Exploring the possibility of Alternative Splicing as a path to the regulation of LINE-1 elements in human and mouse

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Overview

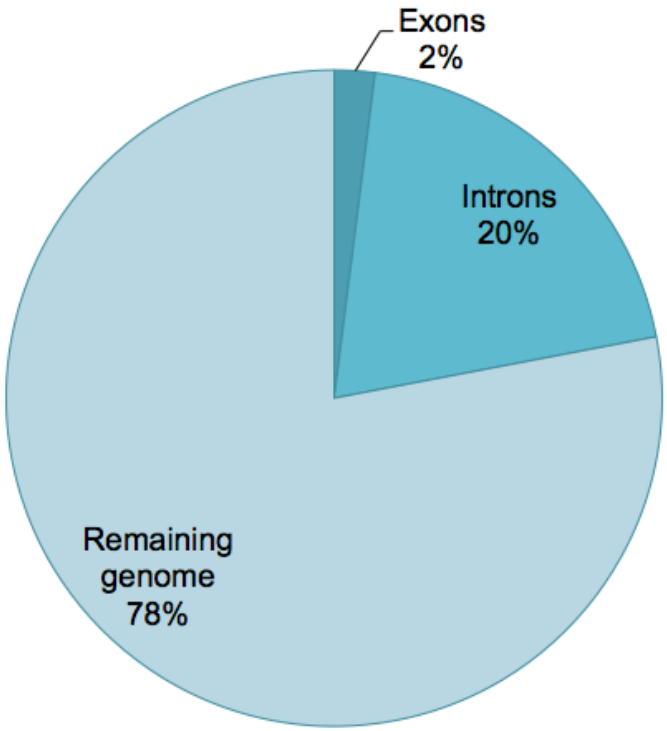
- Background: Transposable Elements (TEs) including L1s

- Regulation of TEs
- Alternative splicing
- Alternative splicing in L1s
- Project Aims

Background

The human genome

Repetitive elements are abundant in the human genome



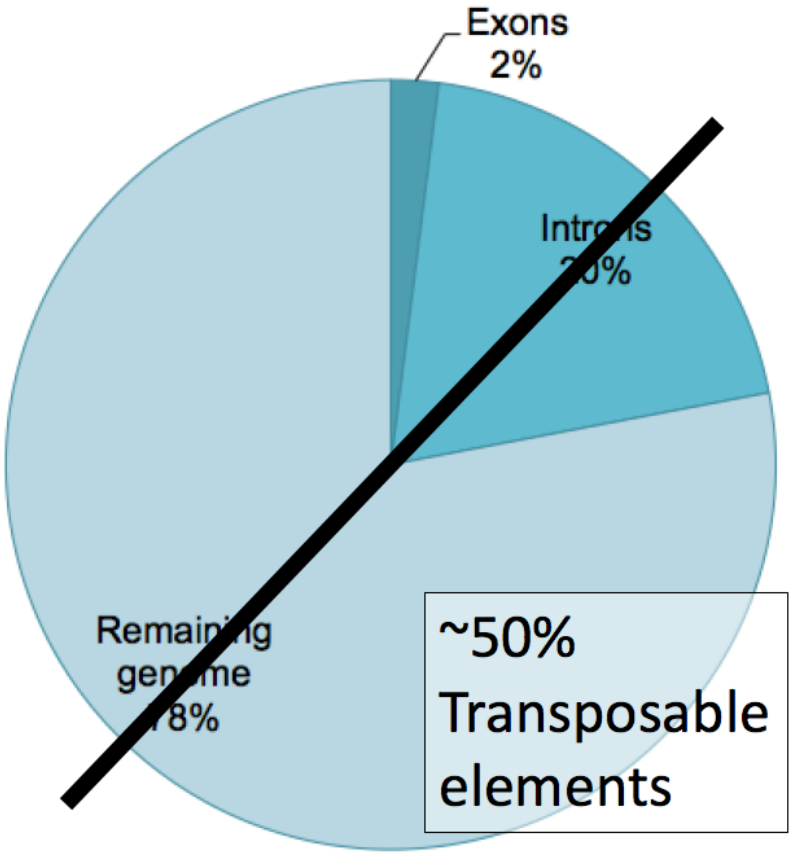
Xu et al. 2010, Singer et al. 2010

Other genome content:

Tandem repeats
Intergenic regions
Duplications
Transposable elements

The human genome

Repetitive elements are abundant in the human genome



The human genome

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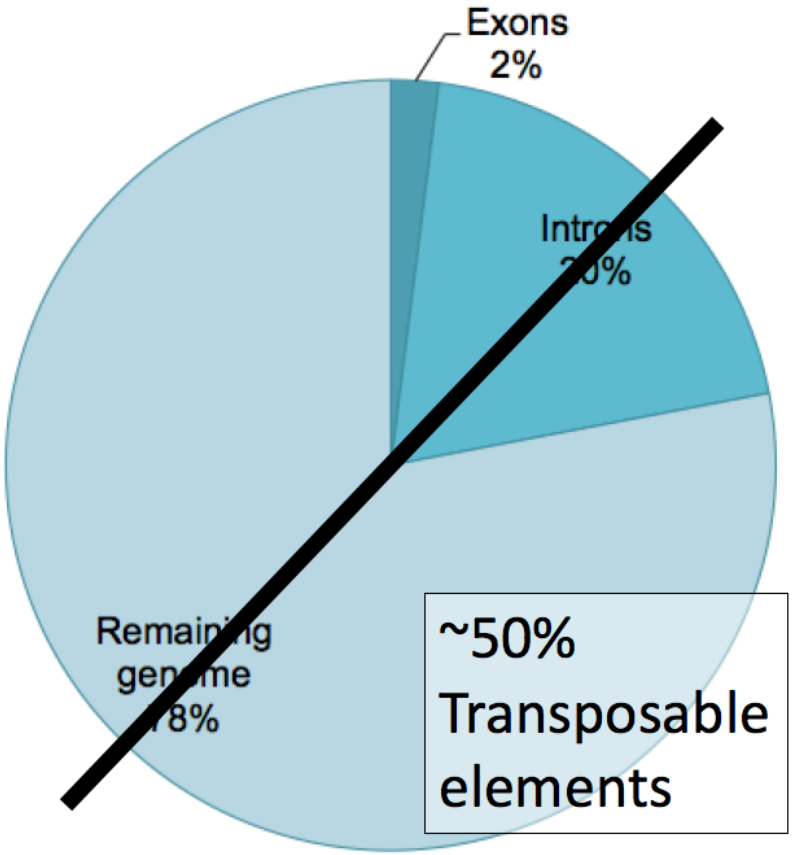
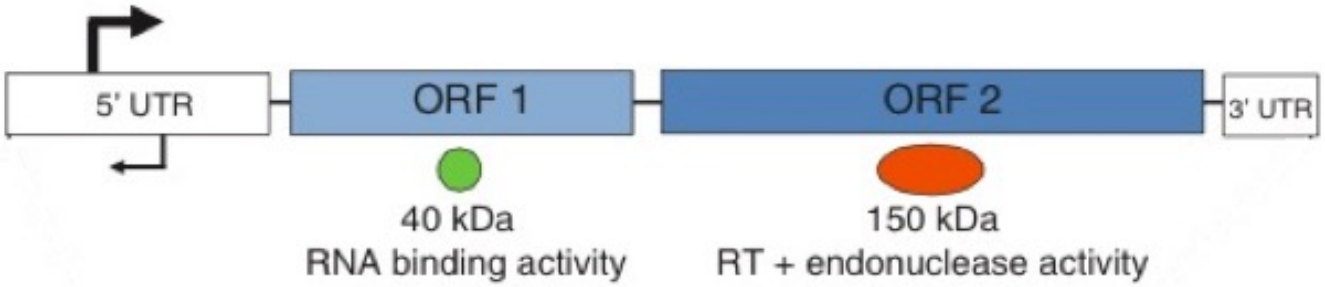


