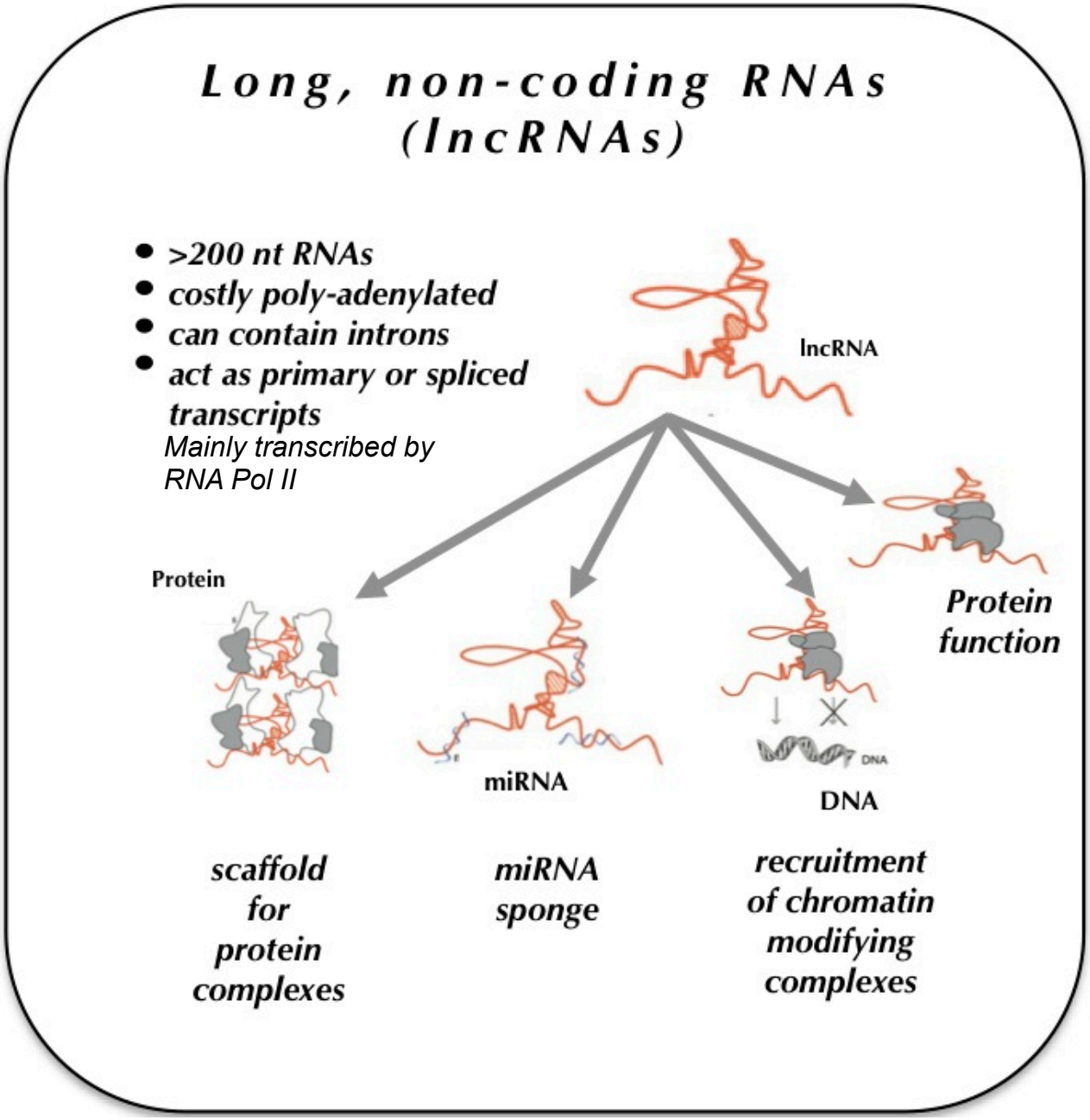
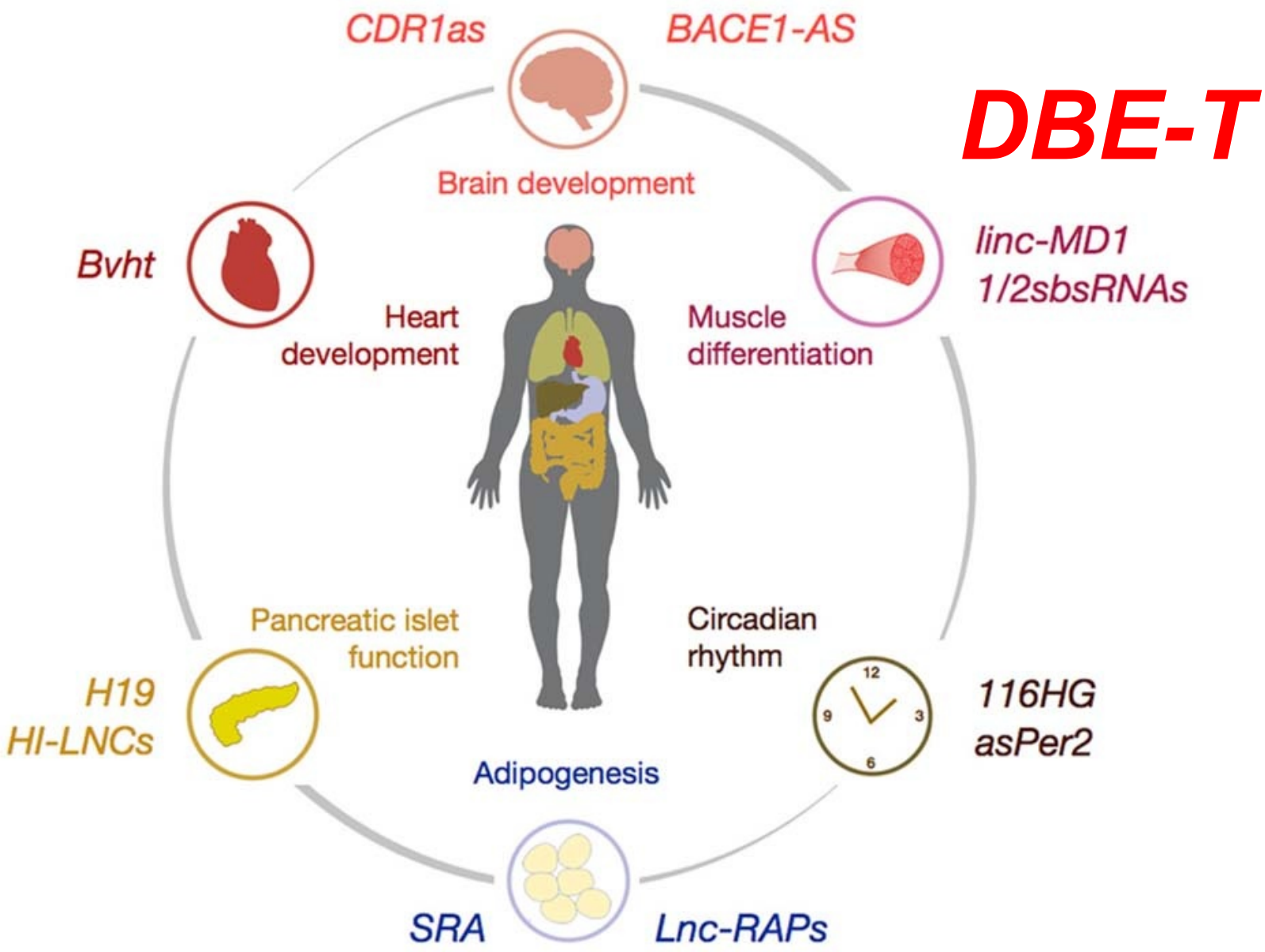
lncRNAs

long, non-coding RNAs

Characteristics of lncRNAs



lncRNAs in physiology



COMPARING mRNAs - lncRNAs

Resource

The GENCODE v7 catalog of human long noncoding RNAs: Analysis of their gene structure, evolution, and expression

Thomas Derrien,[1,11] Rory Johnson,[1,11] Giovanni Bussotti,[1] Andrea Tanzer,[1] Sarah Djebali,[1] Hagen Tilgner,[1] Gregory Guernec,[2] David Martin,[1] Angelika Merkel,[1] David G. Knowles,[1] Julien Lagarde,[1] Lavanya Veeravalli,[3] Xiaoan Ruan,[3] Yijun Ruan,[3] Timo Lassmann,[4] Piero Carninci,[4] James B. Brown,[5] Leonard Lipovich,[6] Jose M. Gonzalez,[7] Mark Thomas,[7] Carrie A. Davis,[8] Ramin Shiekhattar,[9] Thomas R. Gingeras,[8] Tim J. Hubbard,[7] Cedric Notredame,[1] Jennifer Harrow,[7] and Roderic Guigó[1,10,12]

[1]Bioinformatics and Genomics, Centre for Genomic Regulation (CRG) and UPF, 08003 Barcelona, Catalonia, Spain; [2]INRA, UR1012 SCRIBE, IFR140, GenOuest, 35000 Rennes, France; [3]Genome Institute of Singapore, Agency for Science, Technology and Research, Genome 138672, Singapore; [4]Riken Omics Science Center, Riken Yokohama Institute, Yokohama, Kanagawa 351-0198, Japan; [5]Department of Statistics, University of California, Berkeley, California 94720, USA; [6]Center for Molecular Medicine and Genetics, Wayne State University, Detroit, Michigan 48201, USA; [7]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1HH, United Kingdom; [8]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA; [9]The Wistar Institute, Philadelphia, Pennsylvania 19104, USA; [10]Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, 08002 Barcelona, Catalonia, Spain

22:1775–1789 © 2012, Published by Cold Spring Harbor Laboratory Press; ISSN 1088-9051/12; www.genome.org

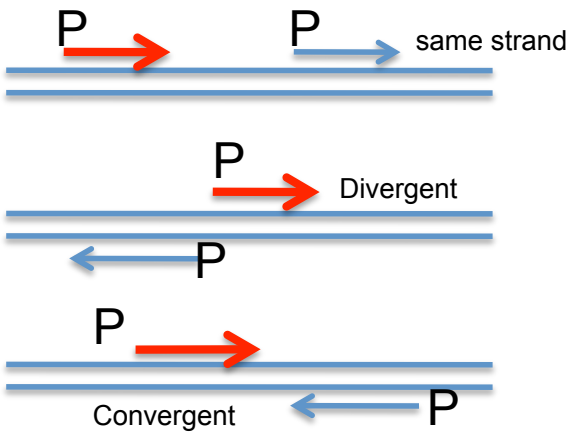
COMPARING mRNAs - lncRNAs

lncRNAs can be

1. Intergenic (lincRNA): Do not intersect with protein coding gene

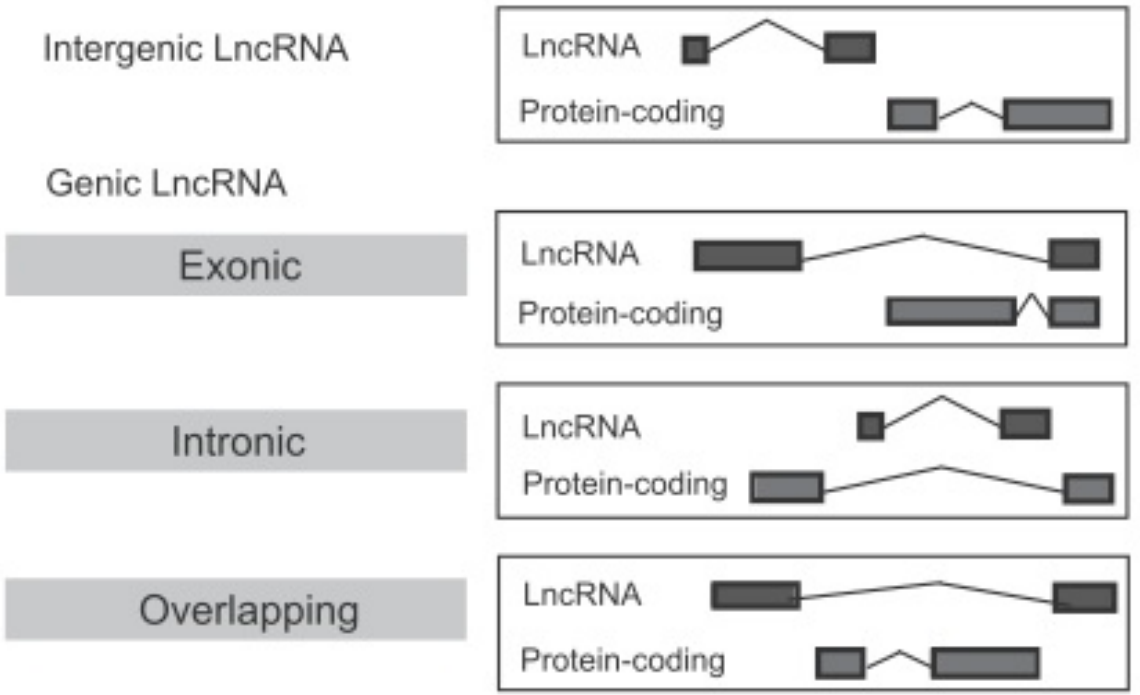
2. Genic: Intersect a protein coding gene

