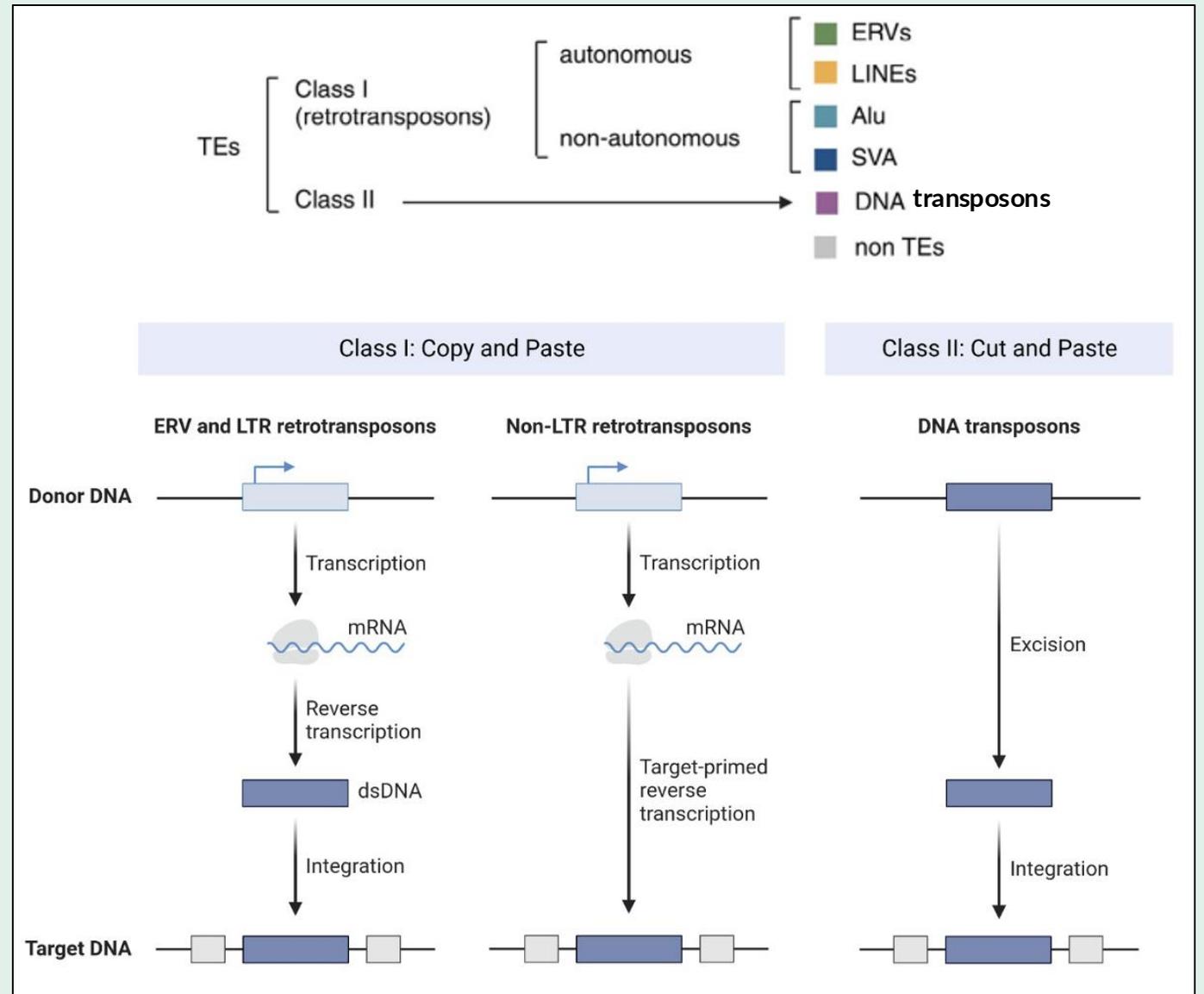
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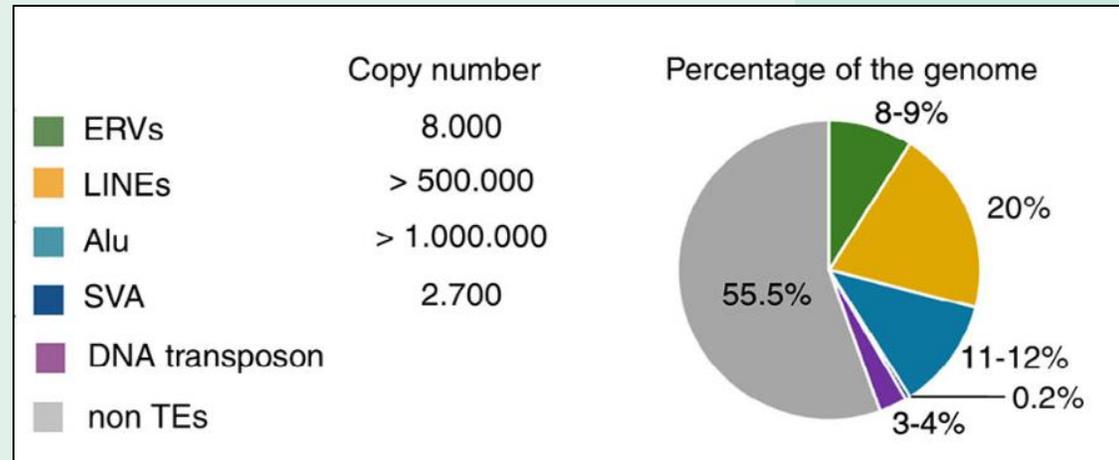
An overview about Transposable Elements (TEs)

Transposable Elements are DNA sequences with the unique ability to **replicate** and **spread** their sequence inside the genome.



An overview about Transposable Elements (TEs)

LINE-1 are the most abundant TEs in mammals.

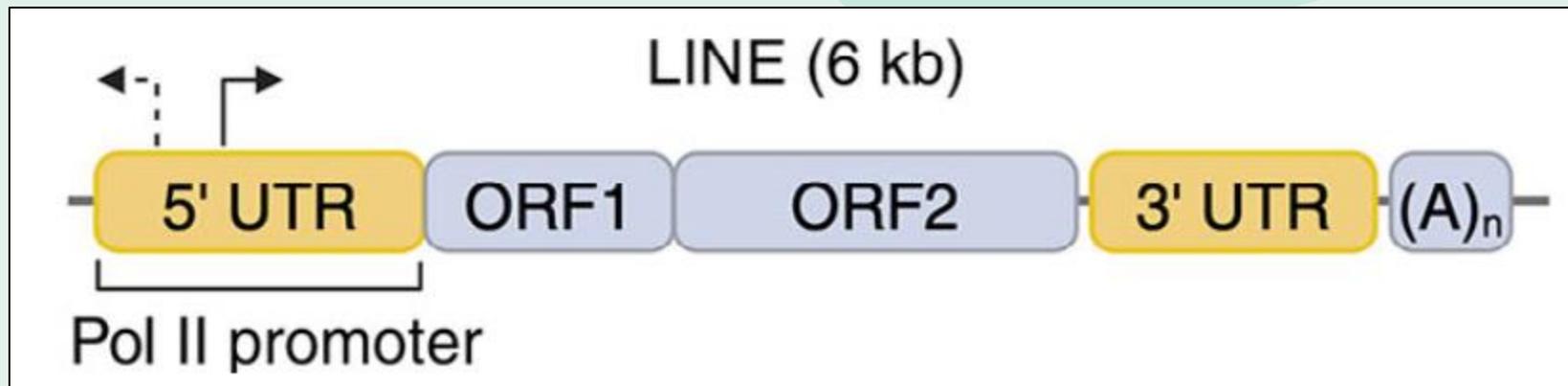


- ❖ They constitute nearly **18%** of the human genome sequence.
- ❖ During evolution, most TEs copies accumulate mutations and become **inactive**.
- ❖ In the human genome, only **80–100 copies** are still **active**.

Something about LINE-1

Characteristics of LINE1s sequences:

- They are 6 kilobases long
- They have 5' UTR containing a Pol II promoter
- They have **two ORFs** coding for:
 - ORF1p: 40 kDa RNA-binding protein which facilitates RNA chaperone activity
 - ORF2p: 150 kDa protein with **endonuclease and reverse transcriptase activity**
- They have 3' UTR with containing transcription termination and polyadenylation sites



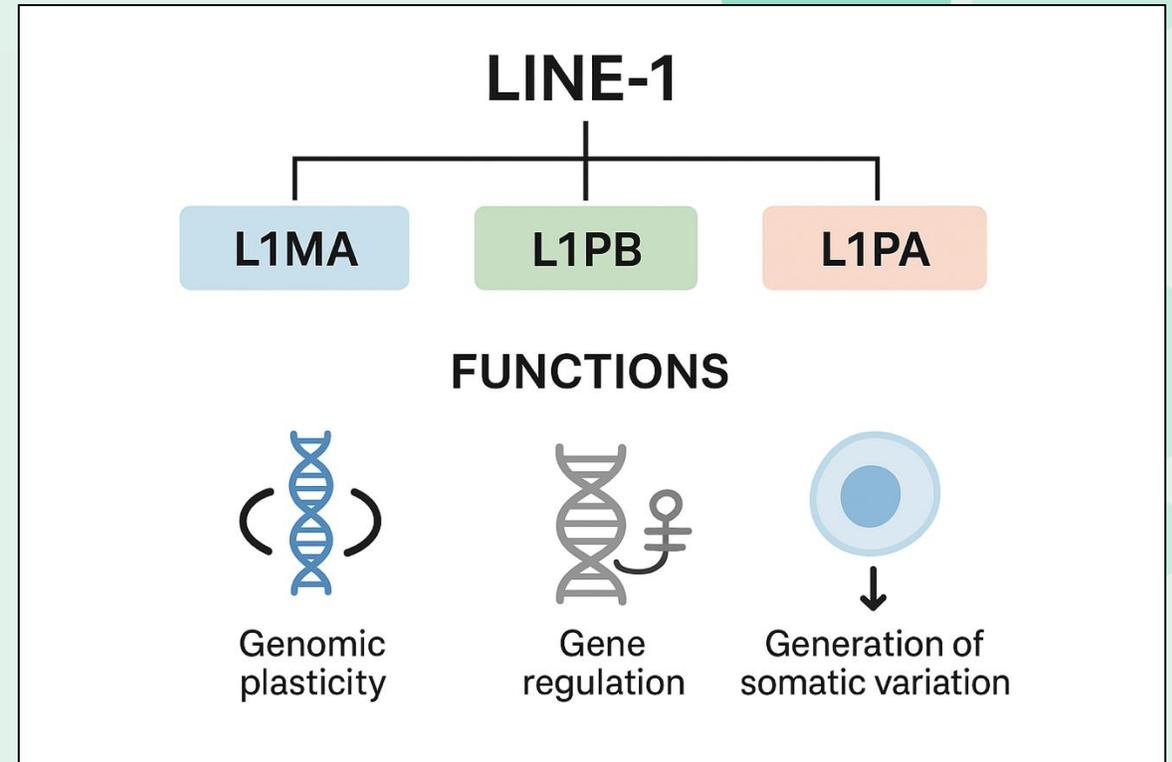
Something about LINE-1

LINE1s Families:

LINE-1s are highly different between their own.