LINE-1 (L1) elements comprise 20% of the human genome

Xu et al. 2010, Singer et al. 2010

LINE-1 (L1) structure

L1s are TEs

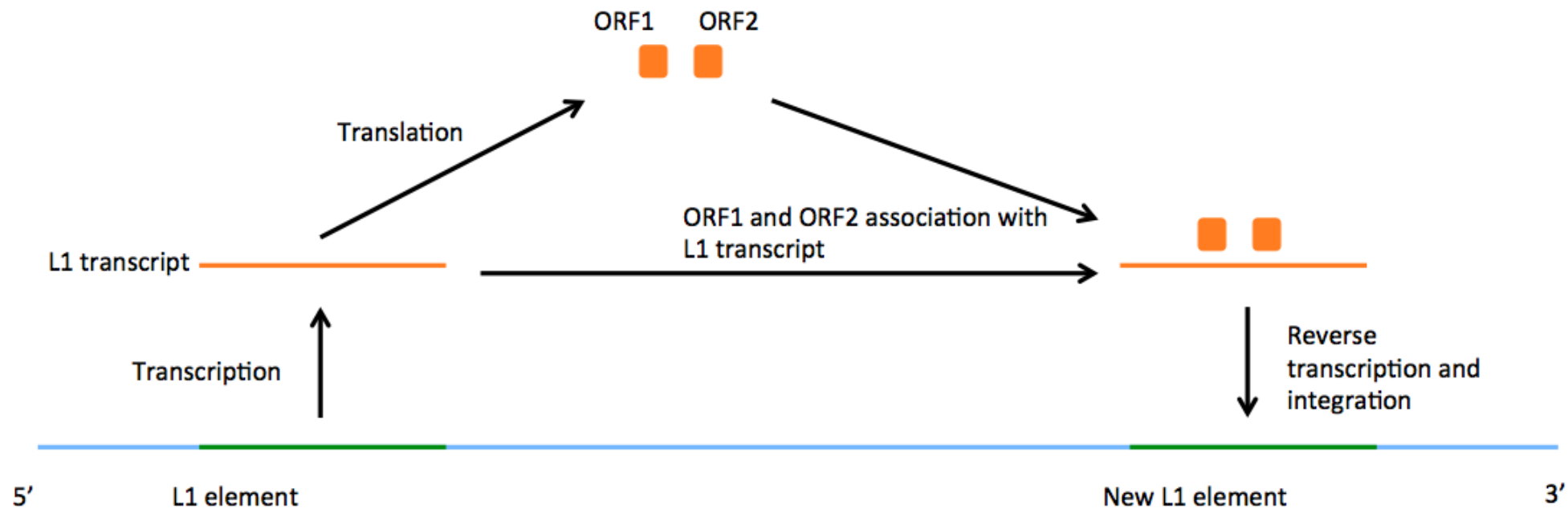


L1 structure

- Full length L1s are 6-7kb
- L1s are often 5' truncated, inverted or degraded
- Some variants are 4kb - HAL1s
  - No ORF2

Retrotransposon replication cycle

LINE-1s are retrotransposons



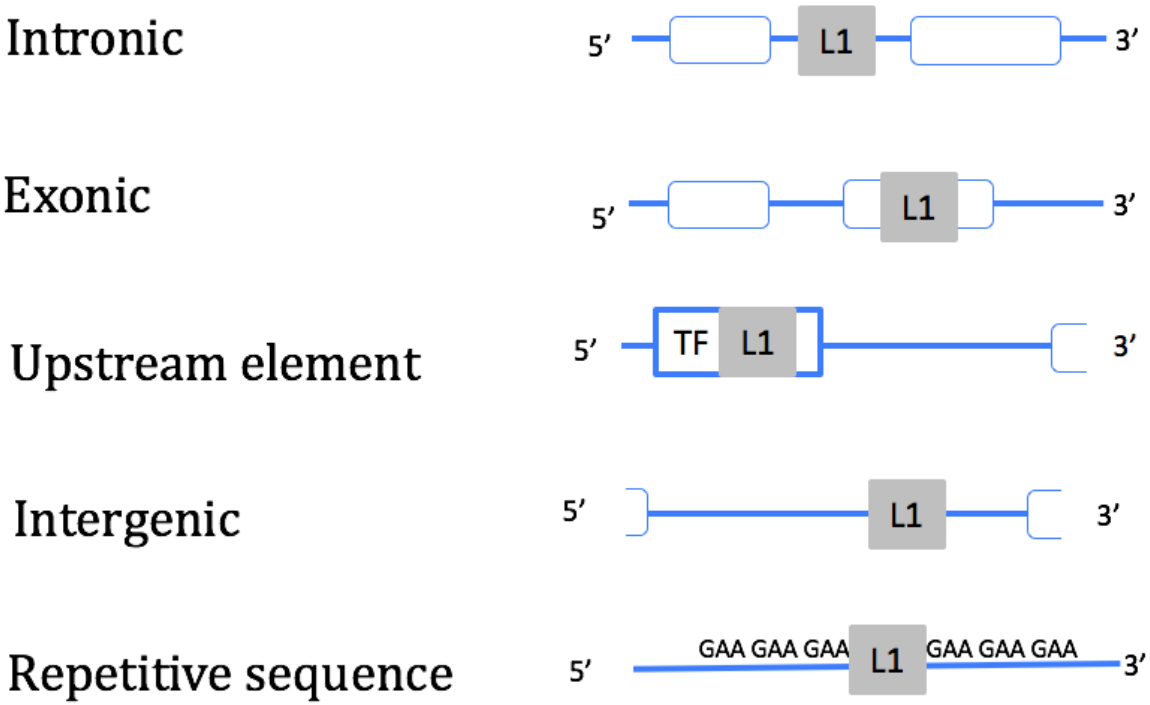
- L1s replicate through an RNA intermediate
- They integrate anywhere in the genome - interspersed repeats

Regulation of TEs

Regulation of TEs

Why is regulation required?