- Exonic
- Intronic
- overlapping



Closest protein coding gene

lncRNA



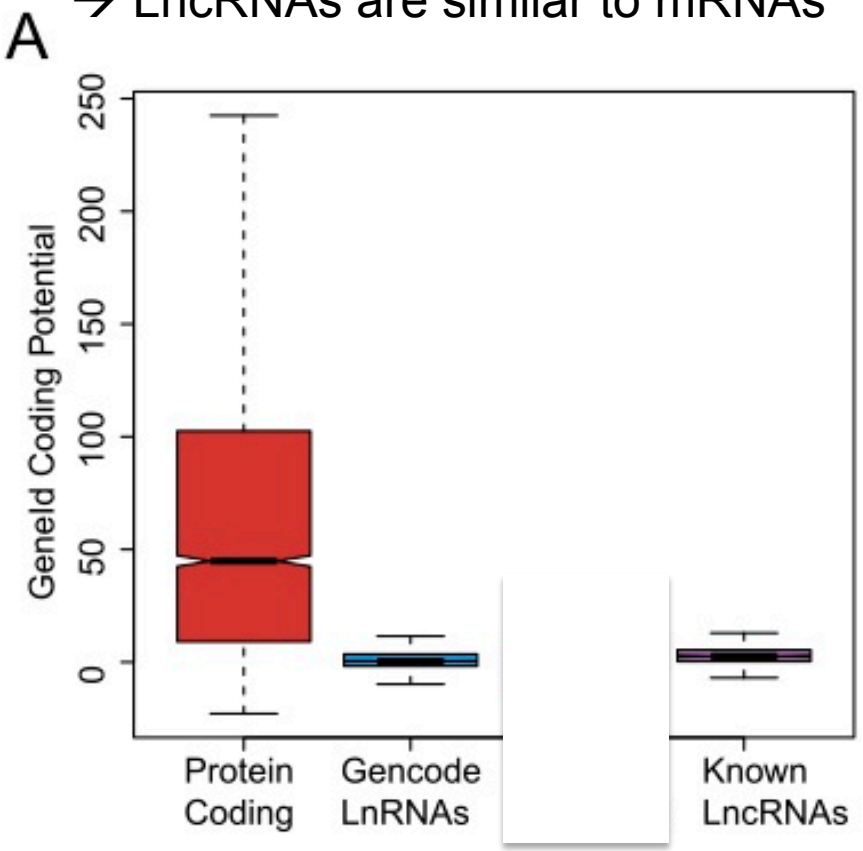
Gencode LncRNAs transcripts (14,880)											
Intergenic (9,518)			Genic (5,362)								
Same Strand	Convergent	Divergent	Exonic (2,411)		Intronic (2,784)		Overlapping (167)				
4,165	1,937	3,416	S	AS	S	AS	S	AS			
			NA	2,411	563	2,221	52	115			

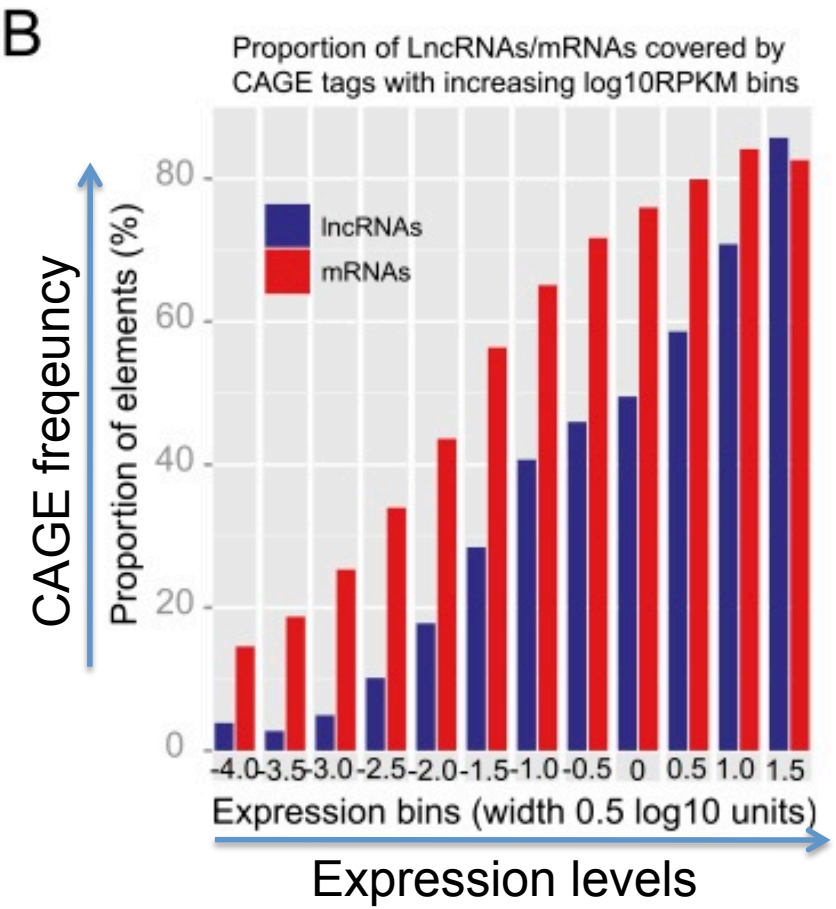
P: promoter

COMPARING mRNAs - lncRNAs

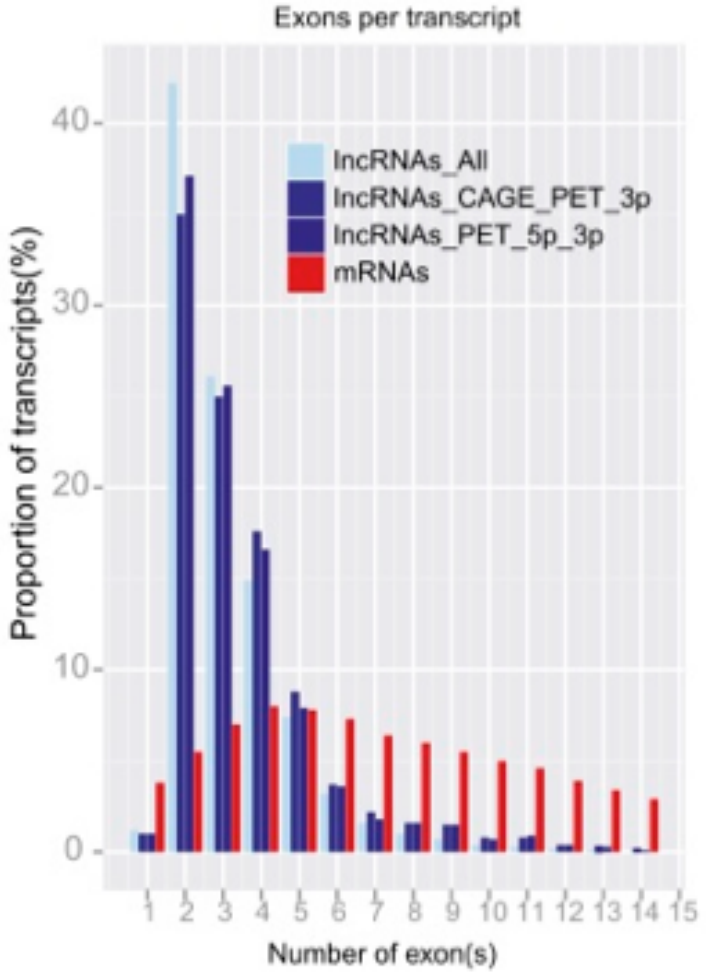
A. LncRNAs do not have coding potential:
→ Longest possible ORF was searched in mRNA/lncRNAs

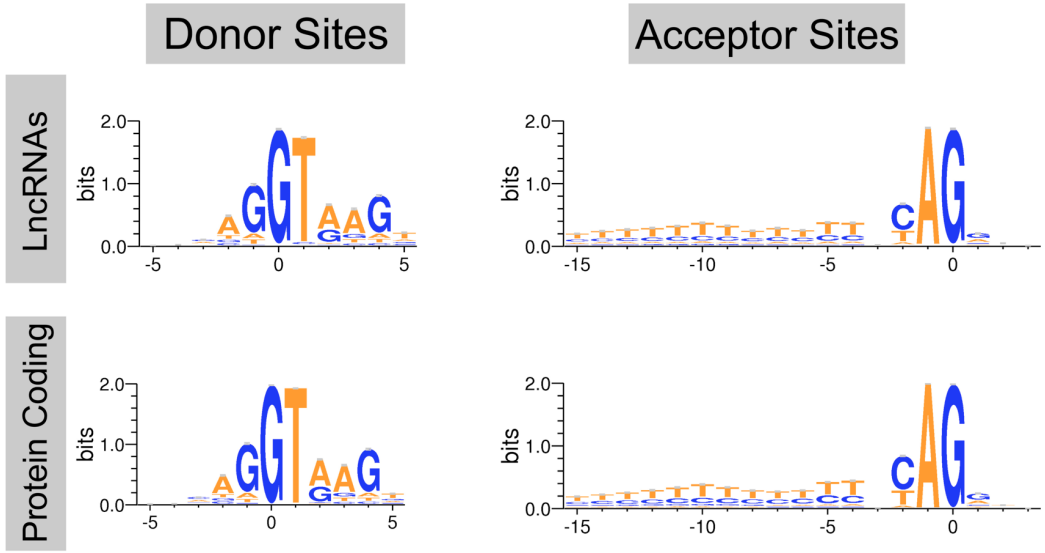
B. CAGE tags can be found in lncRNAs → defined transcriptional start site
CAGE tag freqeuncy increases with increased lncRNA expression levels.
mRNAs are characterized by more CAGE tags
→ LncRNAs are similar to mRNAs