They are divided into **subfamilies** based on several factors:

- sequence similarity
- regulatory components (5'-UTR)
- evolutionary time
- retrotranspositional activity
- abundance
- potential function



L1HS is the only active in the human genome

Something about LINE-1

LINE1s Paradox:

- *Despite the presence of multiple pathways to repress their transcription, L1s are indeed expressed independently of their retrotransposition activity*

L1 activity must be suppressed to avoid:

- genomic instability
- onset of mutations
- replicative stress

Repressed through:

- DNA methylation (CpG)
- epigenetic regulation (H3K9me3/HP1)
- piRNA (especially in germ cells)

Repressed during:

- Late gametogenesis
- Late fetal development
- Ageing

Something about LINE-1

LINE1s Paradox:

- *Despite the presence of multiple pathways to repress their transcription, L1s are indeed expressed independently of their retrotransposition activity*

L1 elements need to be active
(or re-activated) during:

- Early embryonic development
- Germ cells reprogramming
- Neurogenesis
- Response to specific cellular stress

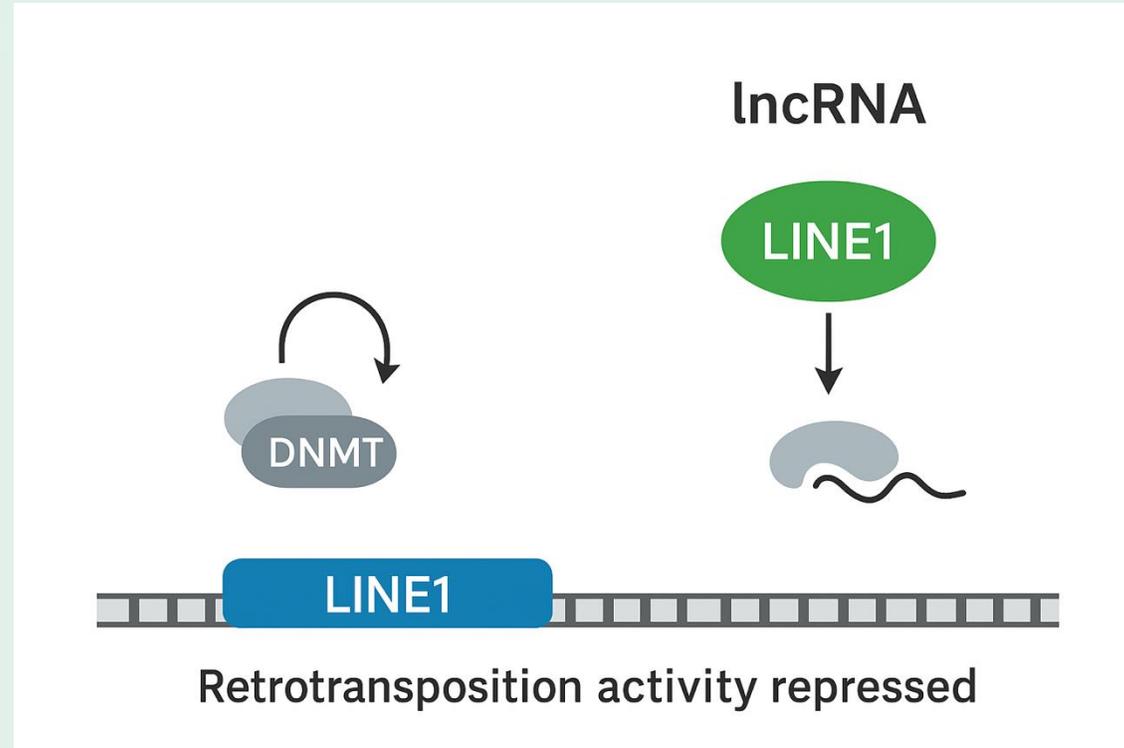
Through:

- DNA Demethylation (CpG)
- Loss of epigenetic repression mechanisms
- Reduction of piRNA pathway



LINE1s Paradox:

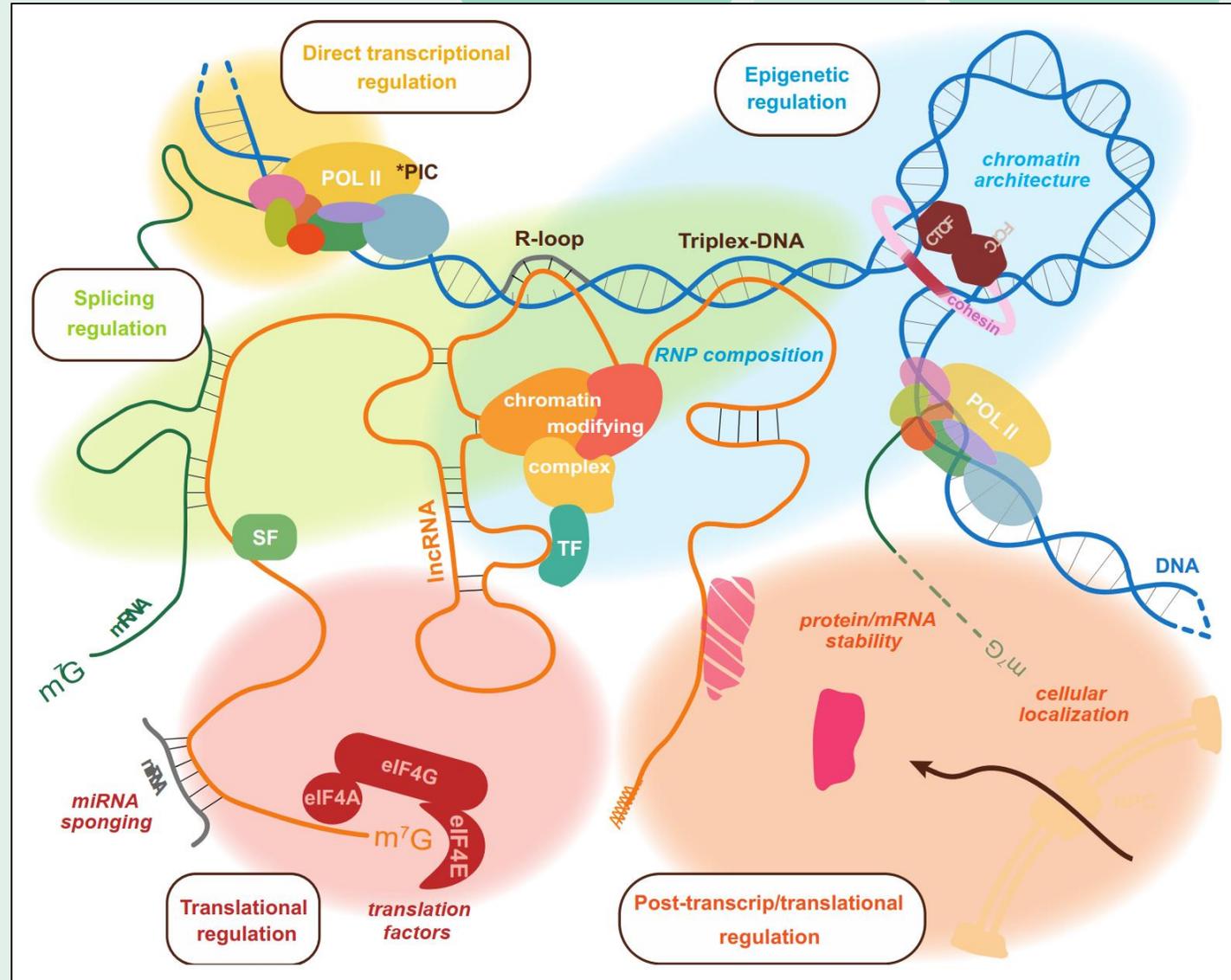
Something about LINE-1



Although the genomic sequence of L1 is repressed, the RNA is still produced, which takes on a regulatory function, typical of lncRNA



More about lncRNAs



More about lncRNAs

There are several ways through which lncRNAs can influence gene expression:

Direct transcriptional control

- Modifying Chromatin
- Forming RNA-DNA Interactions
- Influencing Splicing Factor Activity
- Forming RNA-RNA Hybrids

Epigenetic control of transcription

- Associating with chromatin-modifying complexes
- Modulating spatial chromatin organization

Translational control

- By miRNA Sponging or as Competing Endogenous RNAs (ceRNAs)
- Controlling the subcellular localization of mRNAs
- Regulating translation factors

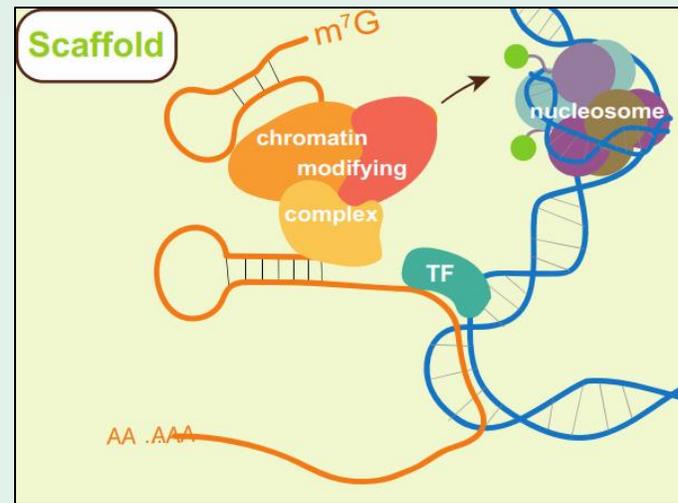
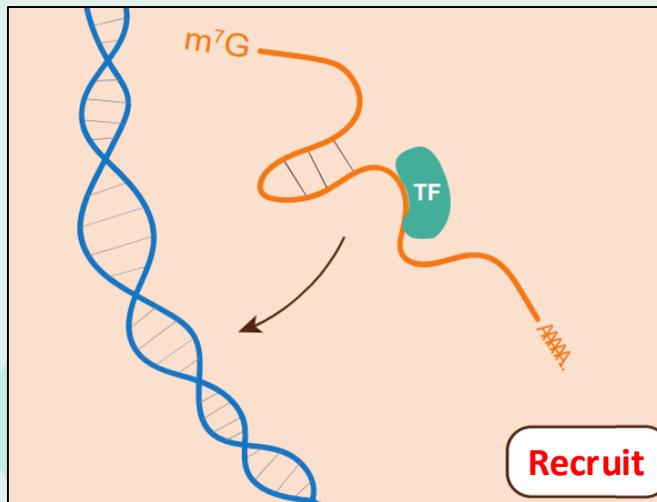
Post-Transcriptional and Post-Translational Control

- Regulating mRNA stability
- Regulating protein stability
- Controlling the subcellular localization of proteins

More about lncRNAs

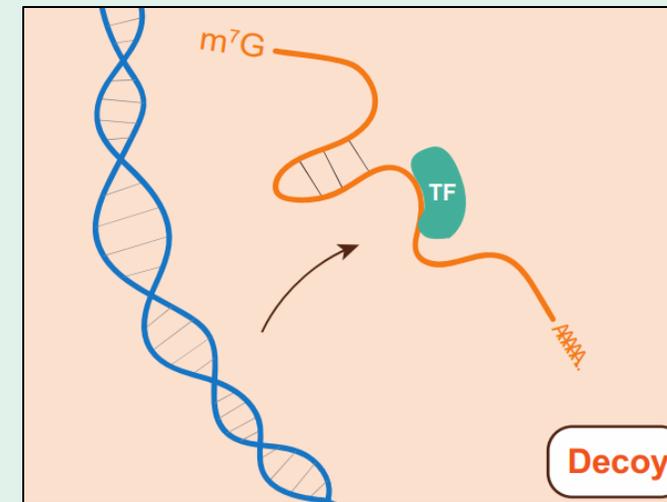
Considering the function of LINE-1 as lncRNA, they act as:

- 1) **Recruit target protein** to regulatory gene sequences



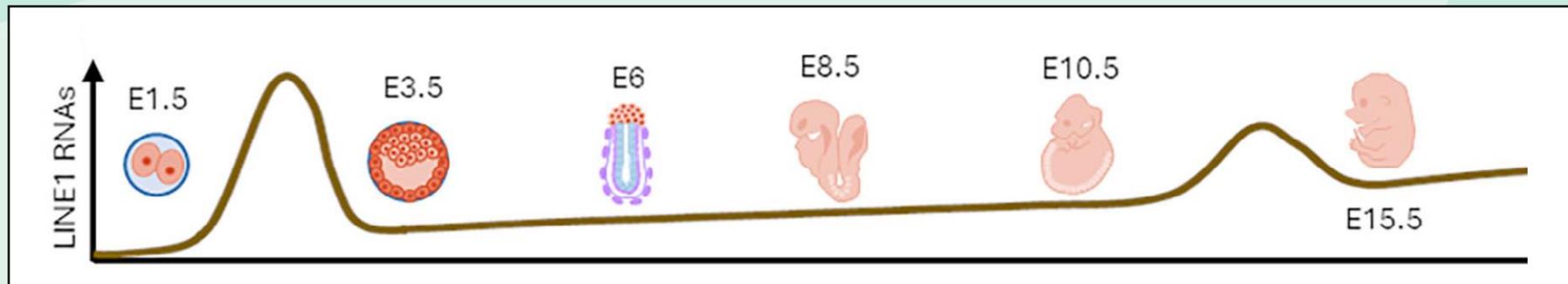
- 2) **Molecular scaffolds for:**
 - assembling protein complexes
 - concentrating proteins in specific physicochemical compartments