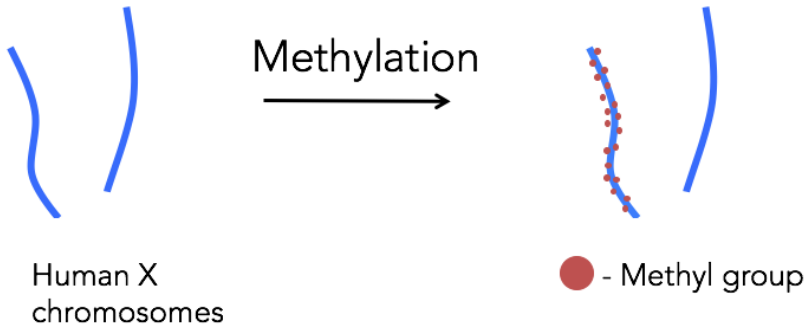
Transposable elements can insert anywhere in the genome



DNA methylation

Methylation of DNA is widespread throughout the genome

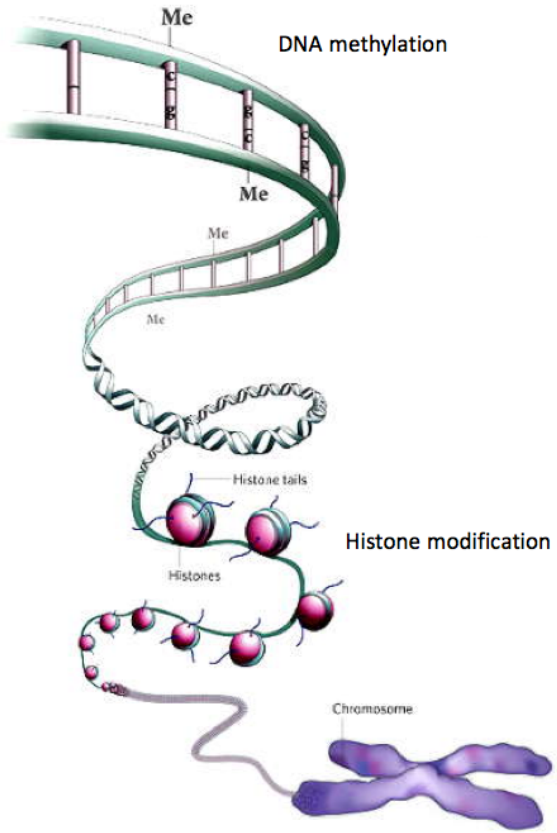
- X chromosome inactivation, TE silencing
- TE accumulation occurs when methylation is impaired
- Levels fluctuate in development
- L1s in the female mature gamete aren't fully methylated



Other regulation

Many other mechanisms have been shown to suppress TEs

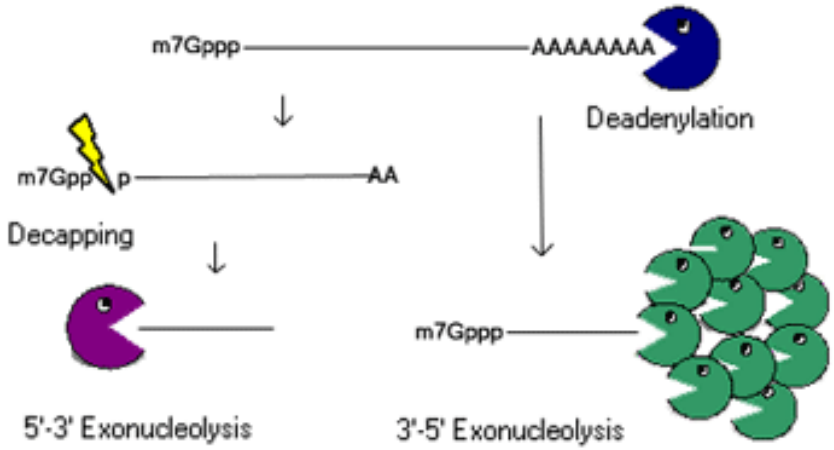
- Histone modifications
  - Methylation - SETB1
  - Ubiquitination
  - Acetylation
- RNA interference
  - miRNAs
  - siRNAs
  - piRNAs
- RNA editases
  - APOBEC