COMPARING mRNAs - lncRNAs

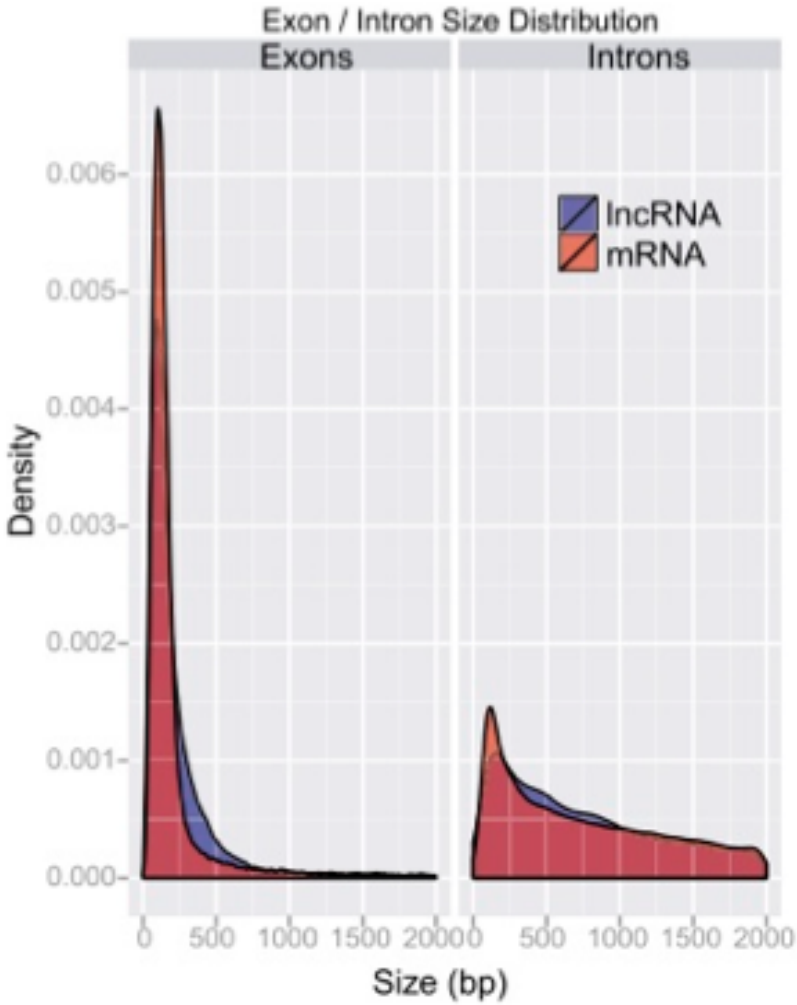




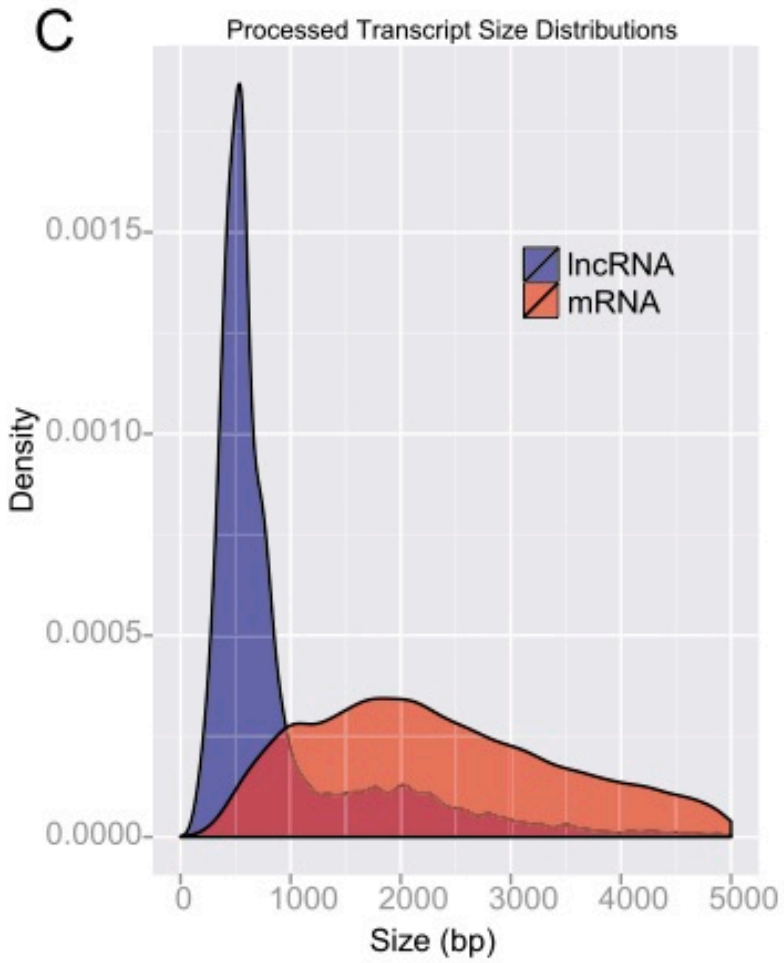
SPLICING: lncRNAs are spliced, relevant
Splice-site prerequisites at splice donor/acceptor
are conserved
BUT: LncRNAs contain fewer INTRONs!! Nost lncRNA
Have only 1 intron!!!; mRNAs 4-7

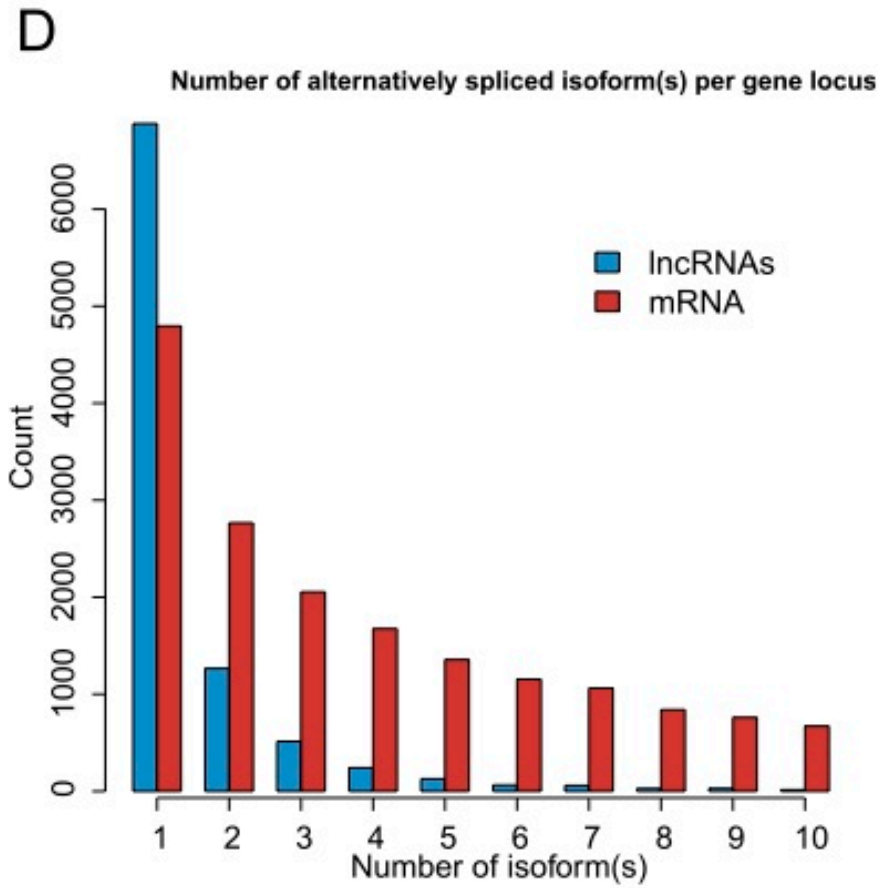
COMPARING mRNAs - lncRNAs

Introns/Exons from lncRNAs are slightly longer



COMPARING mRNAs - lncRNAs





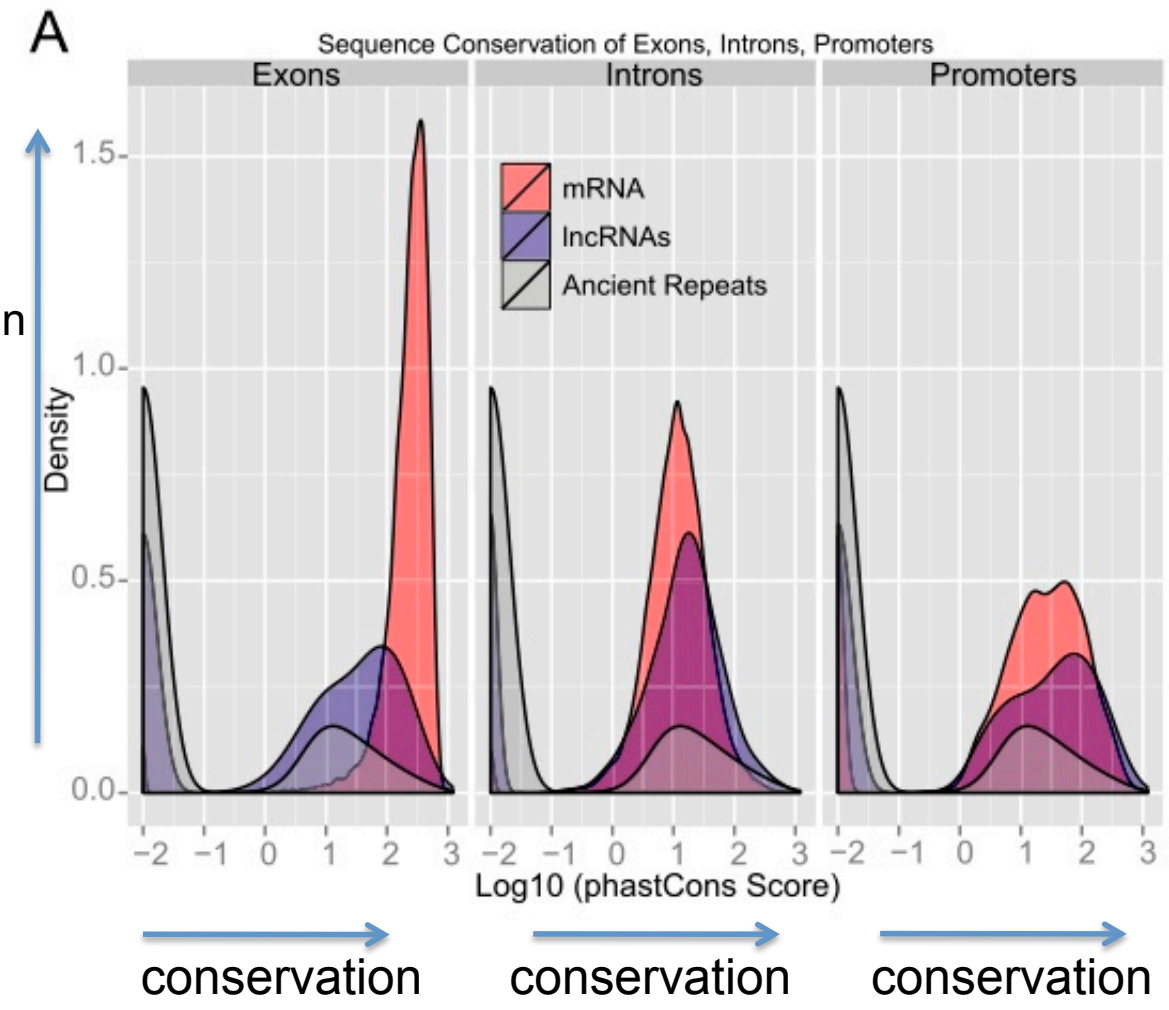
lncRNAs are on average much shorter: Ca 500nt
mRNAs are longer and have wider size distribution

lncRNAs are uniform → little alternative Splicing
mRNAs: large variety of alternative splicing

COMPARING mRNAs - lncRNAs

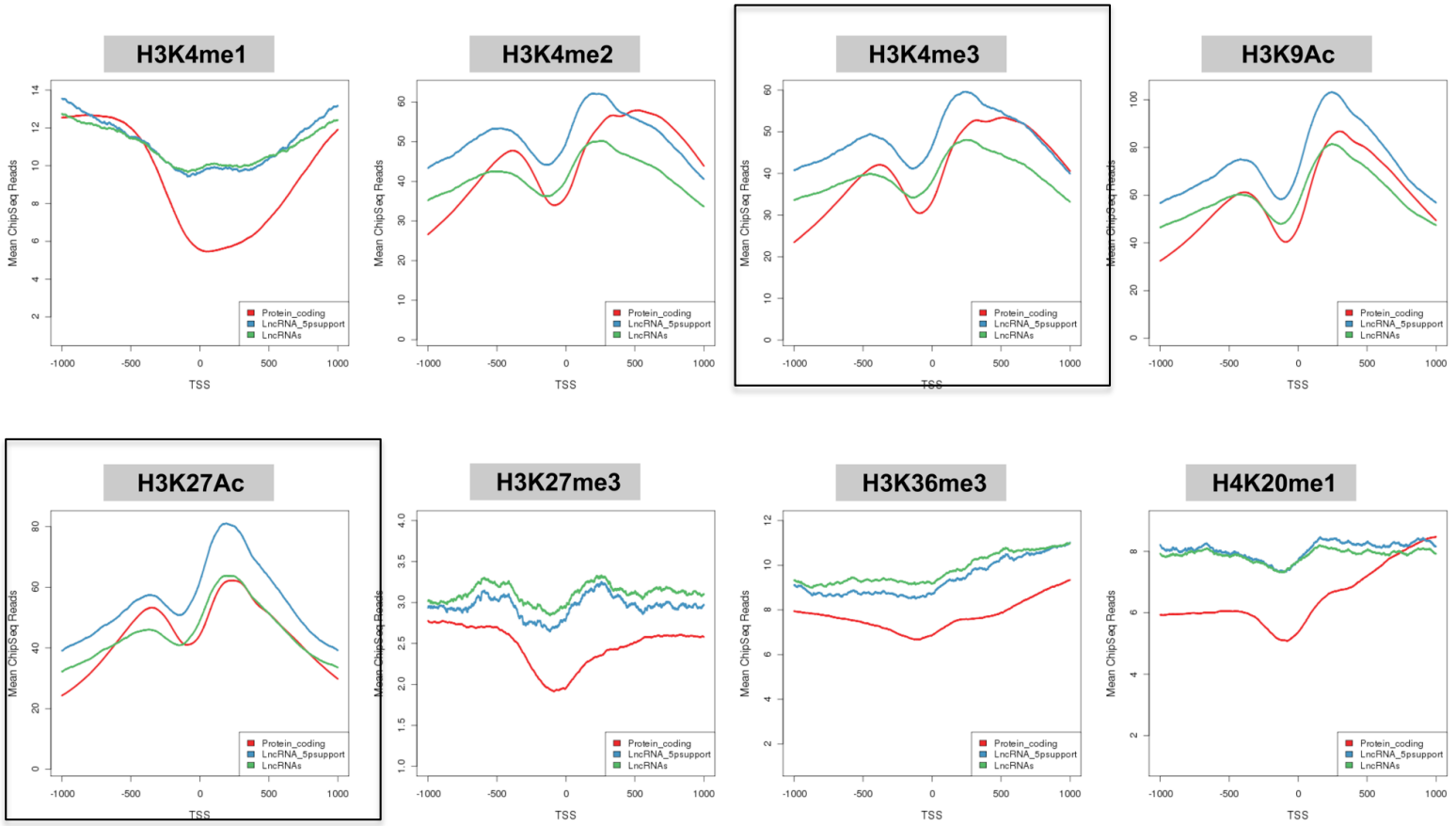
EXONS:
mRNA: high conservation
lncRNA: reduced conservation
But: conservation is higher than mRNA intron conservation