- 3) **Decoys**, to prevent proteins from binding to their target DNA sequence



LINE-1 expression's during development

The expression of L1 RNAs is crucial for proper mouse embryogenesis, not with his retrotransposition activity, but acting as **regulatory lncRNA**



- ❖ Promote the transition from totipotency to pluripotency
- ❖ Promote genome stabilisation
- ❖ Avoid genomic instability
- ❖ Promote blastocyst formation

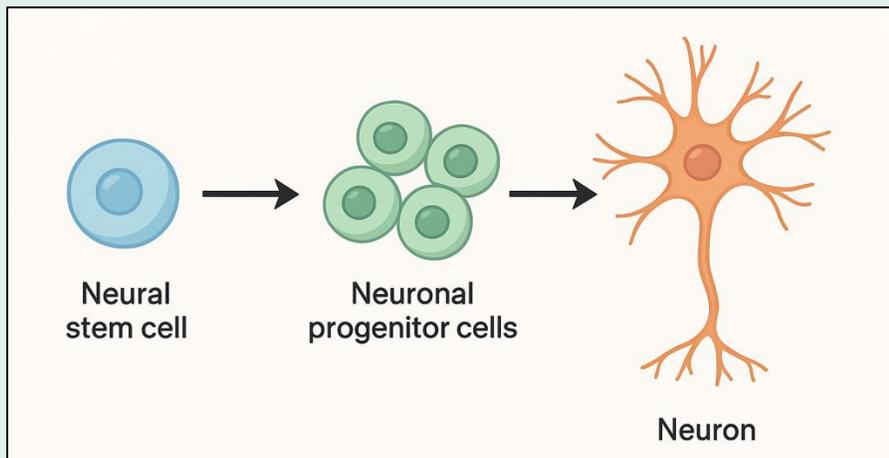
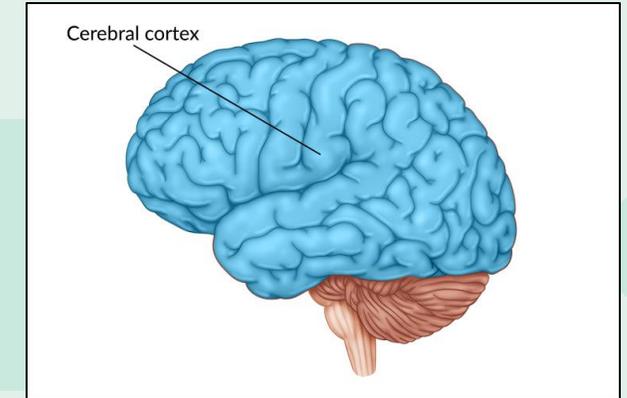
- ❖ Embryonic tissues enter a phase of proliferation and differentiation

Cortical development

The cerebral cortex is a brain structure that is responsible for higher-order **cognitive functions**. Its development is **highly dynamic** and complex. This process includes:

- change in gene expression
- signaling pathway activation
- cell migration
- Intercellular interactions

It contains hundreds of distinct cell types distributed across several anatomical and functional areas. These cells emerge from a **limited set of progenitor cell types** during early development.

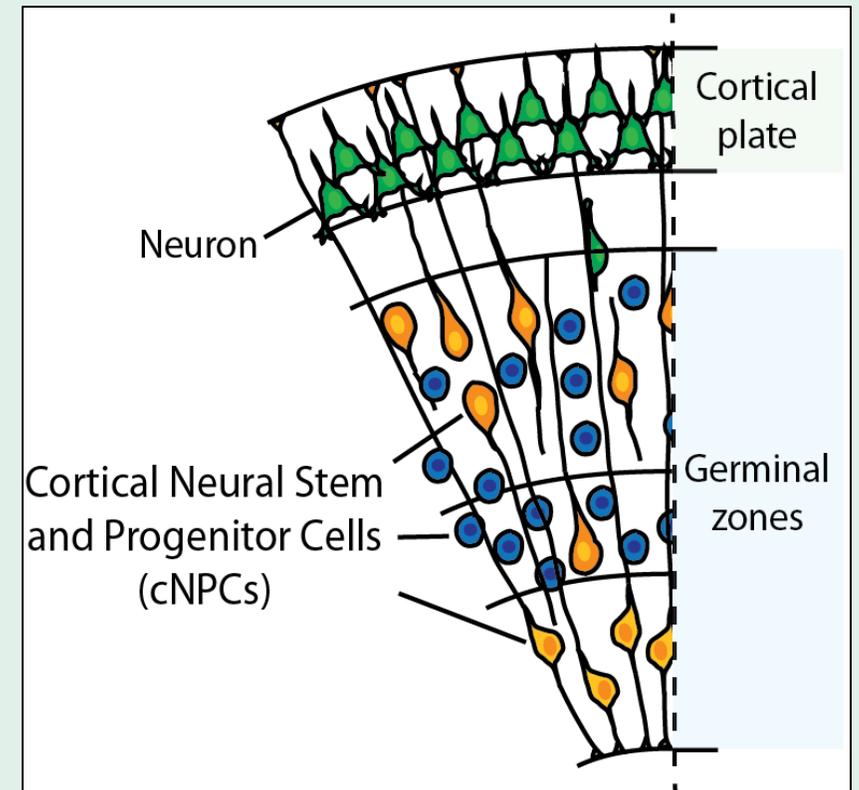


At E10 of mice neuroepithelial stem cells downregulate genes involved in tight junctions in favor of adherens junctions and give rise to radial glia. This transition is concurrent with **changes in gene expression** and the ability for radial glia to give rise to neurons.

Cortical development

Radial glia have the capacity to divide both **symmetrically** and **asymmetrically**

The differentiating daughter cell upregulates high levels of the **proneural transcription factor NGN2**. Also postmitotic neurons express high levels of **DLL1** and promote the activation of **NOTCH receptors**, which are expressed on the surface of radial glia and important for their cellular identity.



Cortical development

A final class of progenitors in the developing cortex are **intermediate progenitor cells** (IPCs). IPCs are typically **neurogenic** and divide symmetrically in the subventricular zones.

In humans a substantial fraction of IPCs can undergo **self-renewal**

Cell class	Cell type	Transcriptomic signature
Progenitors	General	NES, VIM, GFAP, SOX2, PAX6, HES1, MKI67
	NESC	GFAP ⁻ , HMGA2, CDH1, LIX1, OCLN
	vRG	HES1, GPX3, HOPX
	oRG	vRG genes + LIFR, FGFR3, PTPRZ1, PTN, FAM107A, TNC, CD38, high HOPX
	tRG	vRG genes + CRYAB, PALLD, NFATC2, HOPX ⁻
	IPC	EOMES, NEUROD4
	GPC	EGFR, ASCL1, THY1, PDGFR1a ⁻
	qNSC	MKI67 ⁻ , MEX3A
Neurons	General	NEUN, TUJ1
	Migrating newborn	DCX
	Mature	MAPT
Excitatory neurons	General	NEUROD2, NEUROD6
	Subplate	TLE4, NR4A1, CTGF, CRYM, CPLX3
	Deep layer	CTIP2, TBR1, FEZF2, FOXP2, SOX5
	Upper layer	SATB2, CUX2, CUX1, MEFC2, NEFM, POU3F2

The link between LINE-1 and cortical development

LINE-1 retrotransposition activity can be found:

- Germ line → *estimated frequency*: Approximately 1 new LINE1 insertion per 20–200 births in men.
- **Brain** → *estimated frequency*: Approximately 0.2–1 new LINE1 insertion per neuron

Somatic L1 retrotransposition activity, in the brain, have been reported in **neural progenitor cells** (NPCs) and **post-mitotic neurons**. These events lead to **intra-individual genetic variation** and **mosaicism** of neuronal genomes.

Dysregulation of L1s has been found in several neurological conditions as well as in cancer

Methods

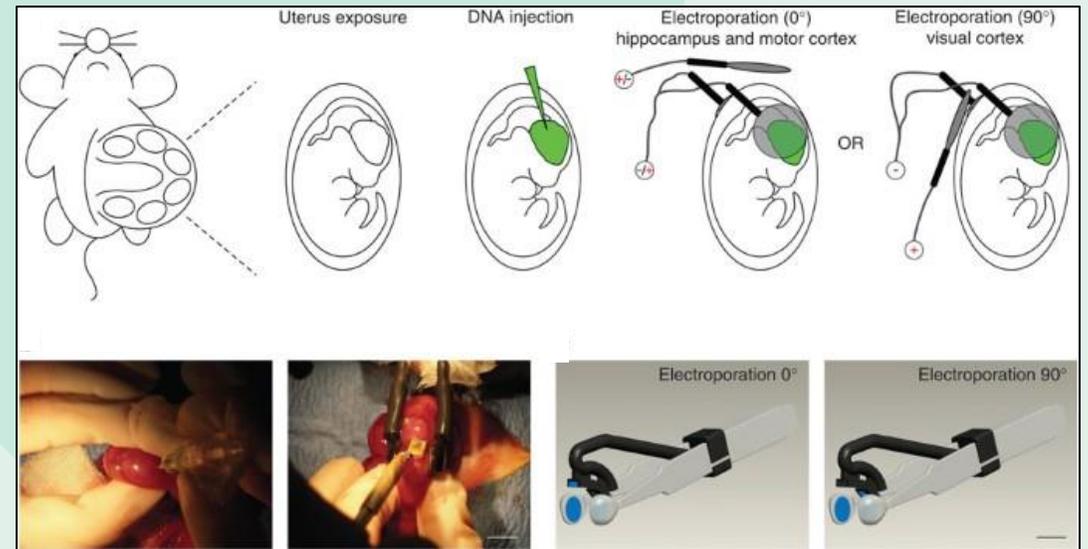
During the paper, various methods will be used, including:

1. In utero electroporation
2. FACS-sorting of GFP⁺
3. Immunofluorescence and imaging
4. RNA purification and RT-qPCR
5. Bisulfite sequencing
6. Chromatin immunoprecipitation
7. RNA immunoprecipitation
8. Subcellular fraction and RNA localization
9. MuSiC analysis
10. Computational characterization of L1- interaction
11. Bioinformatic analysis