mRNA decay

Targeting the L1 RNA intermediate

- Targets aberrant transcripts
- Nonsense mediated decay

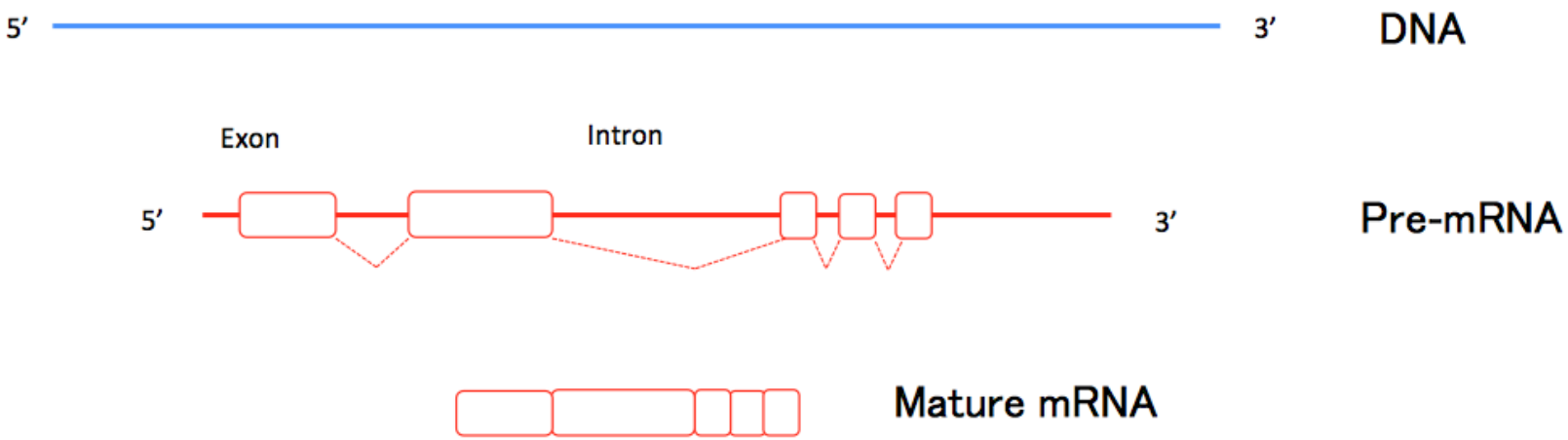


- Alternative splice event → Premature Termination Codon → Target for NMD
- The act of splicing means that decay is a possible regulatory mechanism