INTRONS:
mRNA: higher conservation than lncRNAs



COMPARING mRNAs - lncRNAs

Chromatin signatures at transcriptional start sites are conserved between mRNAs And lncRNAs: MOST lncRNAs ARE TRANSCRIBED BY RNA Pol II



Chromatin signatures around TSS of protein-coding and lncRNA transcripts expressed in the same cell lines where the signatures were monitored by ChipSeq. Shown on the y-axis is the average density of reads covering the TSS of various gene sets, with position plotted on the x-axis (bp relative to positive strand TSS). Protein coding genes are plotted in red, Gencode v7 lncRNAs in green, and lncRNAs with 5′ experimental support (n=2,793) in blue. N.B. A more extensive analysis of histone modifications in multiple cell types is available at http://big.crg.cat/bioinformatics_and_genomics/lncrna_data.

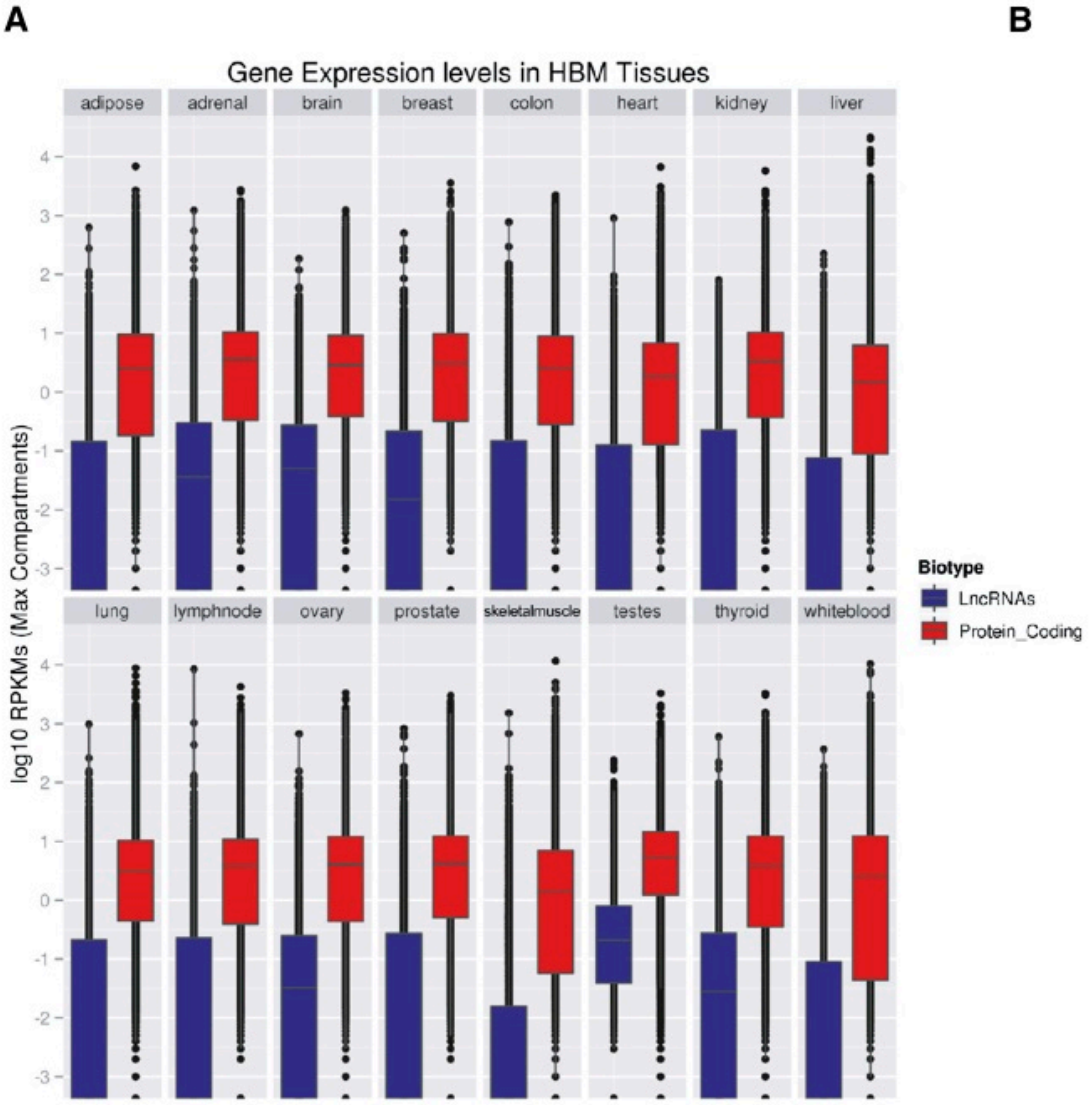
K562

COMPARING mRNAs - lncRNAs

Absolute expression of lncRNAs is much lower than mRNA expression

Most lncRNAs localize to the Nucleus and chromatin

Many lncRNAs (30%) are PRIMATE SPECIFIC
→ Evolutionary advantage



COMPARING mRNAs - lncRNAs

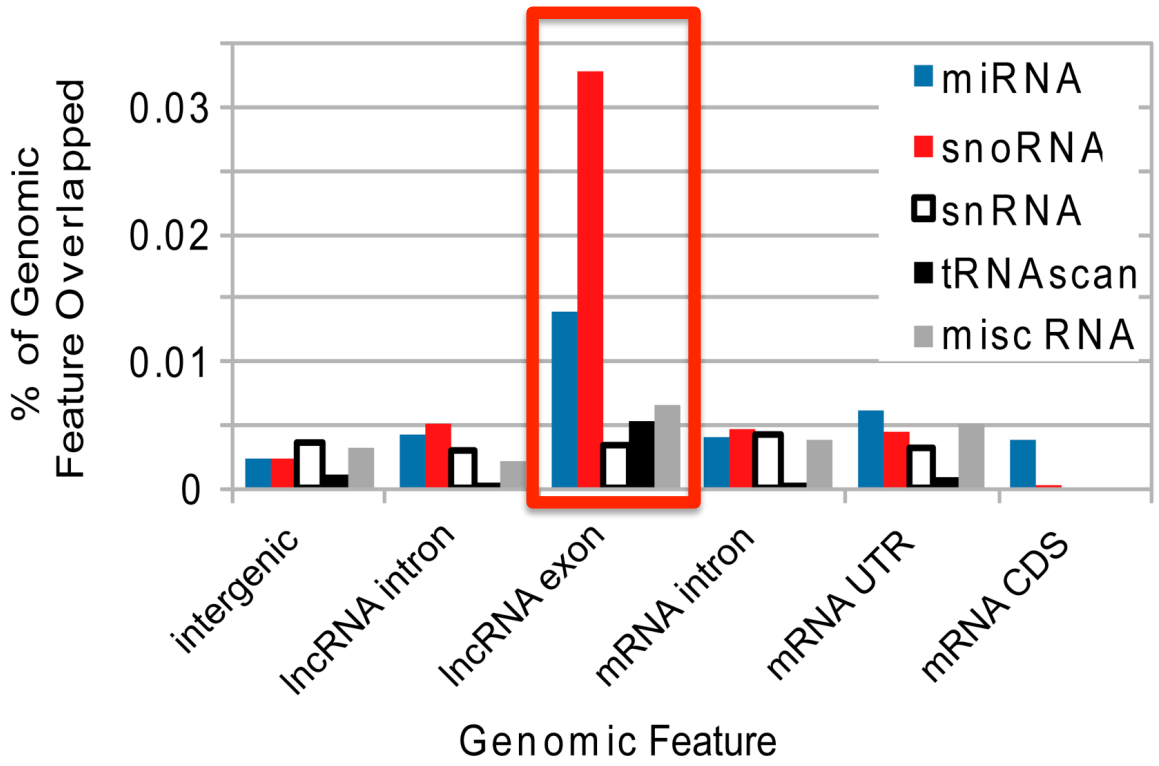


Figure S7: LncRNAs as a source of small RNAs. Shown is the proportion of nucleotides in exons/introns/(UTRS) from protein-coding genes and lncRNAs that overlap different classes of annotated small RNAs. Note that all values refer to cases where the small RNA is on the same strand as the indicated lncRNA/mRNA.

lncRNA exons
Have a remarkable
Potential to encode
functional small RNAs
(sRNAs)
HIGHER THAN EXONS OF
mRNAS!!!!

snoRNA:Small nucleolar RNAs (snoRNAs) are a class of small RNA molecules that primarily guide chemical modifications of other RNAs, mainly ribosomal RNAs, transfer RNAs and small nuclear RNAs.
snRNA: Small nuclear ribonucleic acid (snRNA), also commonly referred to as U-RNA, is a class of small RNA molecules that are found within the splicing speckles and Cajal bodies of the cell nucleus in eukaryotic cells.
tRNA: translation
miscRNAs: MiscRNA is short for miscellaneous RNA, a general term for a series of miscellaneous small RNA. It serves a variety of functions, including some enzyme-like catalysis and processing RNA after it is formed. Besides, some of these small RNAs may serve as switches.

COMPARING mRNAs - lncRNAs

Gencode LncRNAs transcripts (14,880)									
Intergenic (9,518)			Genic (5,362)						
Same Strand	Convergent	Divergent	Exonic (2,411)		Intronic (2,784)		Overlapping (167)		
4,165	1,937	3,416	S	AS	S	AS	S	AS	
			NA	2,411	563	2,221	52	115	

lncRNAs represent a big class of functional elements that
- controlled gene expression
- are processed
- lack protein coding potential
- defined localization
- frequently encode sRNAs
- low conservation
- 35% of lncRNAs are primate specific
- expression is rather low – but controlled!
- mostly transcribed by RNA Pol II

FUNCTION: FOR THE VAST MAJORITY OF lncRNAs THE BIOLOGIVAL FUNCTION IS UNKNOWN!!!!

Examples of lncRNAs

DBE-T AND GENETIC DISEASE

LETTER

doi:10.1038/nature11508

Long non-coding antisense RNA controls *Uchl1* translation through an embedded SINEB2 repeat

Claudia Carrieri[1]*, Laura Cimatti[1]*, Marta Biagioli[1,2], Anne Beugnet[3], Silvia Zucchelli[1,2], Stefania Fedele[1], Elisa Pesce[3], Isidre Ferrer[4], Licio Collavin[5,6], Claudio Santoro[7], Alistair R. R. Forrest[8], Piero Carninci[8], Stefano Biffo[3,9], Elia Stupka[10] & Stefano Gustincich[1,2]

THE ROLE OF THE lncRNA DBE-T IN FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY (FSHD)

FSHD: http://www.omim.org/entry/158900

Facioscapulohumeral muscular dystrophy-1 (FSHD1) is associated with contraction of the D4Z4 macrosatellite repeat in the subtelomeric region of chromosome 4q35.

The genetics of FSHD is, however, complex and detection of a D4Z4-reduced allele may not be sufficient for diagnosis.