Methods

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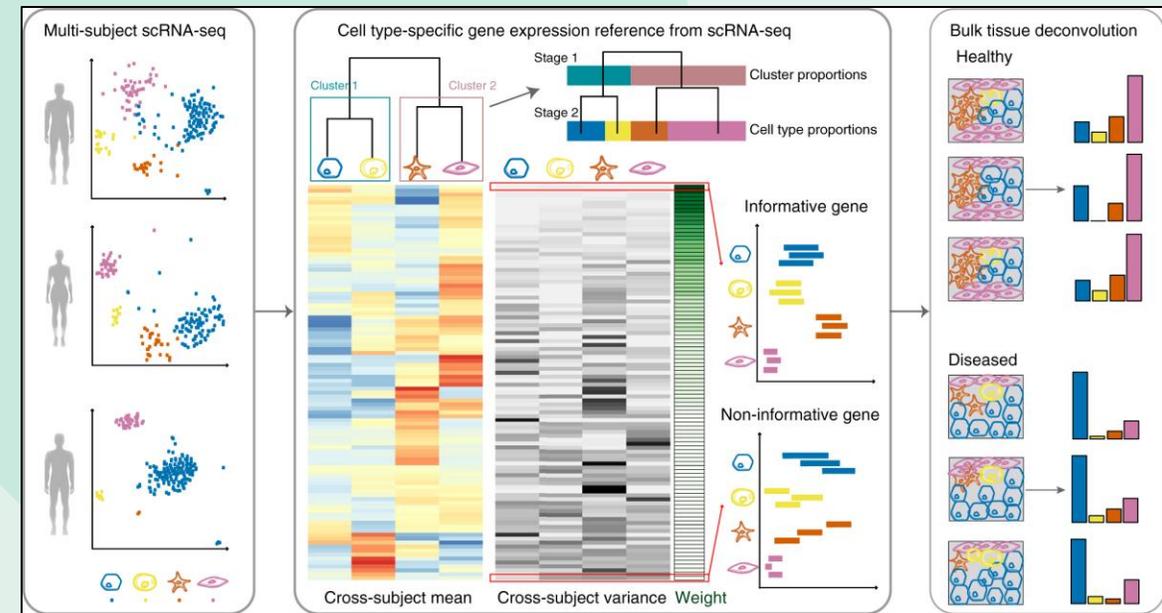
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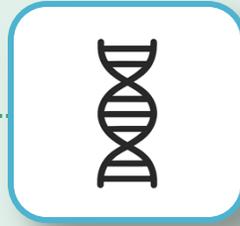
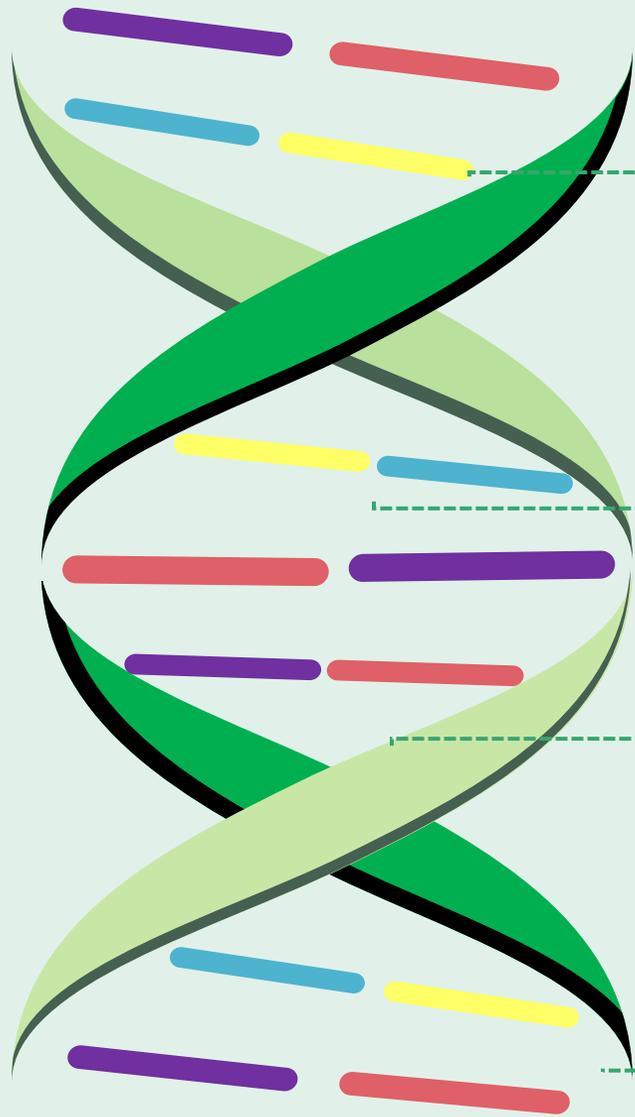


Methods

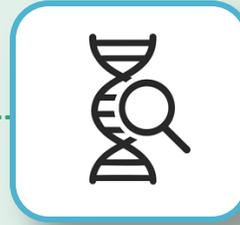
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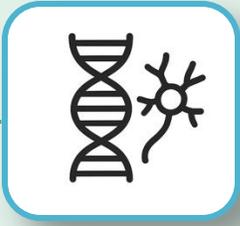




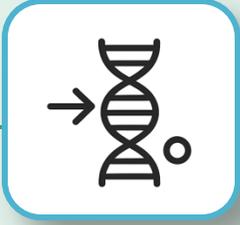
INTRODUCTION



EXPERIMENT



CONCLUSIONS



FUTURE OUTLOOK

LINE-1 regulates cortical development by acting as long non-coding RNAs

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Damiano Mangoni ¹, Alessandro Simi ¹, Pierre Lau¹, Alexandros Armaos¹, Federico Ansaloni¹, Azzurra Codino ¹, Devid Damiani¹, Lavinia Floreani², Valerio Di Carlo ¹, Diego Vozzi¹, Francesca Persichetti³, Claudio Santoro³, Luca Pandolfini ¹, Gian Gaetano Tartaglia ¹, Remo Sanges^{1,2}  & Stefano Gustincich ¹ 

Long Interspersed Nuclear Elements-1s (L1s) are transposable elements that constitute most of the genome's transcriptional output yet have still largely unknown functions. Here we show that L1s are required for proper mouse brain corticogenesis operating as regulatory long non-coding RNAs. They contribute to the regulation of the balance between neuronal progenitors and differentiation, the migration of post-mitotic neurons and the proportions of different cell types. In cortical cultured neurons, L1 RNAs are mainly associated to chromatin and interact with the Polycomb Repressive Complex 2 (PRC2) protein subunits *enhancer of Zeste homolog 2* (Ezh2) and *suppressor of zeste 12* (Suz12). L1 RNA silencing influences PRC2's ability to bind a portion of its targets and the deposition of tri-methylated histone H3 (H3K27me3) marks. Our results position L1 RNAs as crucial signalling hubs for genome-wide chromatin remodelling, enabling the fine-tuning of gene expression during brain development and evolution.

5 Questions

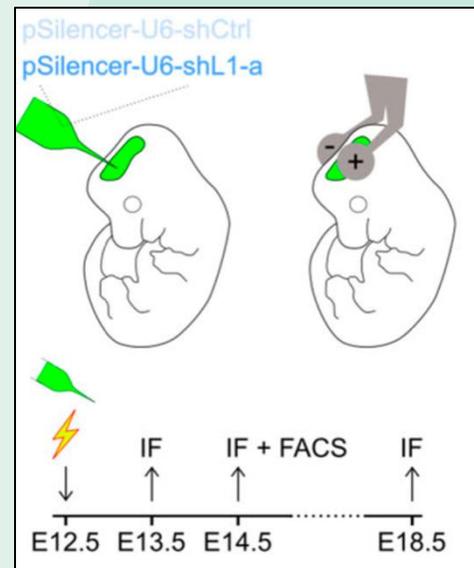
1. *Is LINE-1 RNA required for proper cortical neurogenesis during embryonic development in vivo?*
2. *Does LINE-1 RNA regulate proliferation, differentiation, and maturation of cortical neurons in vitro?*
3. *Are LINE-1 RNAs associated with chromatin, and do they modulate the deposition of the epigenetic mark H3K27me3?*
4. *Do LINE-1 RNAs directly interact with the PRC2 complex?*
5. *Is LINE-1 RNA required to regulate Ezh2 catalytic activity and its targeting to specific genes?*

1. Is LINE-1 RNA required for proper cortical neurogenesis during embryonic development in vivo?

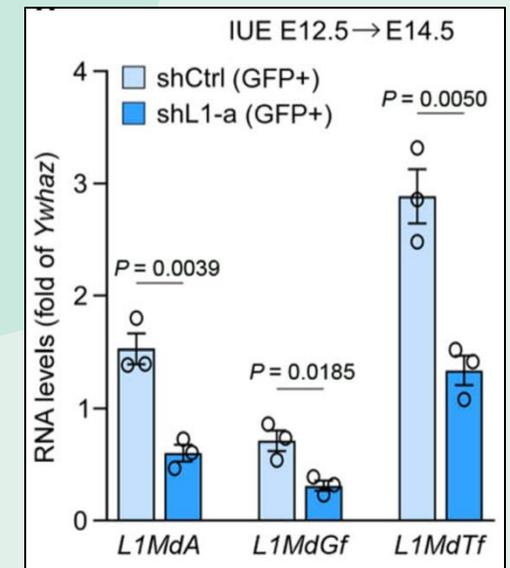
3 subfamilies of L1 was characterized during mouse brain corticogenesis:

1. L1MdA
2. L1MdGf
3. L1MdTf

To study the function of those, they did L1 RNA silencing

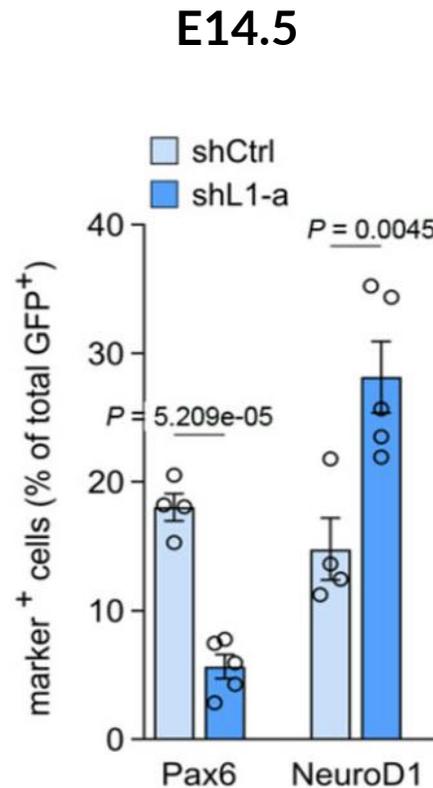
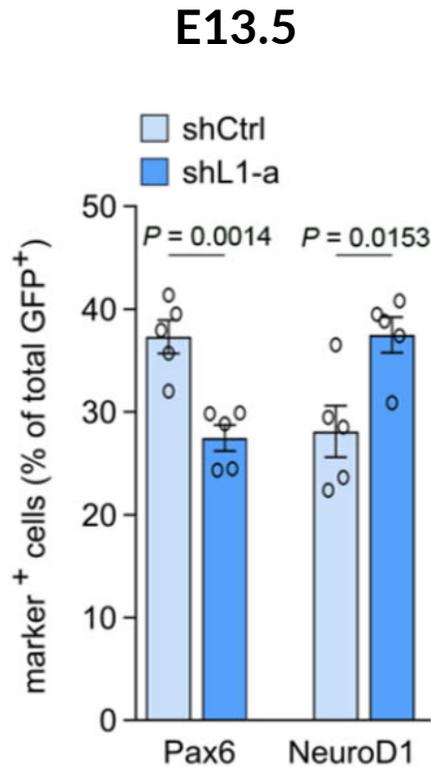


Thanks to RT-qPCR, we can observe the level of L1 transcripts (at different days)



1. Is LINE-1 RNA required for proper cortical neurogenesis during embryonic development in vivo?

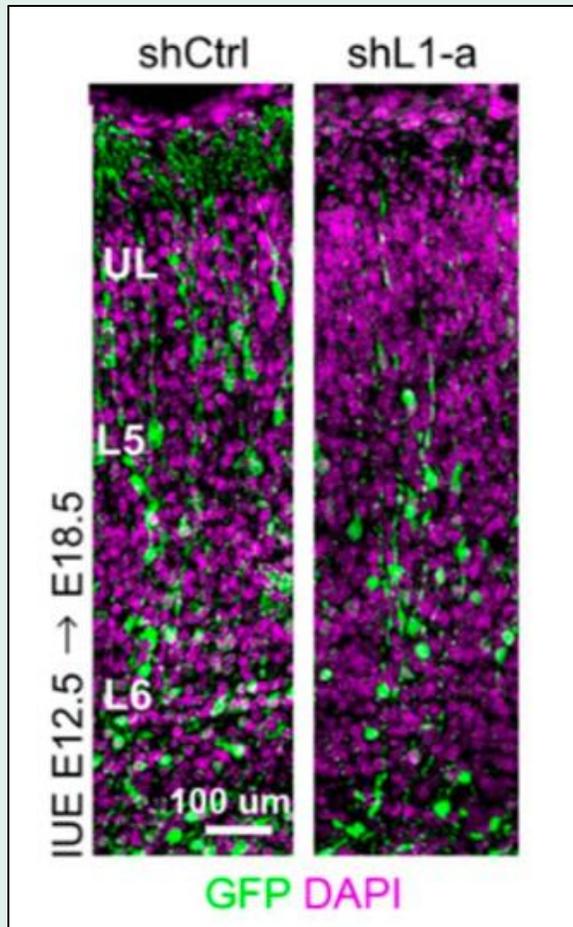
How do L1 RNAs silencing change the proportion of different cell populations in the cortex ?