Alternative Splicing

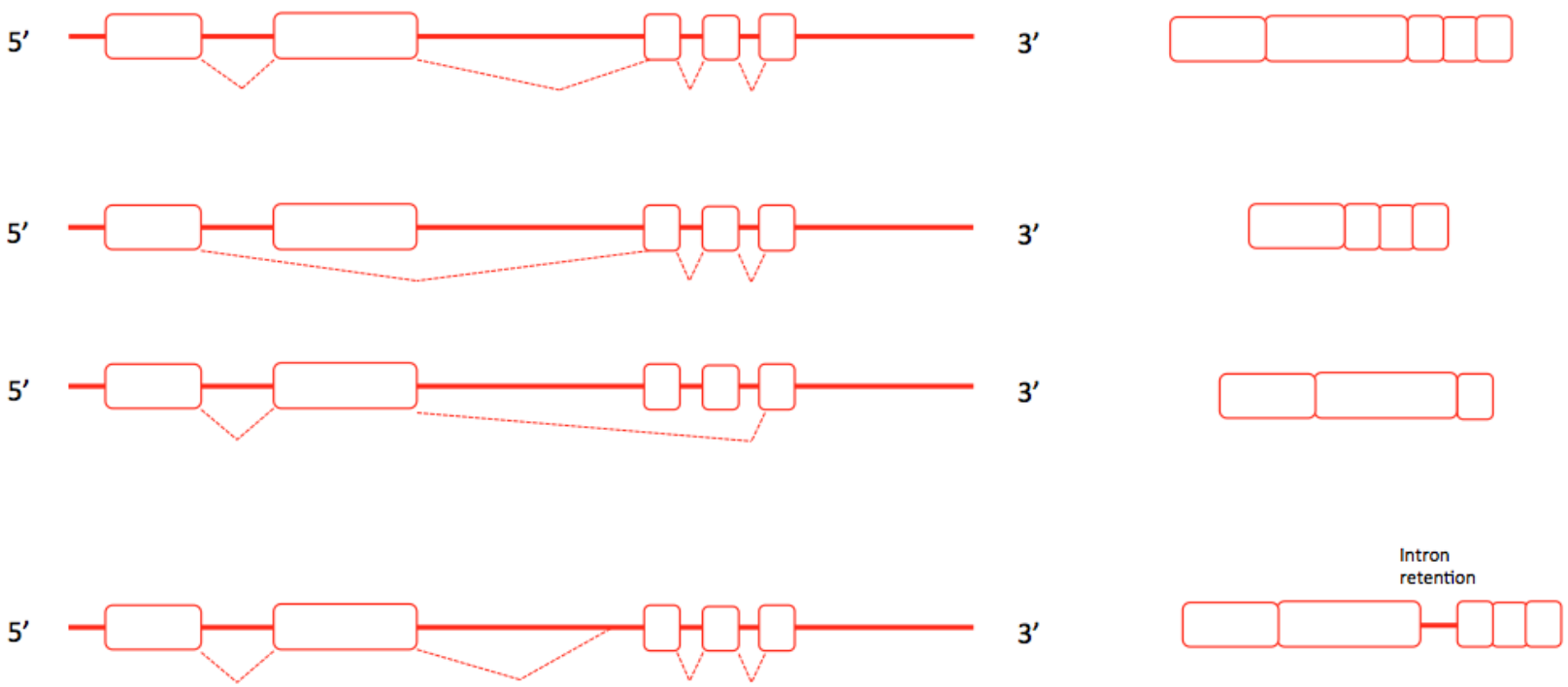
mRNA processing

DNA is transcribed to RNA, which is processed to form mature mRNA



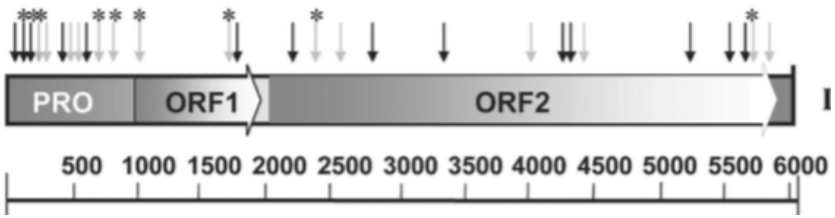
mRNA processing

Alternative splicing can form multiple splice variants



Project Motivation

Belancio et al. (2006) found candidate splice sites in L1 elements and showed evidence of splicing in transfection studies



HAL1s could potentially have resulted from an alternative splicing event

Project Aims

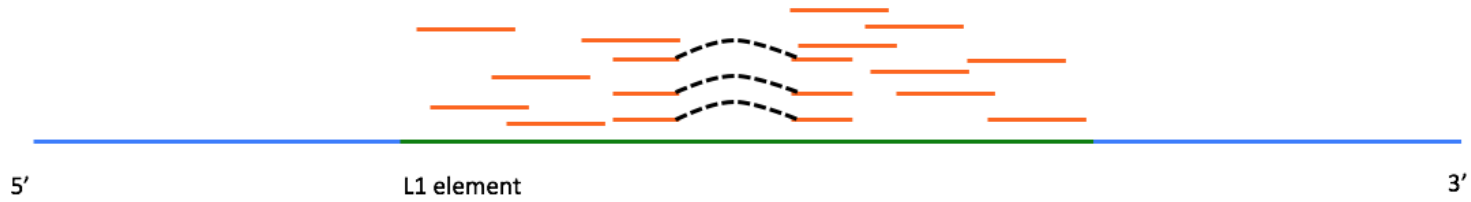
Aim 1

Is AS detectable in the mouse transcriptome?