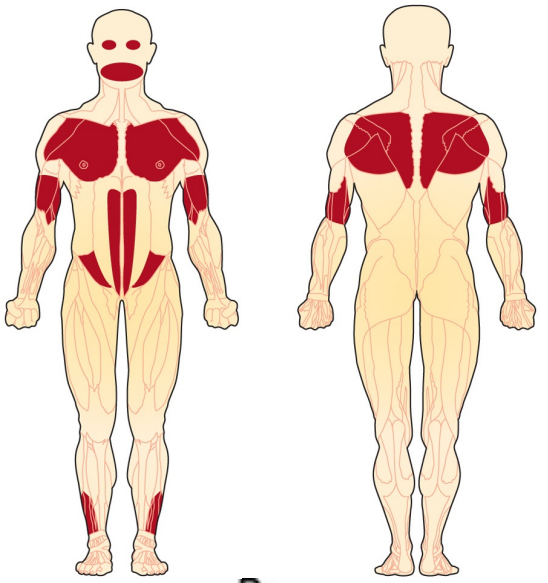
Facioscapulohumeral muscular dystrophy (FSHD) is a genetic muscle disorder in which the muscles of the face, shoulder blades and upper arms are among the most affected.

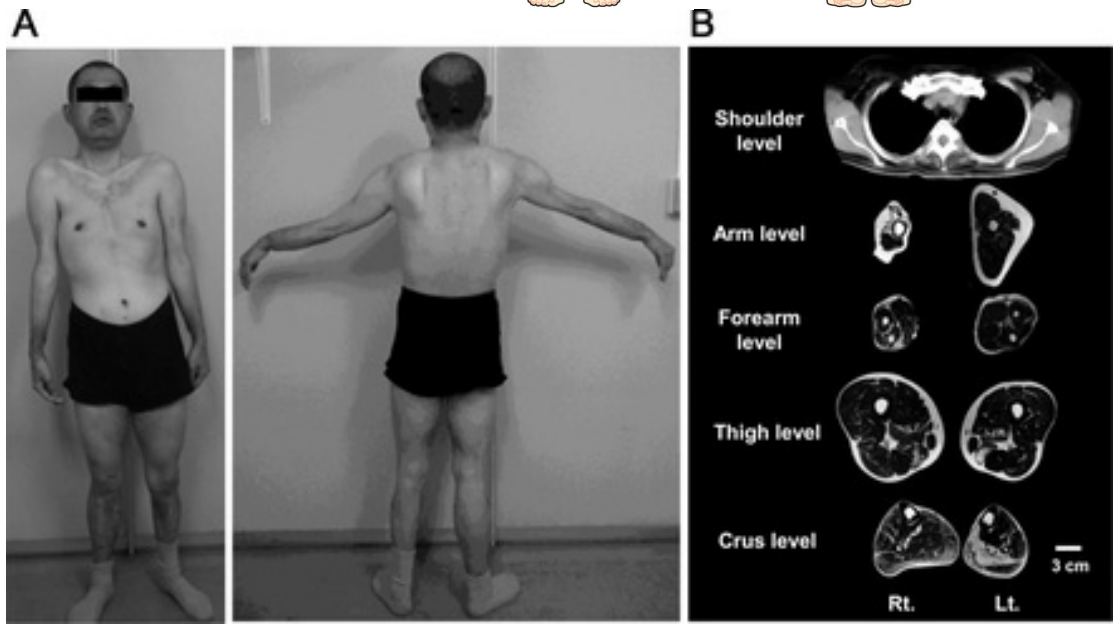
The long name comes from facies, the Latin word and medical term for face; scapula, the Latin word and anatomical term for shoulder blade; and humerus, the Latin word for upper arm and the anatomical term for the bone that goes from the shoulder to the elbow.

The term muscular dystrophy means progressive muscle degeneration, with increasing weakness and atrophy (loss of bulk) of muscles. In FSHD, weakness first and most seriously affects the face, shoulders and upper arms, but the disease usually also causes weakness in other muscles.

Aberrant expression of genes in vicinity to D4Z4 repeats, including DUX4, ANT1, FRG1, FRG2 in FSHD patients are thought to mediate the syndrome (have a "toxic" effect)

Muscles affacted By wasting in FSHD patients

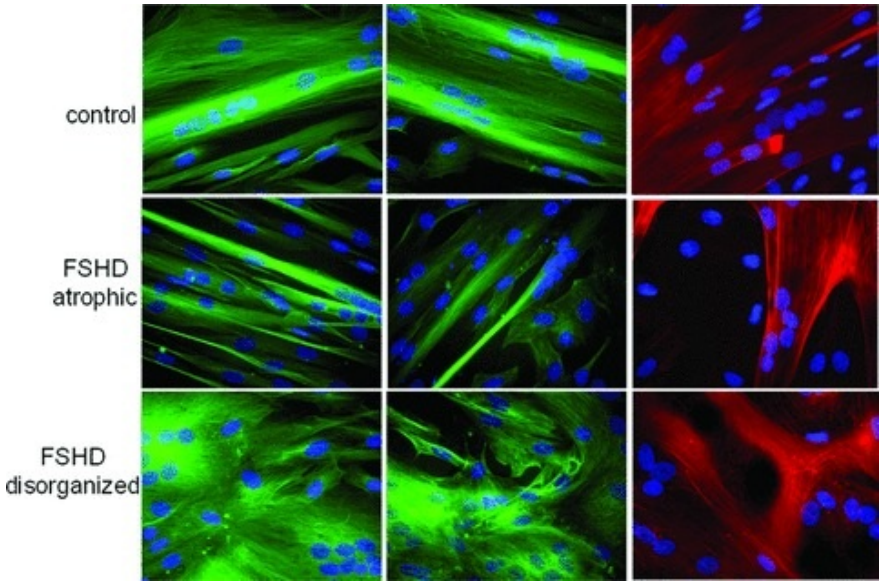




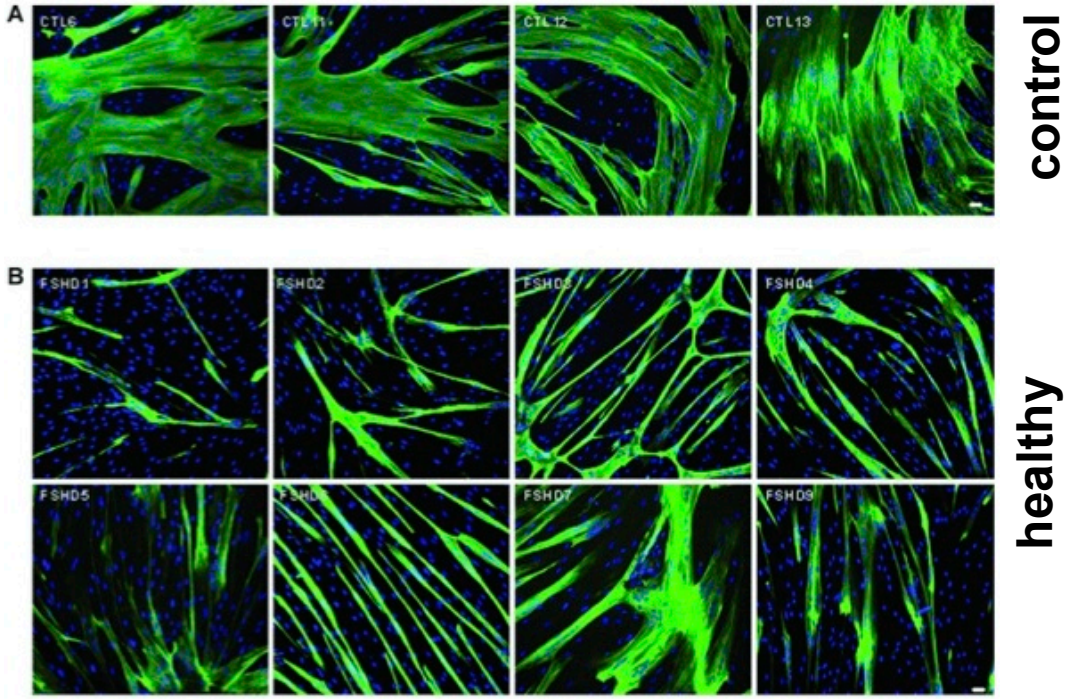
FSDH impairs muscle cell function

Moreover, in patients affected with FSHD, it is quite common to observe the co-existence of affected and apparently healthy muscles. Myoblasts, which were obtained from muscle typically affected in FSHD, manifested an increased susceptibility to oxidative stress during proliferation. Myotubes obtained from patient/healthy myblasts show abnormal morpholgy and muscle marker expression

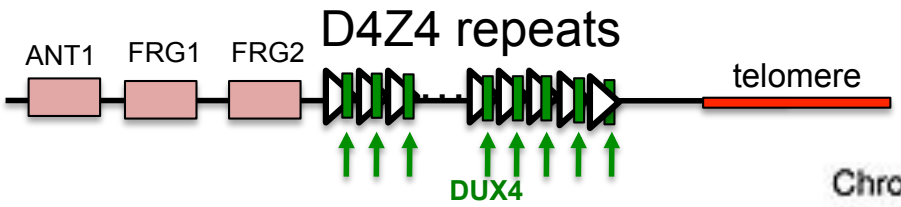
Anti actin/tubulin immunostainingof myotubes obtained by differentiating myblasts cells isolated from healthy or FSHD patients



anti-troponinT immunostainingof myotubes obtained by differentiatin myblasts cells isolated from healthy or FSHD patients

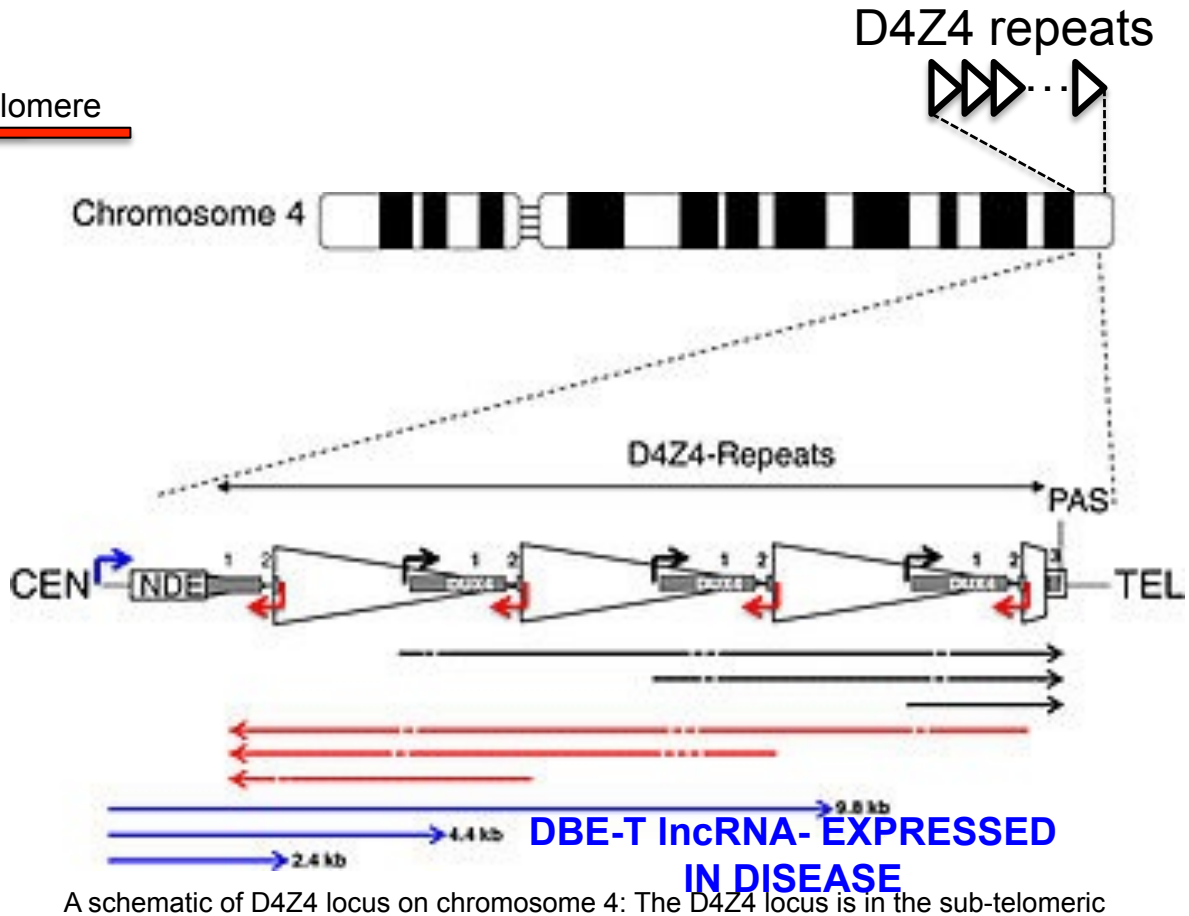


D4Z4 repeats in Chr. 4q subtelomeres give rise to various transcripts



D4Z4 repeat: ca 3kb; extremely GC-rich

Healthy: 11-150 D4Z4 repeats:
ANT1, FRG1, FRG2, DUX4: *repressed*
Diseased: 1-10 D4Z4 repeats:
ANT1, FRG1, FRG2, DUX4: *expressed*



A schematic of D4Z4 locus on chromosome 4: The D4Z4 locus is in the sub-telomeric region of 4q. The figure shows a three repeat D4Z4 array. CEN indicates the centromeric end and TEL indicates the telomeric end. The DUX4 gene is shown as a gray rectangle with exon 1 and exon 2 in each repeat and exon 3 in the pLAM region telomeric to the last partial repeat (numbered 1, 2, and 3). PAS indicates the polyadenylation site on the permissive 4qA allele that is not present on the non-permissive 4qB allele or on chromosome 10. The arrowed lines represent: Blue, DBE-T transcripts (2.4, 4.4, and 9.8 kb) found in FSHD cells and reported to de-repress DUX4 expression; Black and red, transcripts in the sense and antisense direction were detected in both FSHD and control cells and might originate from the mapped sense promoters (black) and anti-sense promoters (red) with dashed lines indicating areas that might be degraded or produce si-like small RNAs. NDE, non-deleted element identified as the transcription start site for the DBE-T transcripts.