L1 silencing increases the neurogenic marker (*NeuroD1*) and decreases the progenitor marker (*Pax6*).

This is because progenitors exit the cell cycle earlier in order to differentiate

1. Is LINE-1 RNA required for proper cortical neurogenesis during embryonic development in vivo?



With another progenitor marker (Trb2+) they saw that it didn't disappear but **accumulates in VZ and SVZ zone**.

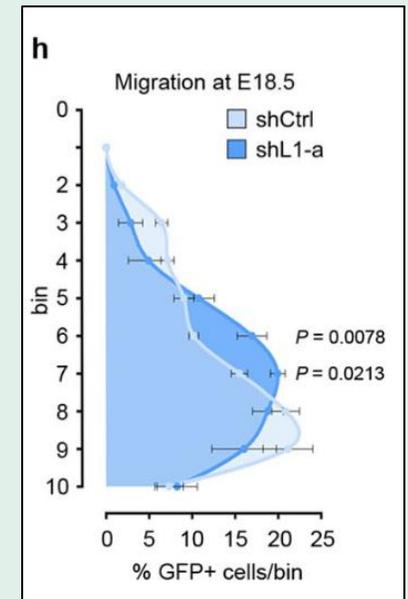
This suggest that the silencing of **L1 is involved also in migration of cells**.

To test this hypothesis, they did another experiment:

Other markers for deep-layer neurons (Trb1+, Ctip2+ and other) decrease with L1 silencing.

Neurons are blocked in the deep layer of the neocortex, they aren't able to migrate to the superficial layer

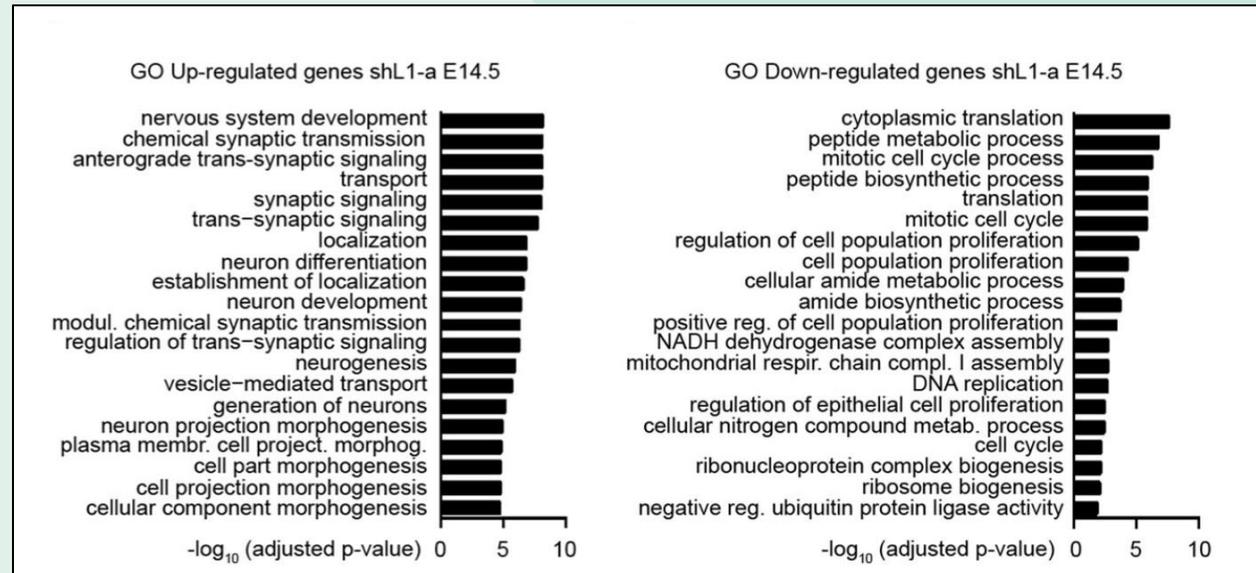
L1 RNA is necessary for maintaining the correct progenitor / neuron balance and for cortical migration during development



1. Is LINE-1 RNA required for proper cortical neurogenesis during embryonic development in vivo?

Authors study the molecular pathway altered by the silencing. They found:

1. **Upregulated** 3480 genes, involved in: nervous system development, axogenesis and projection development
2. **Downregulated** 2705 genes, involved in: cell metabolism, mitochondrial activity, ribosome assembly and cell division



1. Is LINE-1 RNA required for proper cortical neurogenesis during embryonic development in vivo?

YES! We have seen that silencing L1 RNA in the embryonic cortex alters normal neurogenesis:

1. Increasing the number of NeuroD2+ neurogenic cells
2. Reducing the number of Tbr2+ intermediate progenitors and deep-layer neurons (Tbr1+, Ctip2+)
3. Causing an abnormal accumulation of progenitors and neurons in deeper cortical layers
4. Impairing radial migration of newborn neurons.

L1 RNA is required to maintain correct progenitor dynamics and cortical layering.

2. Does LINE-1 RNA regulate proliferation, differentiation, and maturation of cortical neurons in vitro?

They isolated a cells culture from E17.5 embryonic cerebral cortex and cultured them for 21days, and discover:

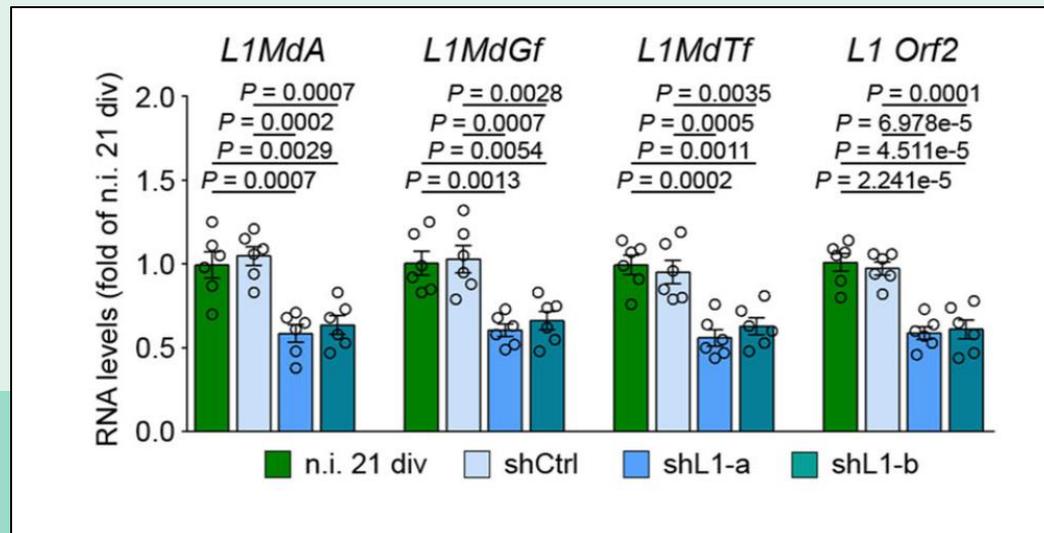
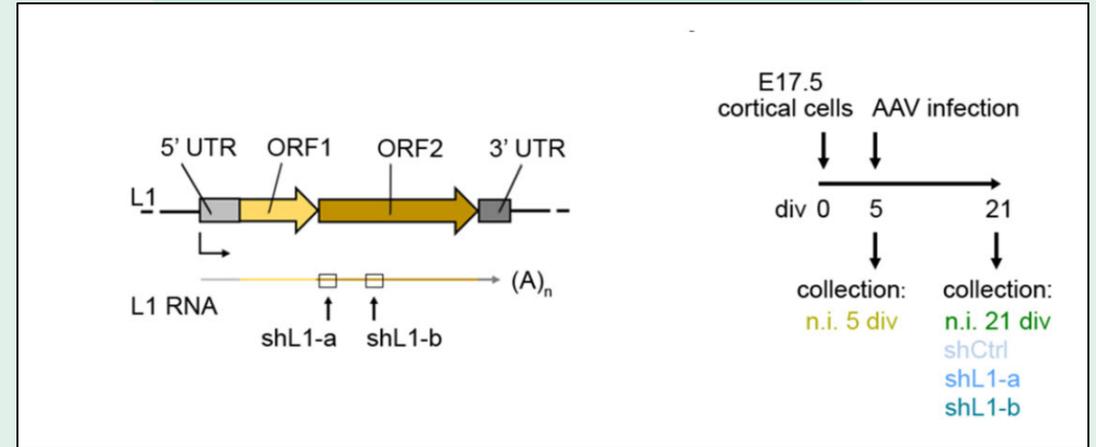
1. L1MdA, L1MdGf, L1MdTf have a **higher transcript level** during the early to the maturation stage, reaching 2-fold upregulation at 21div.
2. The **decrease of the deposition of the H3K9me3** on the L1 promoters

Hypothesis: Neuronal maturation in vitro is associated with increased expression of L1RNAs, due to epigenetic regulator of their promoter.

2. Does LINE-1 RNA regulate proliferation, differentiation, and maturation of cortical neurons in vitro?

To test the hypothesis, they transfected (at the 5 div) the cell culture with Adeno-virus associated expressing shL1a or shL1b, both shRNA targeting the Orf2 of L1.

At the 21 div they examined it.



With a RT-qPCR they found a reduction of the 3 subfamilies of L1 and the conserved sequence of L1 Orf2.

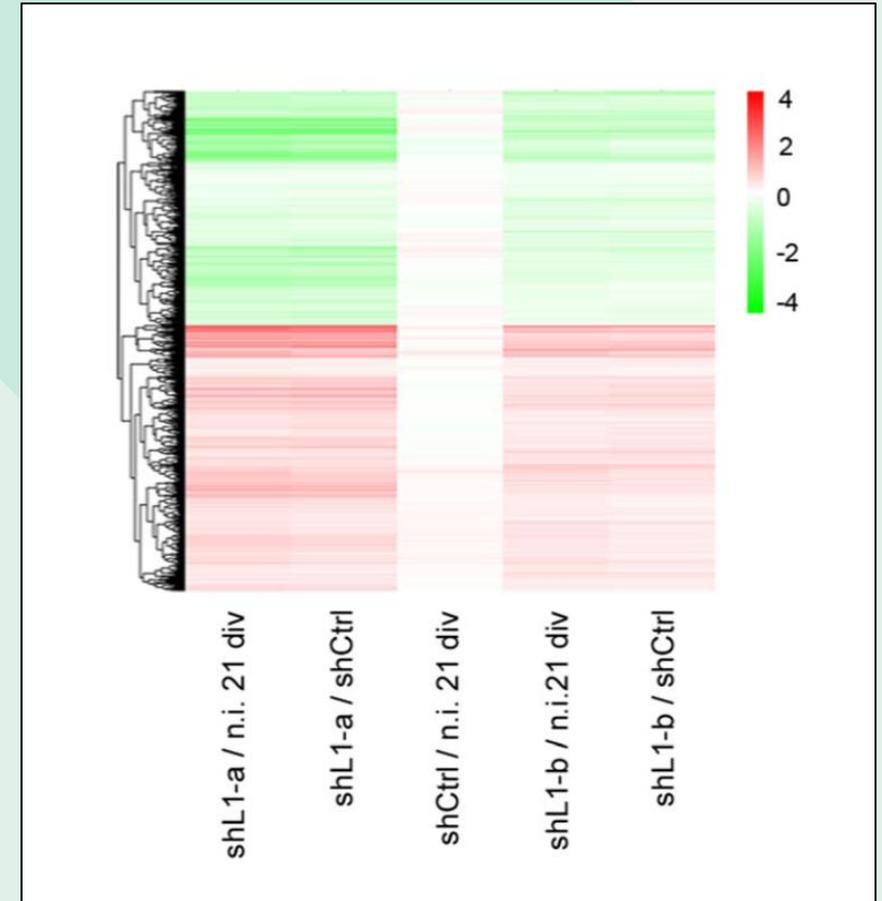
We can see that the shL1a have a better KO efficiency (45%) than shL1b (35%)