There is evidence that AS occurs in L1 elements, so RNA-Seq data will be used to detect it

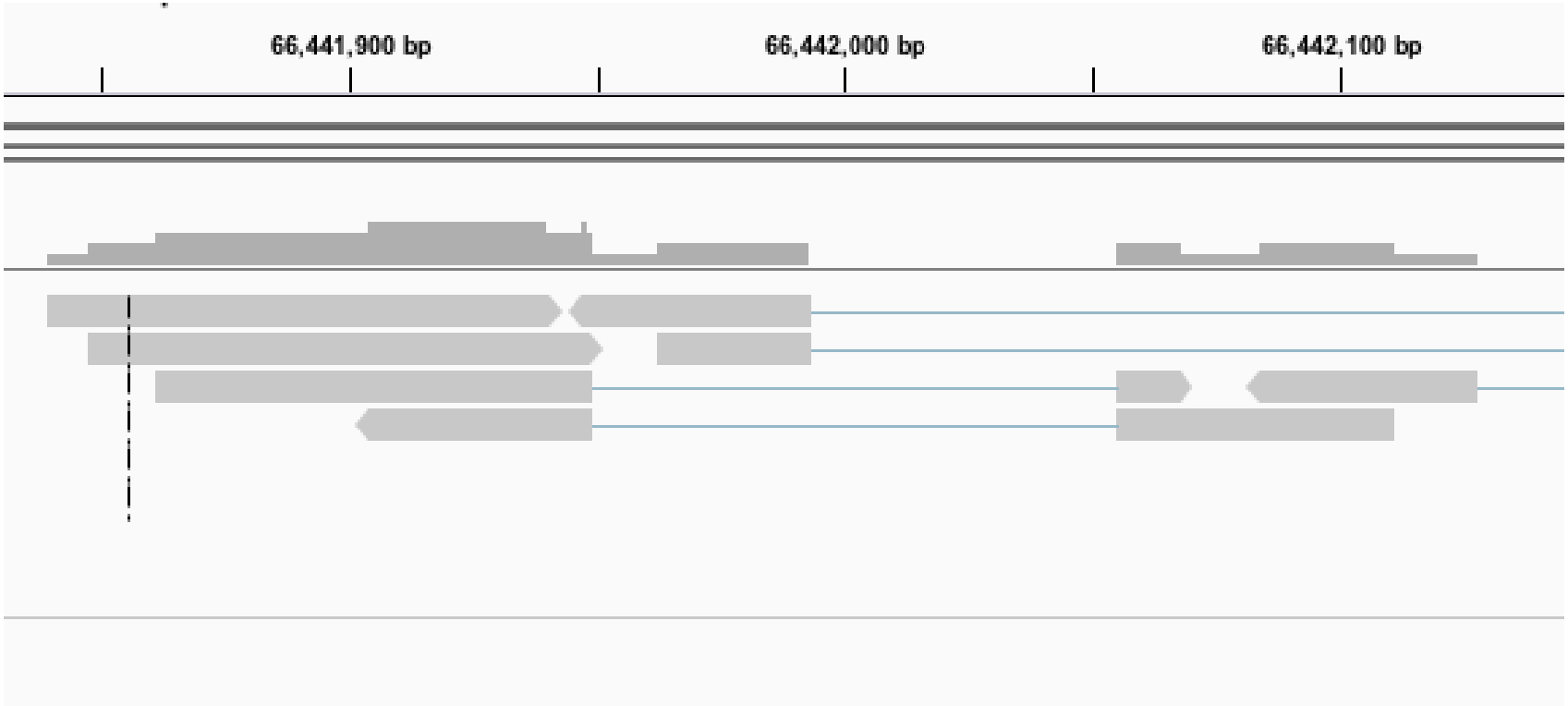
Detecting Alternative Splicing in L1 elements

RNA-Seq reads can be aligned to the genome



- Reads that are split over two locations on the genome with a gap indicate splicing

Read visualisation with Integrated Genomics Viewer



Further aims

Is AS detectable in the mouse genome?

The genome L1s can be compared to full-length L1 consensus sequences, to detect any splicing