HETEROCHROMATIN AT D4Z4 REPEATS SILENCES LOCAL GENE EXPRESSION

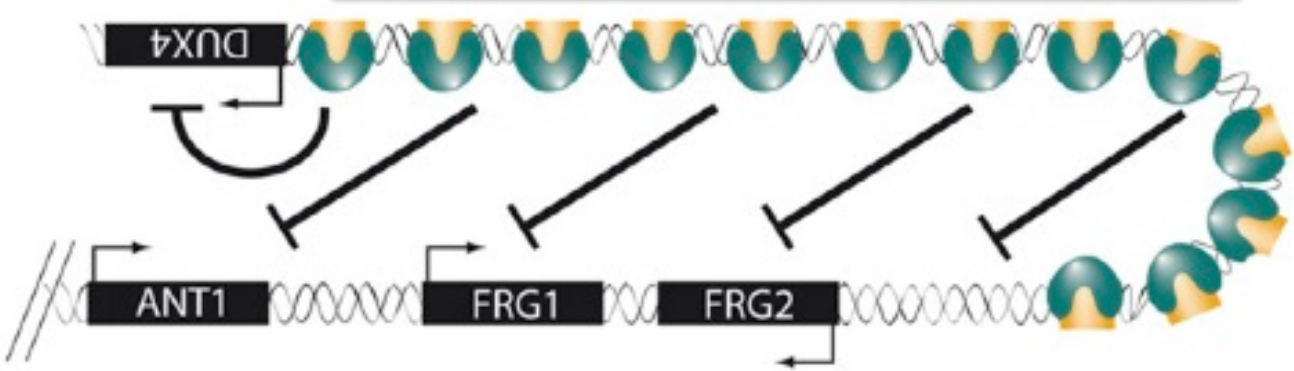
POLYCOMB COMPLEXES

DNA METHYLATION

HEALTHY

11 to 100 D4Z4 repeats

4q35 gene repression



A Long ncRNA Links Copy Number Variation to a Polycomb/Trithorax Epigenetic Switch in FSHD Muscular Dystrophy

Daphne S. Cabianca,[1] Valentina Casa,[1,2] Beatrice Bodega,[3,5] Alexandros Xynos,[1] Enrico Ginelli,[3] Yujiro Tanaka,[4] and Davide Gabellini[1,*]

[1]Dulbecco Telethon Institute at San Raffaele Scientific Institute, Division of Regenerative Medicine, Stem Cells, and Gene Therapy, 20132 Milan, Italy

[2]Università Vita-Salute San Raffaele, 20132 Milan, Italy

[3]Department of Biology and Genetics for Medical Sciences, University of Milan, 20133 Milan, Italy

[4]Genome Structure and Regulation, School of Biomedical Science and Biochemical Genetics, Medical Research Institute, Tokyo Medical and Dental University, Tokyo 113-8510, Japan

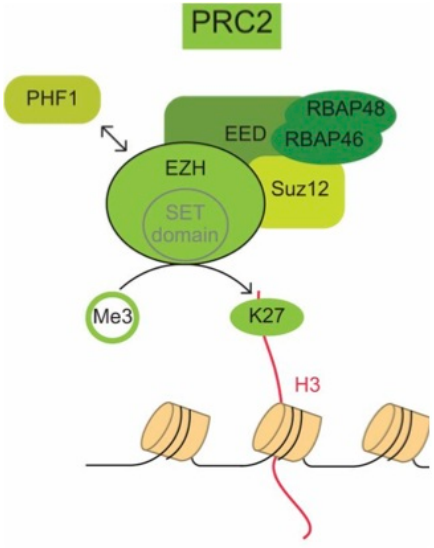
[5]Present address: Dulbecco Telethon Institute at Fondazione Santa Lucia, 00143 Rome, Italy

*Correspondence: gabellini.davide@hsr.it

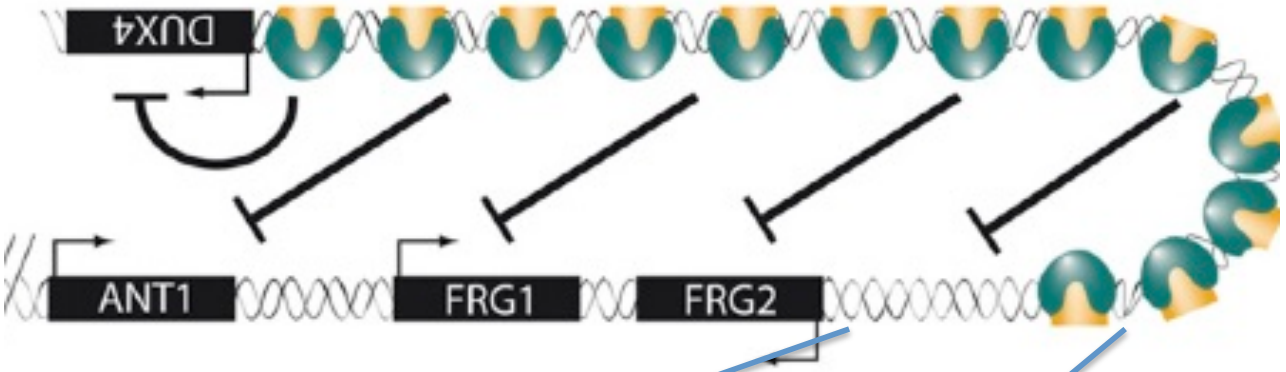
DOI 10.1016/j.cell.2012.03.035

IS SILENCING IMPAIRED IN FSDH PATIENTS??

PRC2 → H3K27me3



POLYCOMB COMPLEX 2



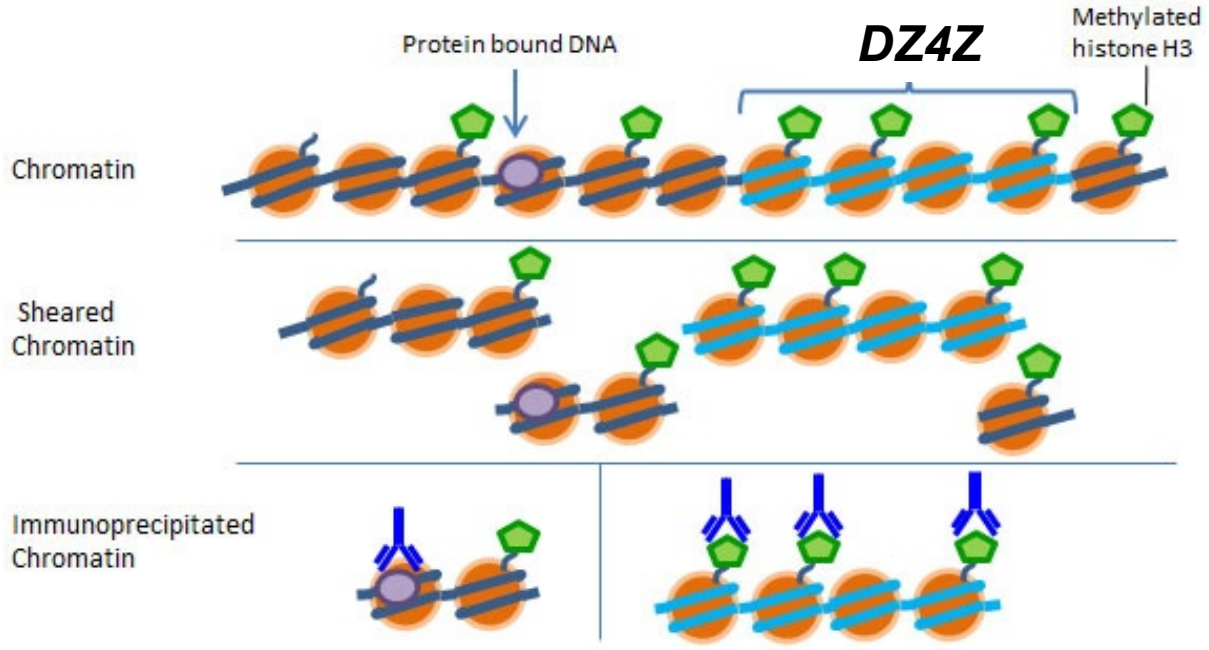
A

1 D4Z4 repeat

IS SILENCING IMPAIRED IN FSDH PATIENTS??

CHEMICALLY CROSSLINKED CHROMATIN ISOLATED FROM

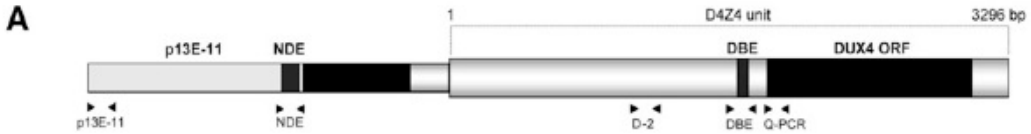
a. **Patient primary muscle cells**
b. **Normal primary muscle cells**



