RNAseq example

description

Cell. 2013
Direct competition between hnRNP C and U2AF65 protects the transcriptome from the exonization of Alu elements.
Zarnack K, König J, Tajnik M, Martincorena I, Eustermann S, Stévant I, Reyes A, Anders S, Luscombe NM, Ule J.

Kathi Zarnack data

Cell

Direct Competition between hnRNP C and U2AF65 Protects the Transcriptome from the Exonization of *Alu* Elements

Kathi Zarnack,[1,8] Julian König,[2,8] Mojca Tajnik,[2,3] Iñigo Martincorena,[1] Sebastian Eustermann,[2] Isabelle Stévant,[1] Alejandro Reyes,[4] Simon Anders,[4] Nicholas M. Luscombe,[1,5,6,7,*] and Jernej Ule[2,*]

[1]European Molecular Biology Laboratory (EMBL) European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK

[2]MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK

[3]Faculty of Medicine, University of Ljubljana, Vrazov trg 2, SI-1104 Ljubljana, Slovenia

[4]EMBL, Genome Biology Unit, Meyerhofstraße 1, 69117 Heidelberg, Germany

[5]UCL Genetics Institute, Department of Genetics, Environment and Evolution, University College London, Gower Street, London WC1E 6BT, UK

[6]Cancer Research UK London Research Institute, 44 Lincoln's Inn Fields, London WC2A 3LY, UK

[7]Okinawa Institute for Science and Technology Graduate University, 1919-1 Tancha, Onna-son, Kunigami-gun, Okinawa 904-0495, Japan

[8]These authors contributed equally to this work

*Correspondence: nicholas.luscombe@ucl.ac.uk (N.M.L.), jule@mrc-lmb.cam.ac.uk (J.U.)

http://dx.doi.org/10.1016/j.cell.2012.12.023

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3629564/

ALU elements and transposomes

- An Alu element is a **short stretch of DNA originally characterized by the action of the Arthrobacter luteus (Alu) restriction endonuclease**.
- Alu elements are **the most abundant transposable elements (*transposon*, jumping gene)**, containing over one million copies dispersed throughout the human genome.
- ***Transposon*** is a DNA sequence that can change its position within a genome, sometimes creating or reversing mutations and altering the

Cell. 2013
Direct competition between hnRNP C and U2AF65 protects the transcriptome from the exonization of Alu elements.
Zarnack K, König J, Tajnik M, Martincorena I, Eustermann S, Stévant I, Reyes A, Anders S, Luscombe NM, Ule J.

Summary of the results

There are ~650,000 ***Alu* elements** in transcribed regions of the human genome. These elements contain **cryptic splice sites**, so they are in constant **danger** of aberrant **incorporation into mature transcripts**. Despite posing a major threat to transcriptome integrity, **little is known** about the molecular **mechanisms preventing their inclusion**.

Here, we present a mechanism for **protecting** the human transcriptome from the aberrant exonization of transposable elements. Quantitative **iCLIP data** show that the **RNA-binding protein hnRNP C competes** with the **splicing factor U2AF65** at many genuine and **cryptic splice sites**.

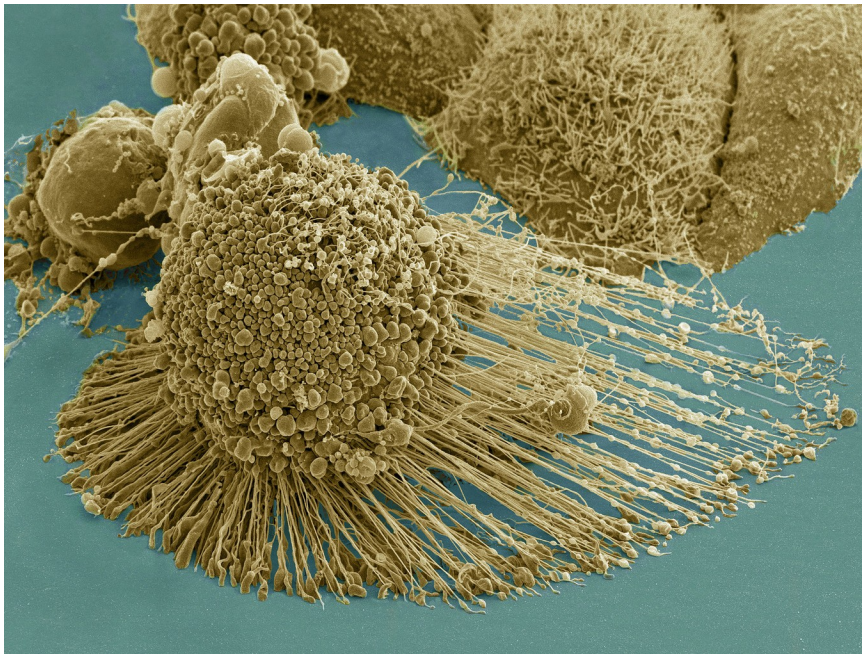
Loss of hnRNP C leads to **formation** of previously **suppressed *Alu* exons**, which severely **disrupt transcript function**. Minigene experiments explain disease-associated mutations in *Alu* elements that hamper hnRNP C binding. Thus, by preventing U2AF65 binding to *Alu* elements, **hnRNP C** plays a **critical role** as a **genome-wide sentinel protecting the transcriptome**. The findings have important implications for human evolution and disease.

Design of the experiment

- The aim was to find out what the HNRNPC gene does
- The experiment performed a knockdown of the **HNRNPC** gene in **HeLa** cells (hnRNP C Stealth Select RNAi siRNAs HSS179304 and HSS179305) as well as control siRNA Stealth RNAi siRNA Negative Control (Invitrogen).
  - SiRNA negative control (control) – 2 samples
  - KD1 – knock-down 1 – 2 samples
  - KD2 – knock-down 2 – 2 samples
  - RNA-seq libraries were sequenced on an Illumina GA-2 (72 cycles, paired end)
  -

- **HeLa**

- is an immortal cell line used in scientific research. It is the oldest and most commonly used human cell line. The line is derived from cervical cancer cells taken on February 8, 1951,from Henrietta Lacks, a 31-year-old African-American mother of five, who died of cancer on October 4, 1951. The cell line was found to be remarkably durable and prolific, which allows it to be used extensively in scientific study.



Design of the experiment

- The experiment performed a knockdown of the **HNRNPC** gene in **HeLa** cells (hnRNP C Stealth Select RNAi siRNAs HSS179304 and HSS179305) as well as control siRNA Stealth RNAi siRNA Negative Control (Invitrogen).
  - SiRNA negative control (control) – 2 samples
  - KD1 – knock-down 1 – 2 samples
  - KD2 – knock-down 2 – 2 samples
  - RNA-seq libraries were sequenced on an Illumina GA-2 (72 cycles, paired end)
  -

Goal of the practical

Get from the **raw sequencing data** to the **gene expression** (**RNA-Seq**)
Analyze **RNA-Seq** data and get **differential gene expression** and **expression** of individual **exons** (example at gene CD55 gene)

Show **coverage cryptic exon(s)** (example at gene CD55)