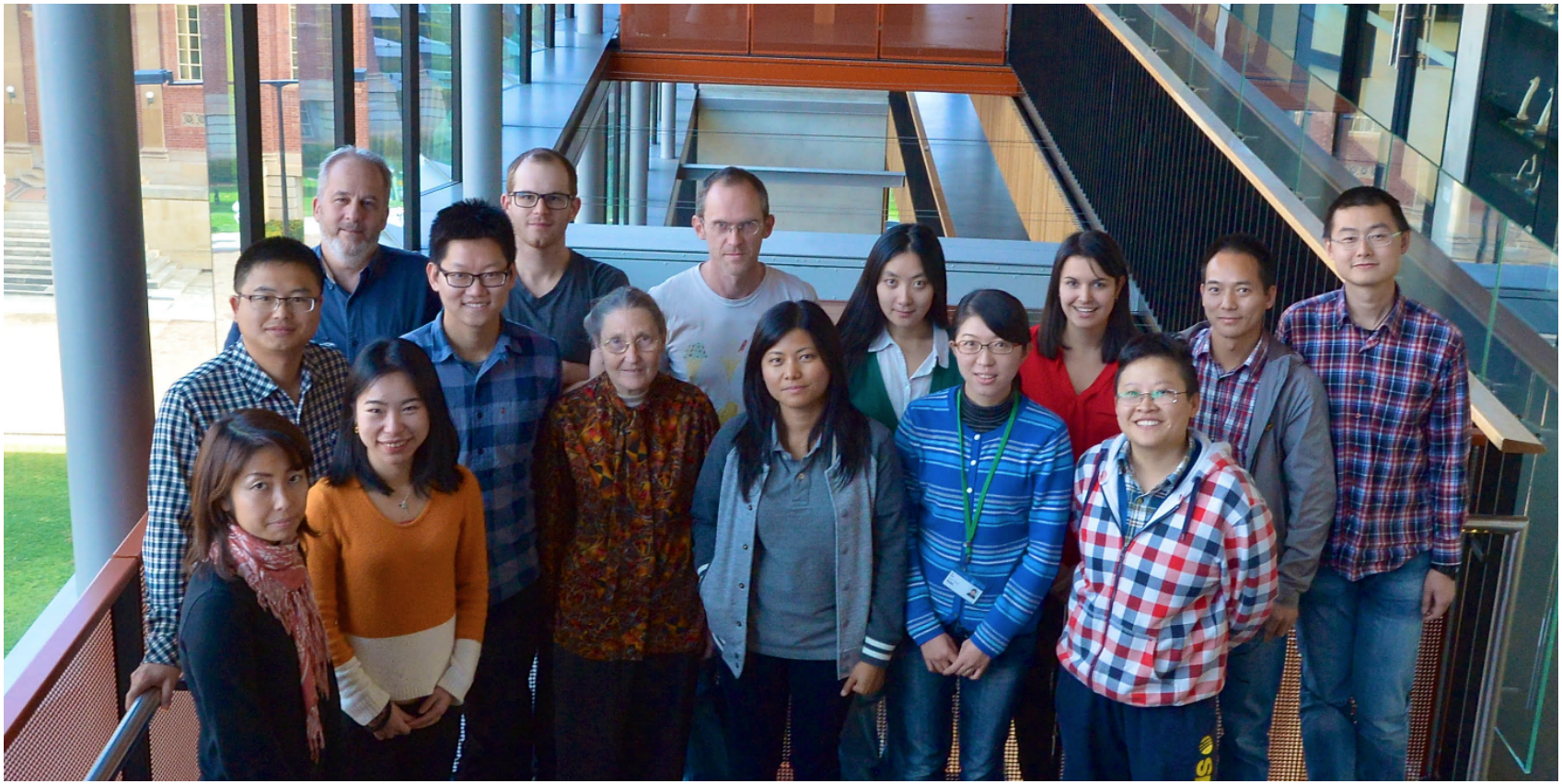
Is AS detectable in the human transcriptome or genome?

If AS is found in both the human genome, further comparative analysis is possible, such as if the same splice donor and acceptor sites are used

Summary

- L1s are the most abundant TEs in the human genome, and we are using them as a candidate for TE regulation
- We have some evidence that they are alternatively spliced
- The mouse and human transcriptome and genome will be used for detection

Thank you



References

Belancio, V. P., Hedges, D. J., and Deininger, P. 2006. LINE-1 RNA splicing and influences on mammalian gene expression. Nucleic Acids Research, 34(5):15121521.

Xu, A. G., He, L., Li, Z., Xu, Y., Li, M., Fu, X., Yan, Z., Yuan, Y., Menzel, C., Li, N., Somel, M., Hu, H., Chen, W., P aa bo, S., and Khaitovich, P. 2010. Intergenic and repeat transcription in human, chimpanzee and macaque brains measured by RNA-Seq. PLoS computational biology, 6:e1000843.

Singer, T., McConnell, M. J., Marchetto, M. C. N., Coufal, N. G., and Gage, F. H. 2010. Line-1 retrotransposons: mediators of somatic variation in neuronal genomes? Trends in neurosciences, 33(8):34554.