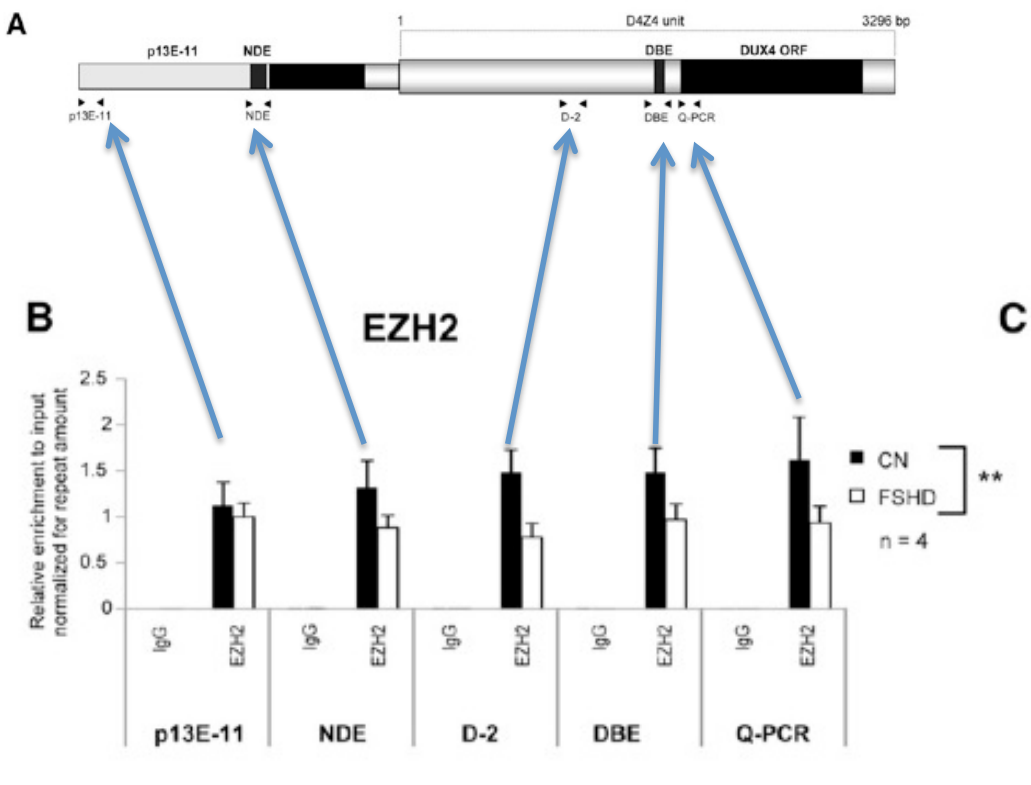
crosslinked chromatin

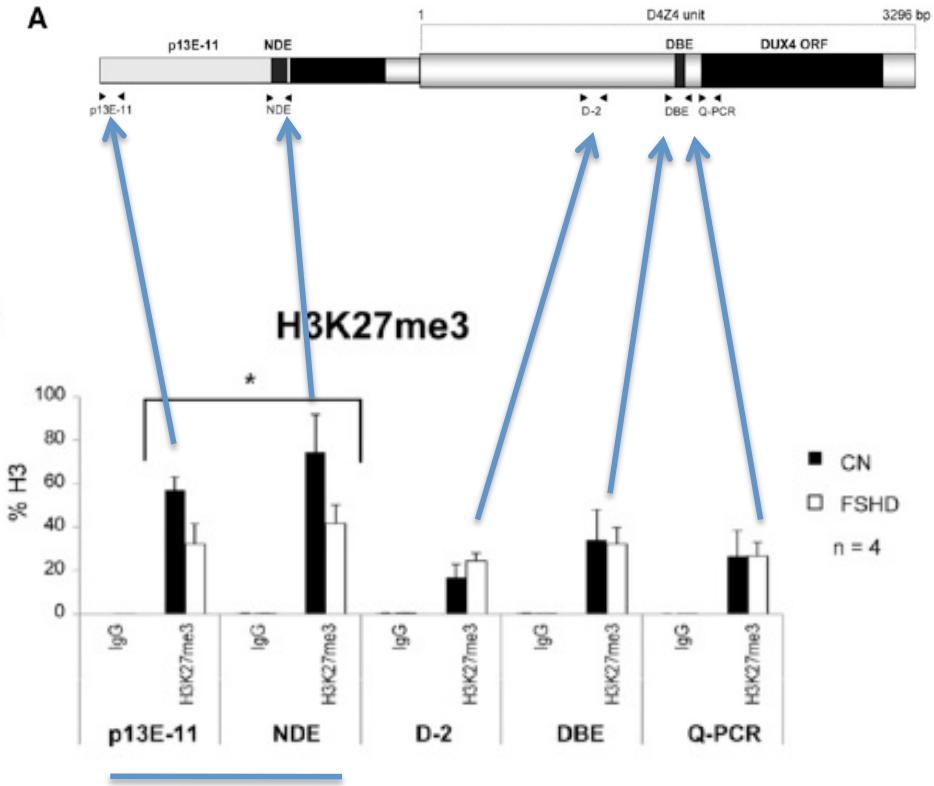
Precipitate-Ab-Chromatin complex with beads that bind heavy chain of antibody

Make PCR with primers that amplify specific regions in D4Z4 repeats

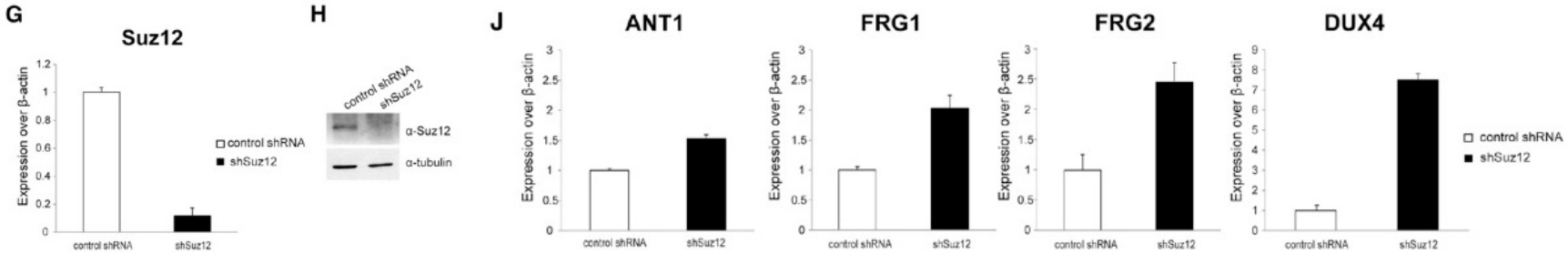


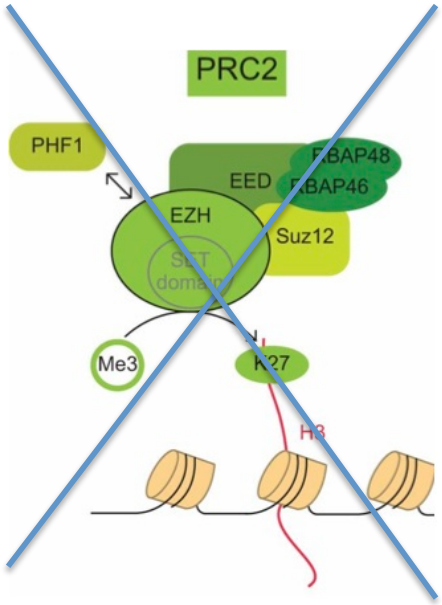
FSDH IS LINKED WITH LOSS OF PRC2 FUNCTION AT DZ4Z REPEATS

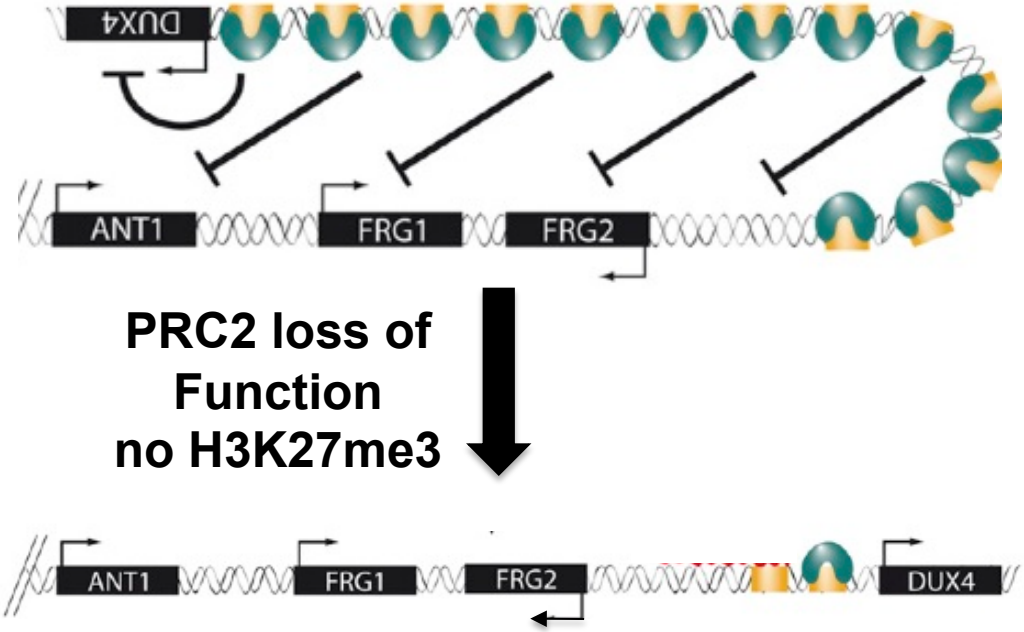




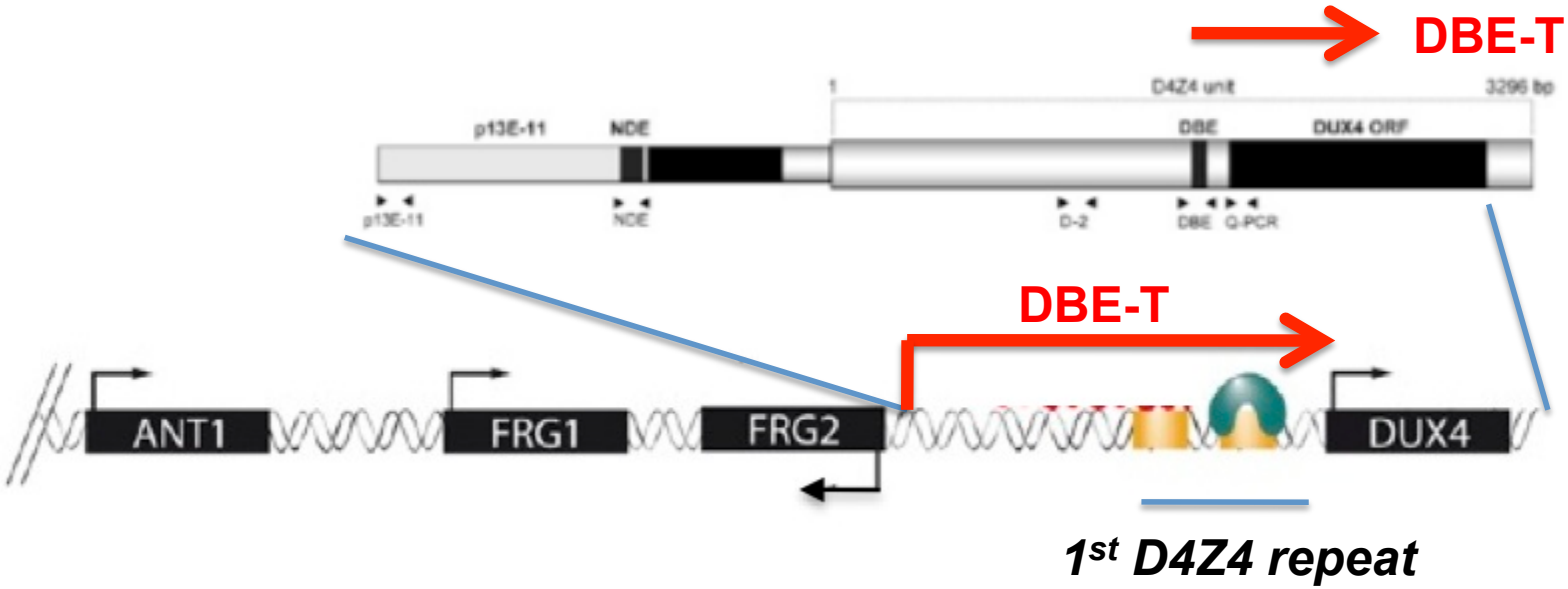
LOSS OF PRC2 FUCNTION INCREASES ANT1---DUX4 EXPRESSION



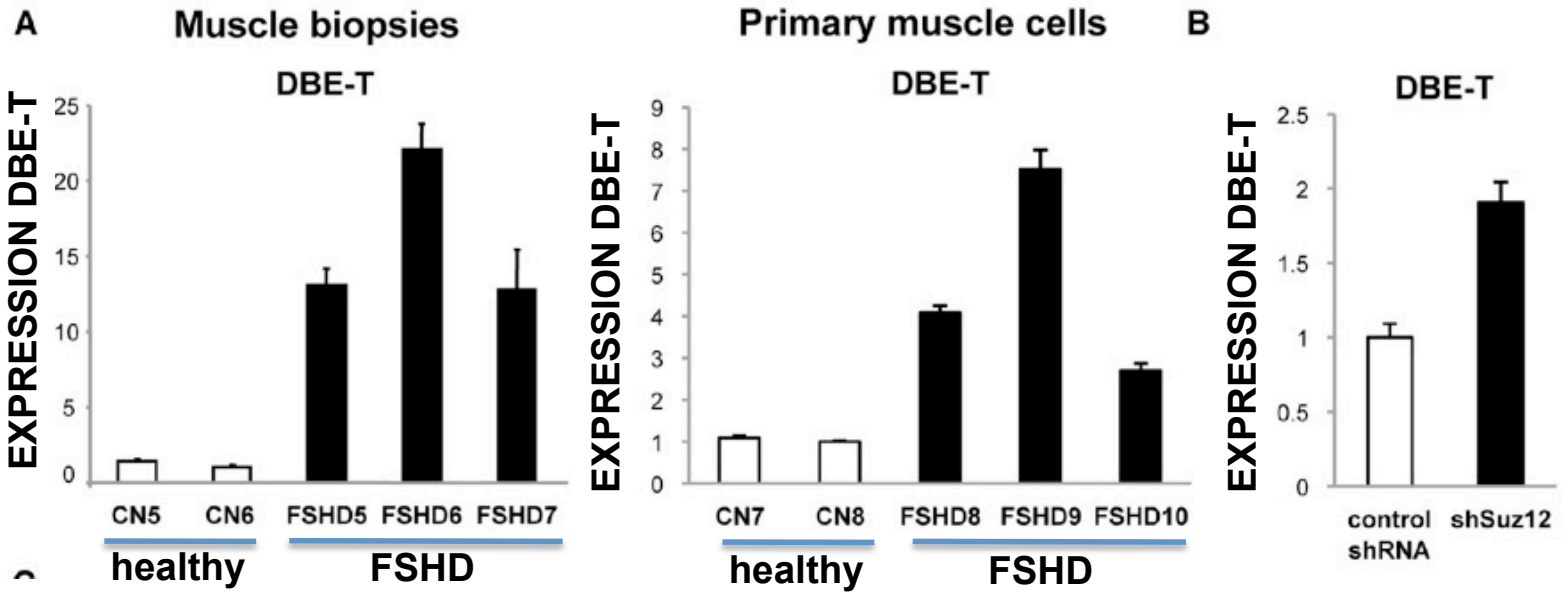




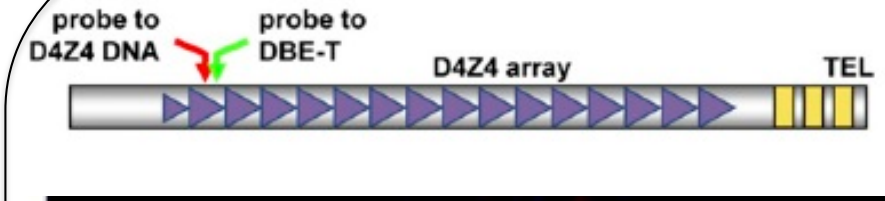
LOSS OF PRC2 FUCNTION CAUSES AN UPREGUALTION OF A NOVEL lncRNA – DBE-T