2. Does LINE-1 RNA regulate proliferation, differentiation, and maturation of cortical neurons in vitro?

Both shRNA have influenced gene expression:

1. **shL1a** → upregulated gene: 2968 & downregulated gene: 3933
2. **shL1b** → upregulated gene: 4758 & downregulated gene: 2958

Comparing these all gene, they found that both shRNA have 3028 **DEG** (dysregulated expression of gene) → upregulated gene: 1601 & downregulated gene: 1422

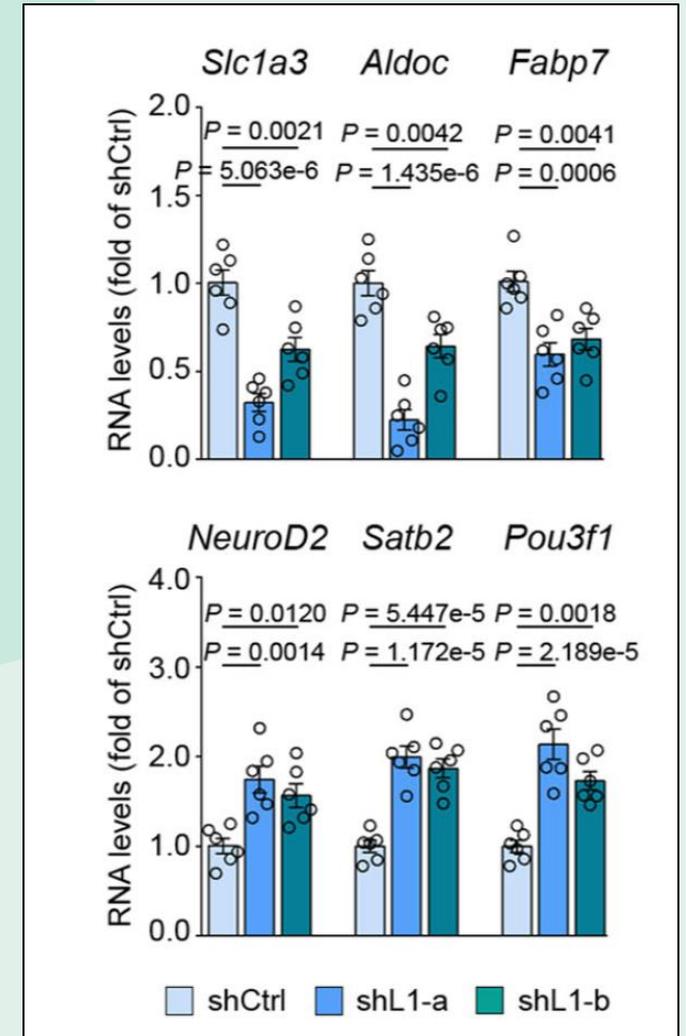


2. Does LINE-1 RNA regulate proliferation, differentiation, and maturation of cortical neurons in vitro?

The L1 silencing can change the cell type composition of cortical cultures?

Thanks to MuSiC analysis, they discover some markers whose expression change with shL1a, these marker are corresponding of 2 cell cluster:

1. **Astrocytes immature** (*Slc1a3*, *Aldoc*, *Fabp7*) that **decrease** in shL1-treated sample
2. **Upper-layer late-born neurons** (*NeuroD2*, *Satb2*, *Pou3f1*) that **increase** shL1-treated sample



2. Does LINE-1 RNA regulate proliferation, differentiation, and maturation of cortical neurons in vitro?

YES! In vitro L1 silencing causes:

1. Reduced proliferation of neural progenitors
2. Impaired neuronal differentiation
3. Defective maturation

L1 RNA supports cortical neuron development and maturation also in vitro.

3. Are LINE-1 RNAs associated with chromatin, and do they modulate the deposition of the epigenetic mark H3K27me3?

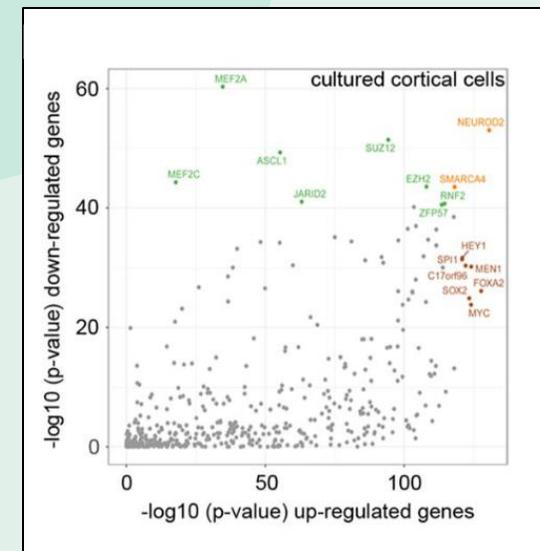
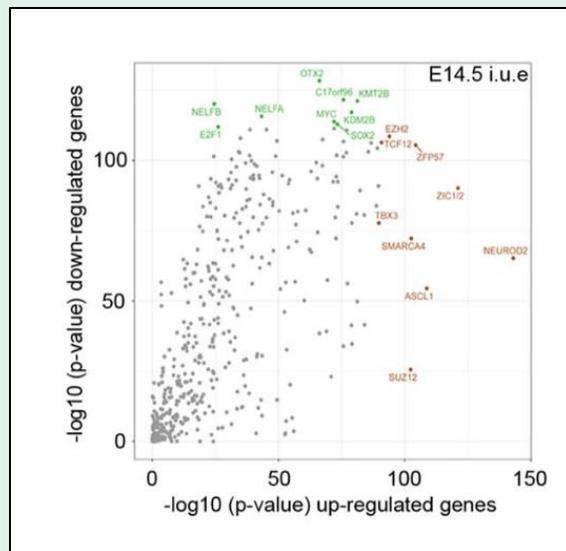
They studies the TFs that are involved in the regulation of DEGs in E14.5 cortical cells in vivo, and in cortical cells in vitro.

1. In vivo at E14.5 the TFBSs are enriched for:

- pluripotency markers
- lysine methyltransferases e demethylases
- neurogenic transcription factors
- components of PRC2

2. In cultured neurons the TFBSs of DEGs are enriched for:

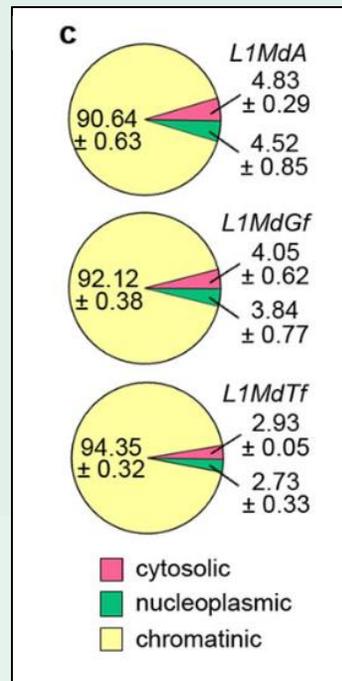
- Post-mitotic neurons markers
- Neuronal progenitor markers
- components of PRC2



3. Are LINE-1 RNAs associated with chromatin, and do they modulate the deposition of the epigenetic mark H3K27me3?

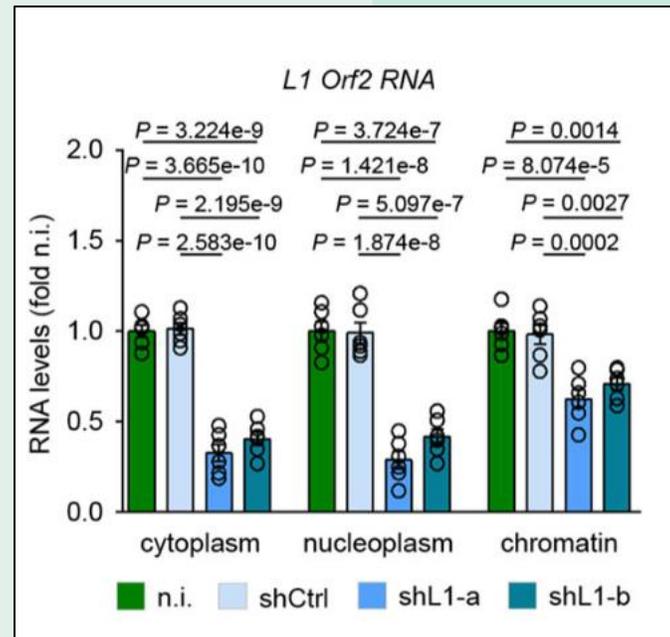
To test the association between L1 and the chromatin, they took the cell culture (21div) and they saw the localization of the L1 RNA.

For all 3 subfamilies of L1 RNAs, the vast majority of L1 transcript were chromatin associated



Then test the ability of shL1a and shL1b to reduce the amount of chromatin bound L1 transcripts.

1. Cytosolic and nucleoplasmic L1 RNA were both decreased of 60-70%
2. Chromatin L1 RNAs decreased by 30-40%

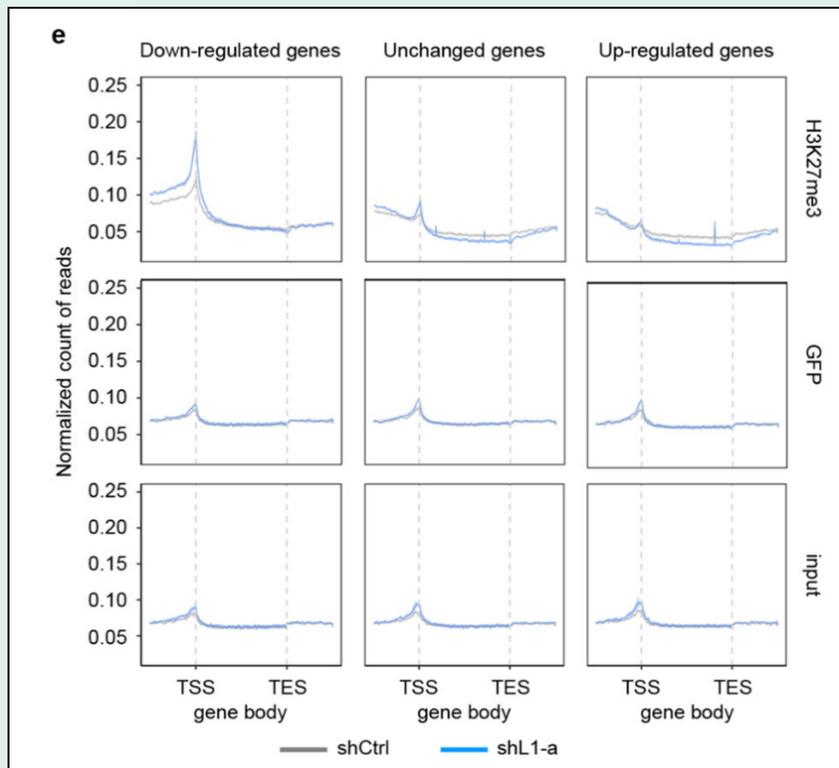


Chromatin transcripts are more difficult to degrade because they are associated with remodeling complexes

3. Are LINE-1 RNAs associated with chromatin, and do they modulate the deposition of the epigenetic mark H3K27me3?

In neuronal development, H3K27me3 deposition regulates cell fate transitions

The silencing of L1 RNA stops the neuronal differentiation, so we want to understand if the L1 RNA is needed for the regulation of the deposition of the H3K27me3 mark.



With the silencing of L1 transcript, they saw that there was an overall higher deposition of H3K27me3 on TSS of downregulated genes

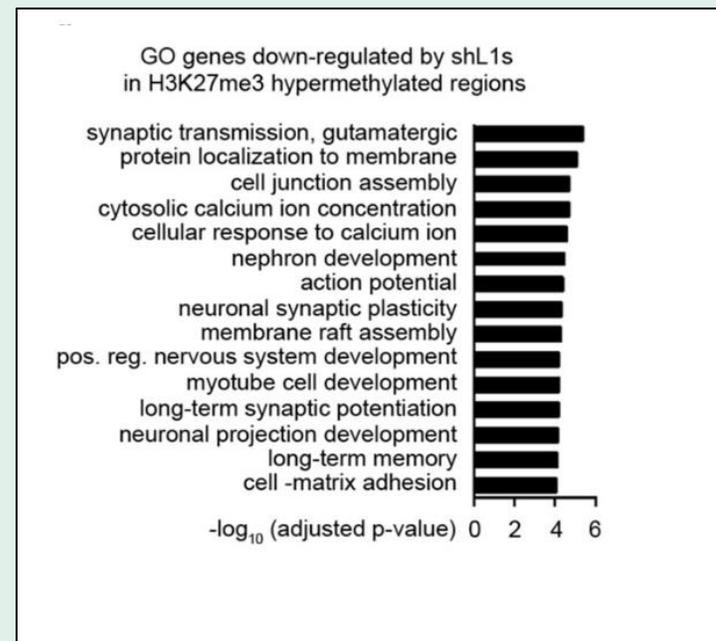
- L1 RNA limits the H3K27me3 deposition, so the progenitors can differentiate in neurons
- L1 RNA silencing increases the H3K27me3 deposition, so the progenitors can't differentiate in neurons

3. Are LINE-1 RNAs associated with chromatin, and do they modulate the deposition of the epigenetic mark H3K27me3?

They select the genes that are downregulated due to H3K27me3 hypermethylation (caused by L1 silencing) and on this pool they performed a Gene Ontology analysis.

The results was an enrichment for biological process related to neuronal cells.

Confirming that differentiation can not happen



3. Are LINE-1 RNAs associated with chromatin, and do they modulate the deposition of the epigenetic mark H3K27me3?

YES! Fractionation experiments show that >90% of L1 transcripts are chromatin-bound.

Upon L1 silencing:

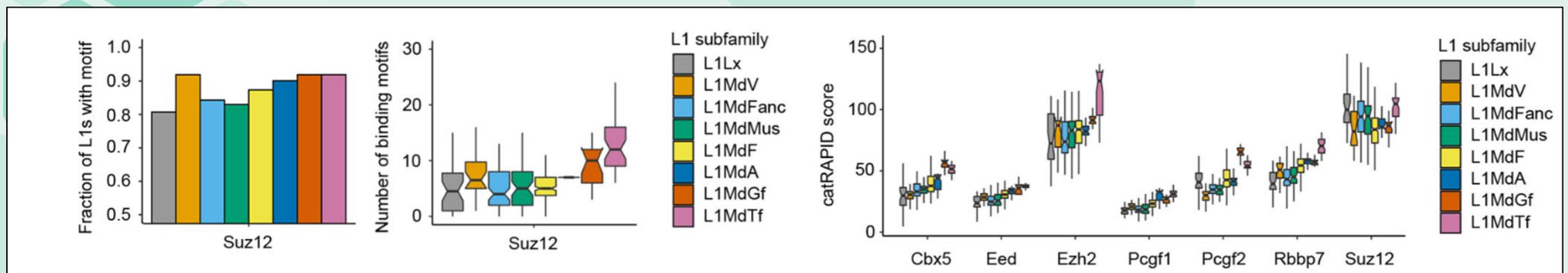
- There is a global increase in H3K27me3 deposition
- ~4600 genomic regions become hypermethylated at H3K27me3

L1 RNA helps to prevent excessive PRC2-mediated gene repression during neuronal development.

4. Do LINE-1 RNAs directly interact with the PRC2 complex?

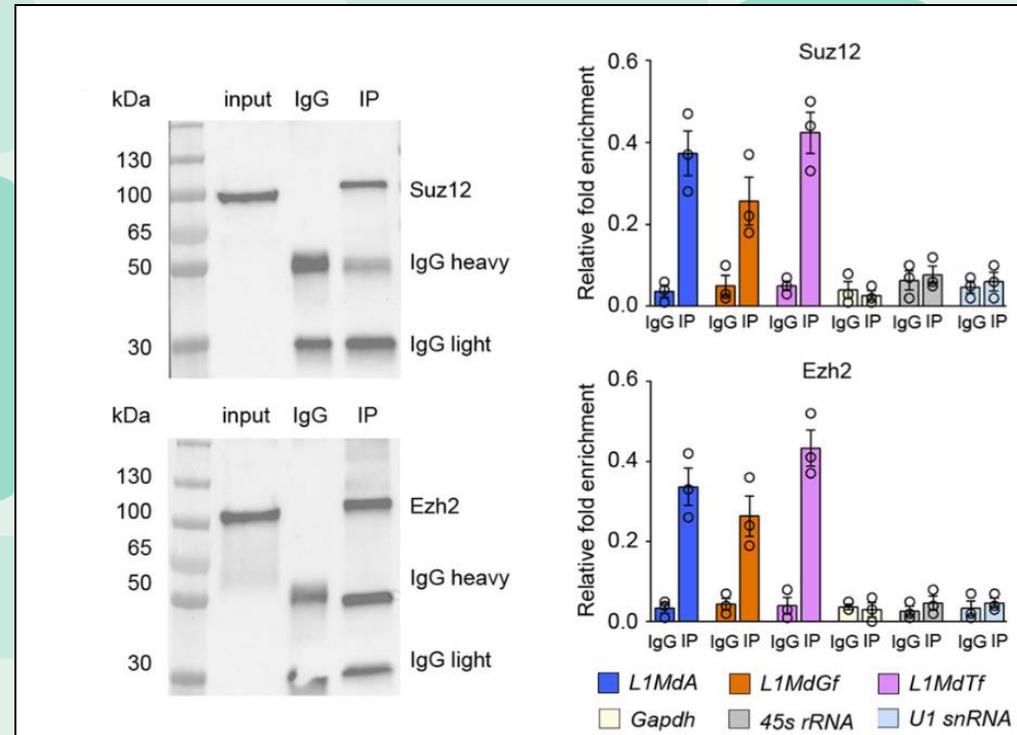
What are the protein that can interact and bind the L1 RNA?

Thanks to *catRAPID* algorithm, the authors can predict that the PRC2 components, **Suz 12** and **Ezh2**, are the protein that have the highest interaction with the **L1RNA**.



4. Do LINE-1 RNAs directly interact with the PRC2 complex?

They validated what the algorithm predicted, thanks a immunoprecipitation of the binding site of Suz12 and Ezh2 to L1RNA, in 21 div cortical cells.



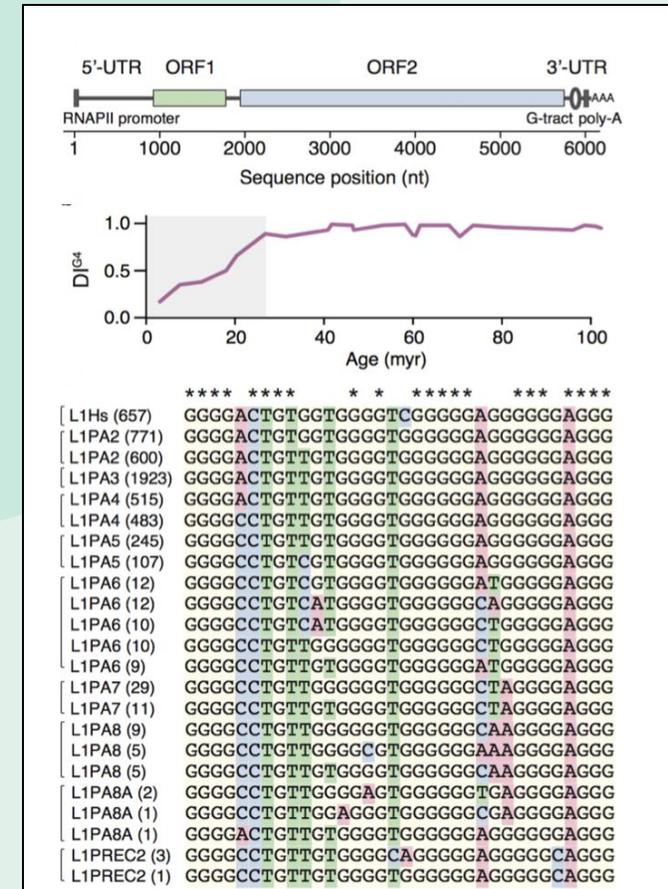
The subfamilies detected for this binding are **L1MdA**, **L1MdGf** and **L1MdTf**

4. Do LINE-1 RNAs directly interact with the PRC2 complex?

PRC2 interact preferably with G-quadruplex secondary structure and G-tracts of the L1 RNA.

This structure leading the displacement of PRC2 from nucleosomes and the depletion of H3K27me3 from genes.

To demonstrate the role of G4s in the binding of Suz12 and Ezh2 to L1RNAs, they take the binding site (discovered with the *catRAPID*) and overlapped with the predict G4 sequences il L1 subfamilies. They found a significant overlap between the binding site of Suz12 / Ezh2



4. Do LINE-1 RNAs directly interact with the PRC2 complex?

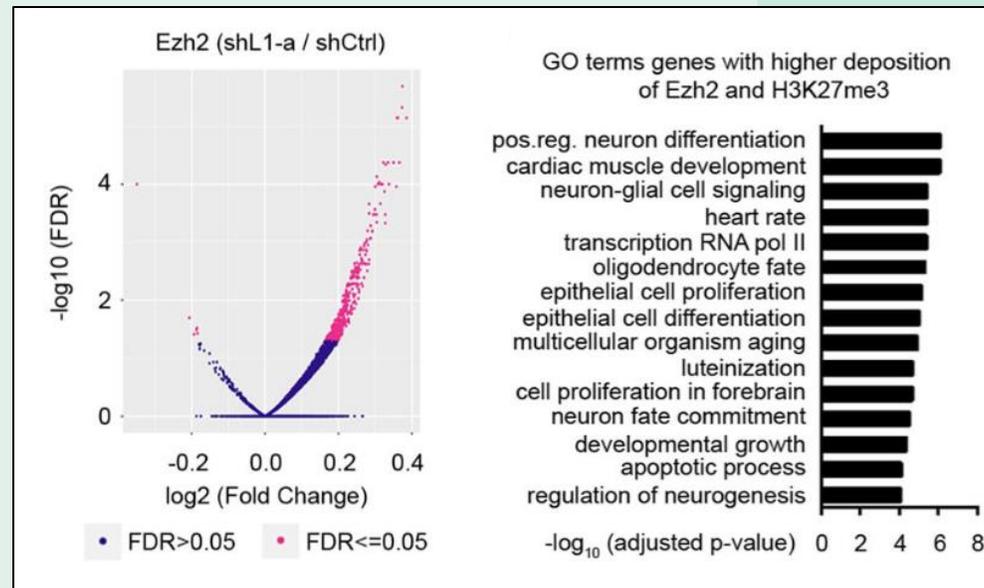
YES! RIP and biochemical assays demonstrate that:

- L1 transcripts physically bind Suz12 and Ezh2
- PRC2 has a high affinity for the structured regions of L1 RNA
- G4 structure is important for PRC2-L1 RNA interaction

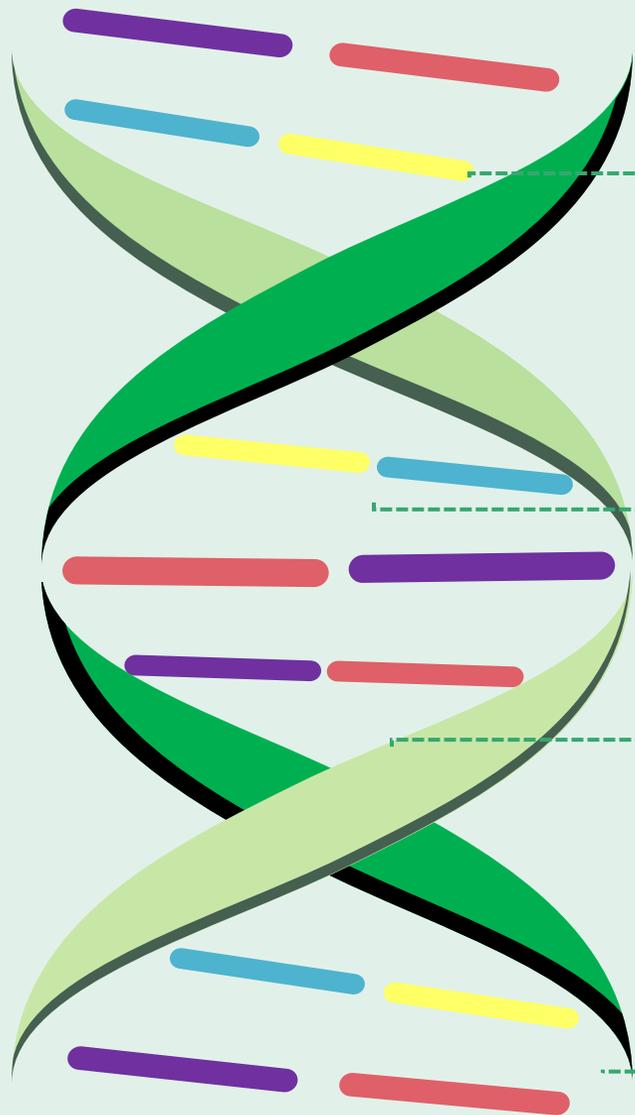
L1 RNA is a direct interactor of PRC2.

5. Is LINE-1 RNA required to regulate Ezh2 catalytic activity and its targeting to specific genes?

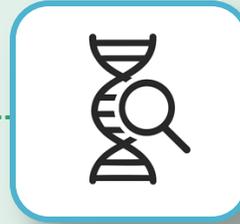
They discovered that L1 silencing cause higher binding of Ezh2 in the same regions that have H3K27me3 deposition. The regions have genes important for transcriptional regulation of neuronal, glial and oligodendrocyte lineage specification.



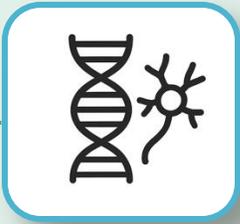
L1 RNA normally restrains Ezh2 activity, preventing excessive gene silencing.



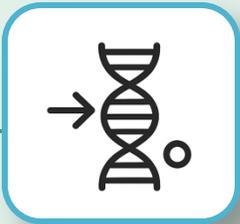
INTRODUCTION



EXPERIMENT



CONCLUSIONS



FUTURE OUTLOOK

In summary...

1. *Is LINE-1 RNA required for proper cortical neurogenesis during embryonic development in vivo?*
 - ✓ **L1 act as regulatory lncRNA to maintain the balance between proliferation and differentiation of neural progenitors.**
2. *Does LINE-1 RNA regulate proliferation, differentiation, and maturation of cortical neurons in vitro?*
 - ✓ **L1 are necessary to initiate the correct transcriptional cascade that leads to: neuronal differentiation; maturation; migration of neurons into the cortical plate**

In summary...

3. *Are LINE-1 RNAs associated with chromatin, and do they modulate the deposition of the epigenetic mark H3K27me3?*

✓ **L1 acts as lncRNA and influence the chromatin remodeling complex**

4. *Do LINE-1 RNAs directly interact with the PRC2 complex?* 5. *Is LINE-1 RNA required to regulate Ezh2 catalytic activity and its targeting to specific genes?*

✓ **L1 physically bind Ezh2 and Suz12**

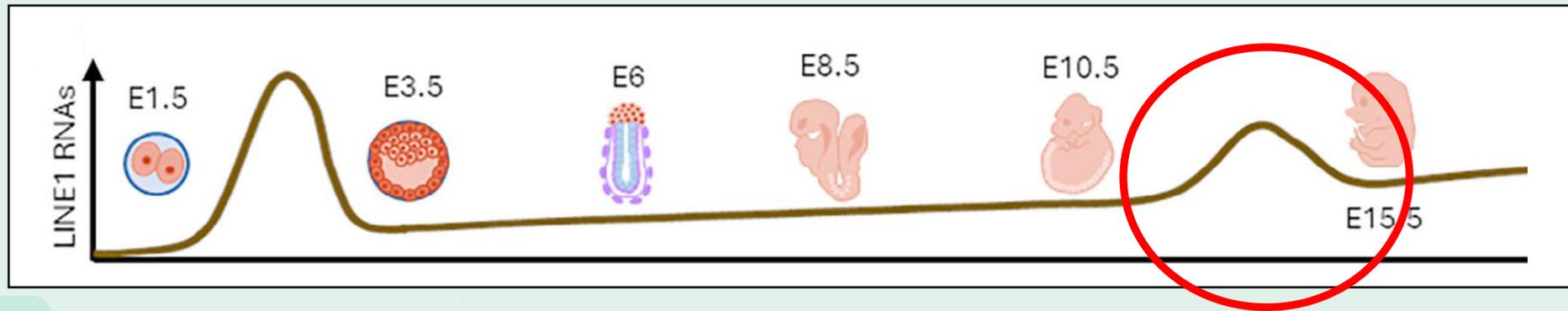
a) **When L1 is expressed → sequesters PRC2 → less repression → cell proliferate**

b) **When L1 is silenced → PRC2 return to gene → more H3K27me3 → altered differentiation**

✓ **The L1-Ezh2 bind is favored by G4 secondary structure**

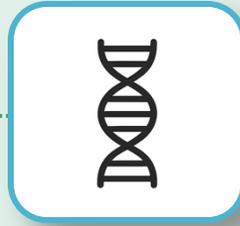
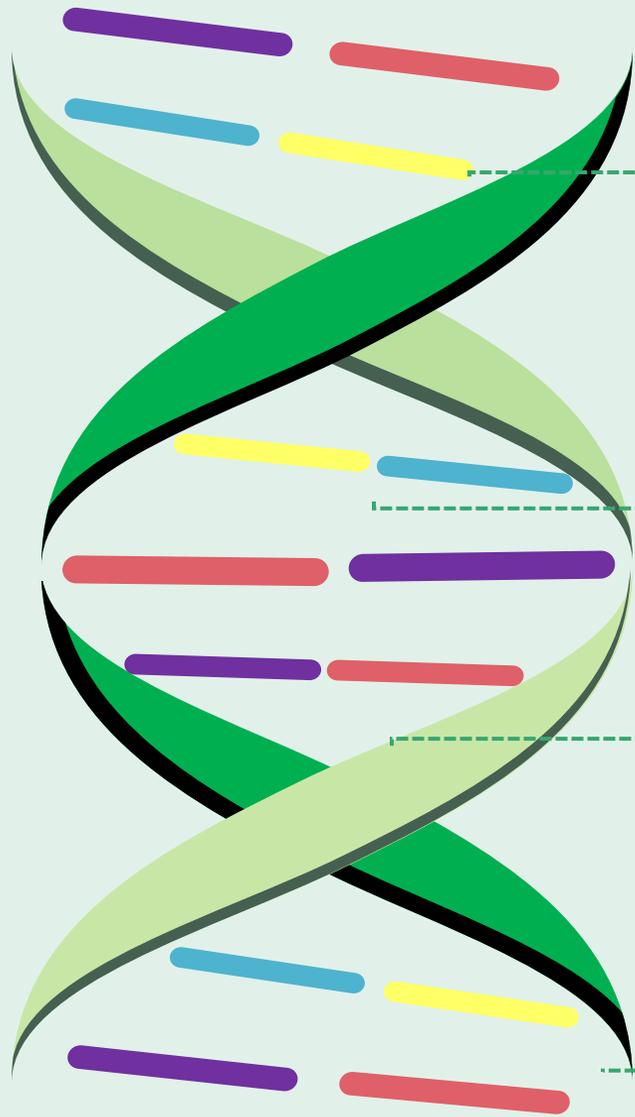
In summary...

All this, WHEN does it occur during cortical development?

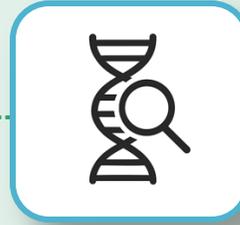


What happen between E10.5 and E15.5?

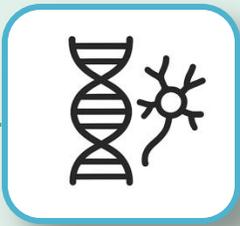
Cortical neurogenesis begins, where it is essential that progenitor cells start producing the first neurons. L1 acts as an «*epigenetic conductor*», triggering the production of neurons that populate the cortex.



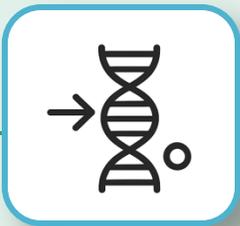
INTRODUCTION



EXPERIMENT



CONCLUSIONS



FUTURE OUTLOOK

Role of L1 in several diseases

Dysregulation of L1s has been found in several **neurological conditions**, including:

- Neurodevelopmental diseases
- Cognitive and neurodegenerative disorders
- Cancer

Increased levels of L1 retrotransposition were observed in neurons from patients affected by Rett syndrome and rare inherited encephalopathies, resulting in severe mental and physical illness.

The contribution of L1s to the etiology of these diseases remains **poorly understood**. These studies provide the basis for future clinical trials and hold promise for innovative strategies to understand better the adverse effects of retrotransposon dysregulation in neurodegenerative disorders.

Role of RNA L1 in stimulating osteogenic activity during bone repair

We can attribute two personalities to L1:

Beneficial LINE1 activity



- Tumor suppression in premalignant cells (Zhao, *et al.*, 2021).
- Tumor suppression in cancer cells via viral mimicry (Mehdipour *et al.*, 2020).



- Regulation of chromatin accessibility during embryonic development following fertilization (Jachowicz *et al.*, 2017).



- Regulation of brain corticogenesis development (Mangoni *et al.*, 2023).
- Mediation of neuronal precursor cell mosaicism (Maciai *et al.*, 2017).



- Activation of osteogenesis following bone fracture (Mangiavacchi *et al.*, 2024).

Pathogenic LINE1 activity



- Driver of sterile, aging-associated inflammation via cGAS/STING activation (Simon *et al.*, 2019; De Cecco *et al.*, 2019).
- Driver of chronic inflammatory signalling in auto-immune disease (Zhang *et al.*, 2020).



- Promotor of tumorigenesis via insertion mutations, Alu element activation, and chromosomal rearrangement (Xiao-Jie *et al.*, 2016).



- Driver of neuroinflammation and numerous neuro-pathologies (Suarez *et al.*, 2018).

Role of RNA L1 in stimulating osteogenic activity during bone repair

L1 is a potential factor in alleviating osteoporotic disease.

This Hypothesis has been demonstrated thank to some experiment:

1. The administration of L1 RNA to osteoblasts resulted in a significant induction of bone mineralization
2. Transcriptome analysis showed that L1 became active following fracture
3. By transfecting exogenous L1 RNA into osteoblasts in vitro, mineralized matrix production increased in a dose-dependent manner.

HOW?

The authors demonstrate that L1 RNA stimulates the **phosphorylation of a PRK kinase**, which in turn stimulates the **phosphorylation of eIF2a to induce bone mineralization in osteoblasts.**

Still without an answer...

- 1) It remains to be determined how L1s can **promote both** neuronal progenitor **proliferation** in vivo and neuronal **differentiation** in vitro
- 2) Future work will address the details of **PRC2 - L1 RNAs interactions** at single gene locus
- 3) L1 RNAs can influence the activity of other **transcriptional networks** by mechanisms similar of G4s once. These interactions may have been relevant in the evolution of the brain. But this is only an idea...

Analyzing L1 transcriptomes has posed challenges due to several **limitations** in standard molecular biology techniques



Possible future prospects

More than 1000 L1s are polymorphic in the human genome, and this number is underestimated too

- **De novo assembly** produced by using **long-read sequencing** approaches, combined with the refinement of **bioinformatic pipelines** represents an opportunity to resolve the heterogeneity of L1 expression. It will be important to understand how many LINE-1s are transcribed, what their function is, and how they contribute to human phenotypic diversity

An important yet underexplored area in cortical development is the question of **interindividual variation**.



THANK YOU

For the attention



**Q&A
session**