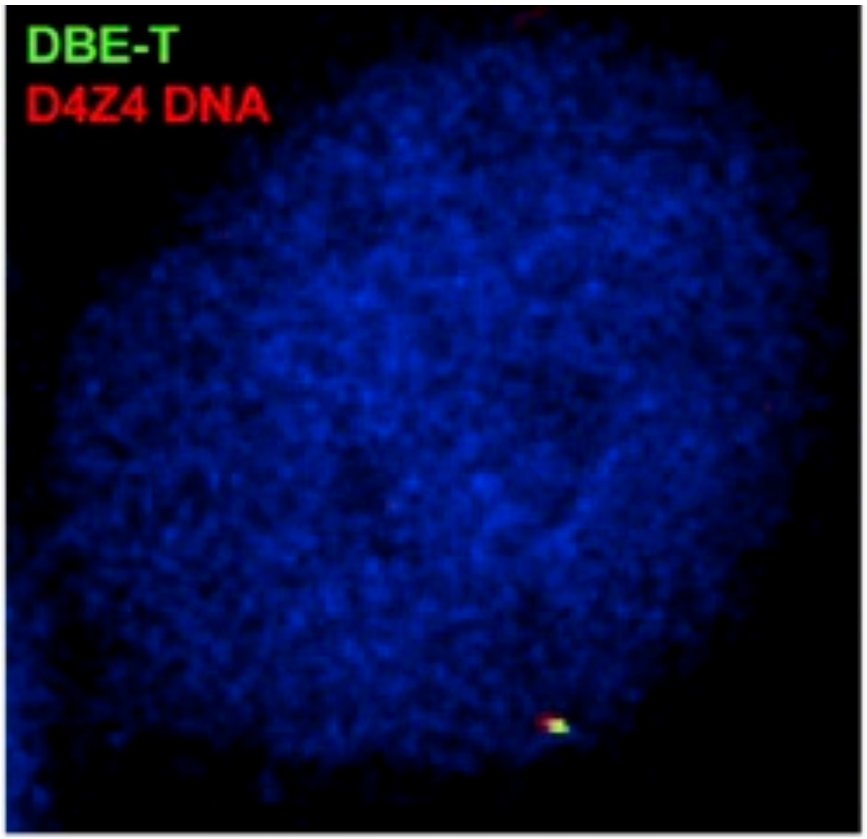


CONDITIONS:
- FSDH
- Loss of PRC2 function



DBE-T lncRNA COLOCALIZES TO D4Z4 REPEATS

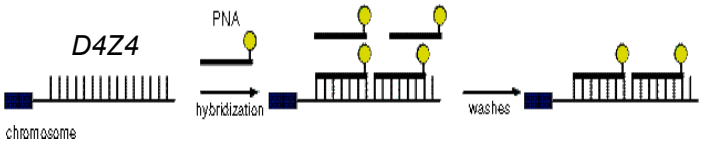




COMBINED DBE-T RNA-FISH AND D4Z4 DNA FISH

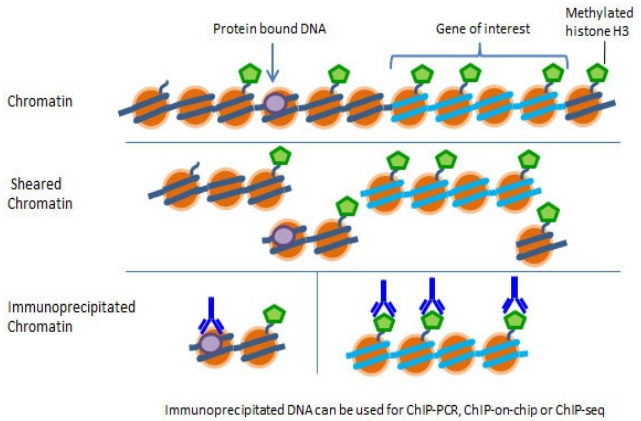
1. FIX CELLS
2. HYBRIDIZE A DBE-T PROBE
(fluorescently labelled green)
2. CAPUTURE IMAGE WITH
3. MICROSCOPE
4. WASH
5. DENATURE DNA (HEAT)
6. HYBRIDIZE D4Z4 PROBE
(fluorescently labelled – red)
7. CAPUTURE IMAGE
8. SUPERIMPOSE IMAGES

Fluorescence in situ hybridization



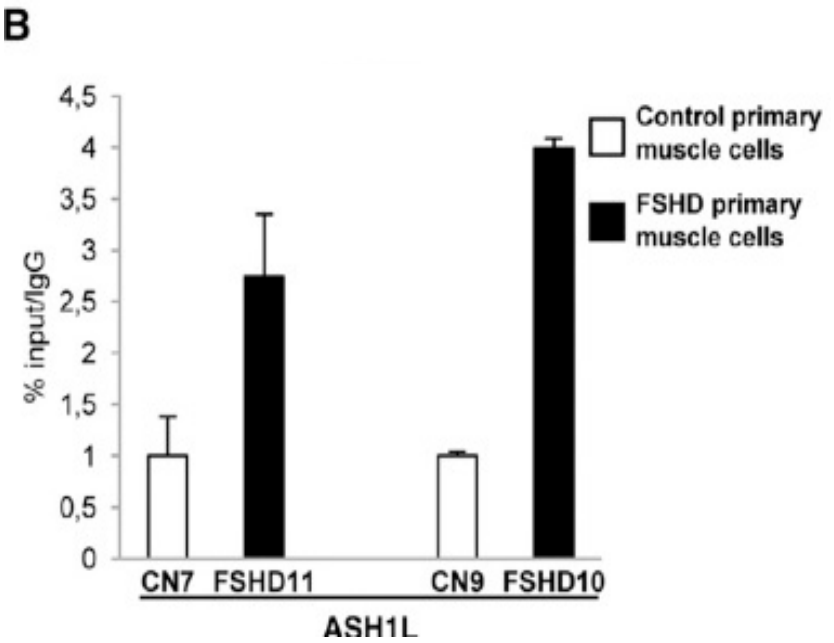
D4Z4 repeats

THE HMTase Ash1L localizes to DZ4Z REPEATS

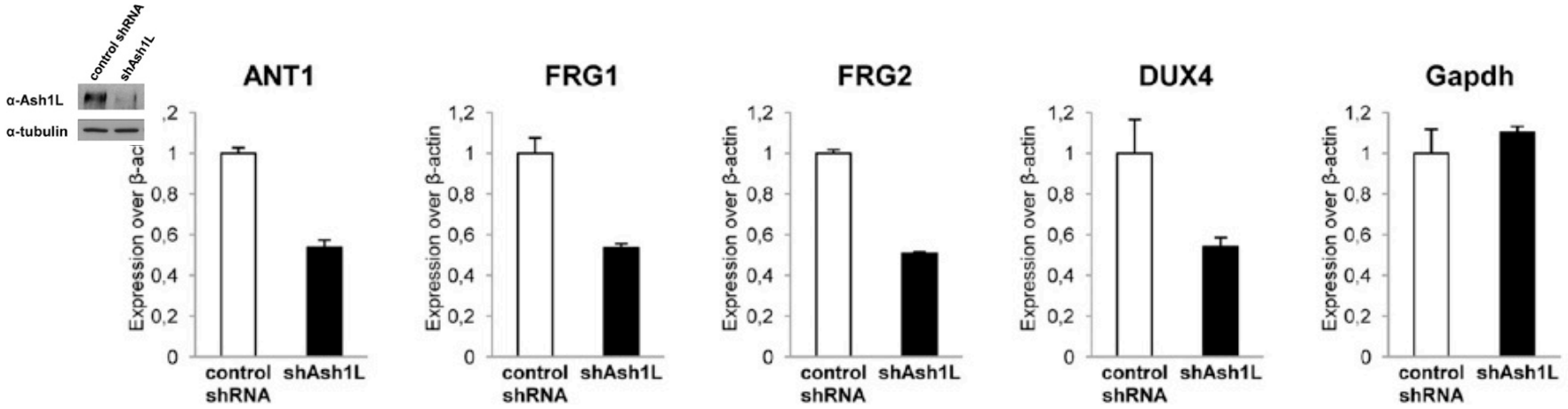


Ashl1: Histone methyltransferase
→H3K4me3
→H3K36m2

ACTIVATES TRANSCRIPTION

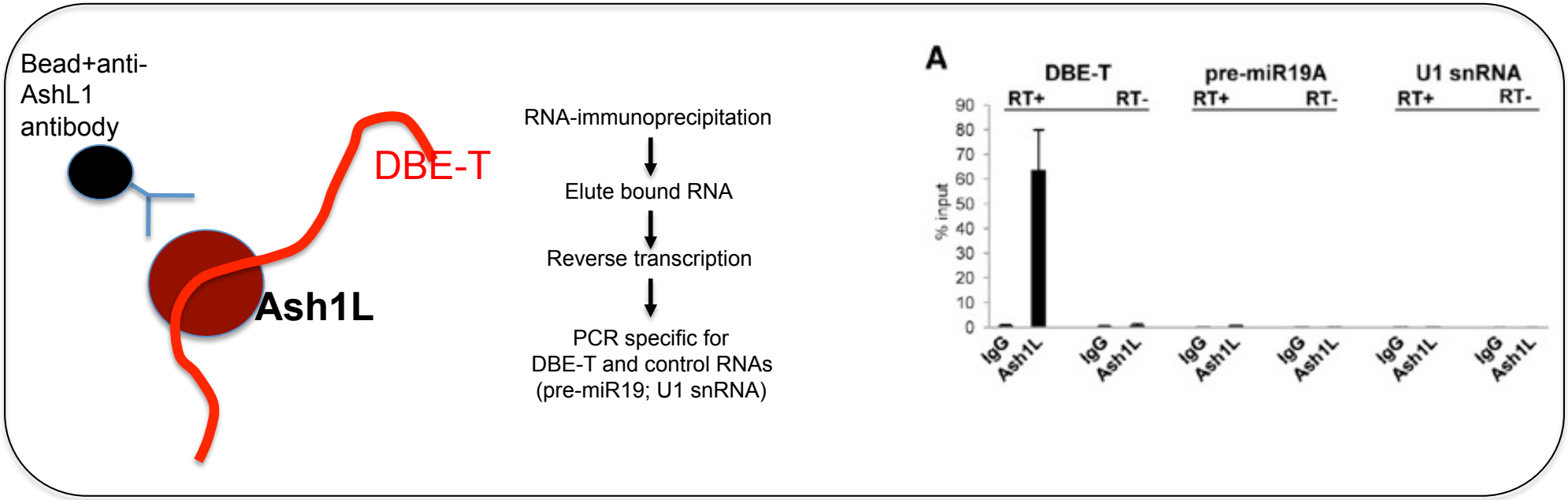


Loss of Ash1L increases the expression of genes close to D4Z4 repeats



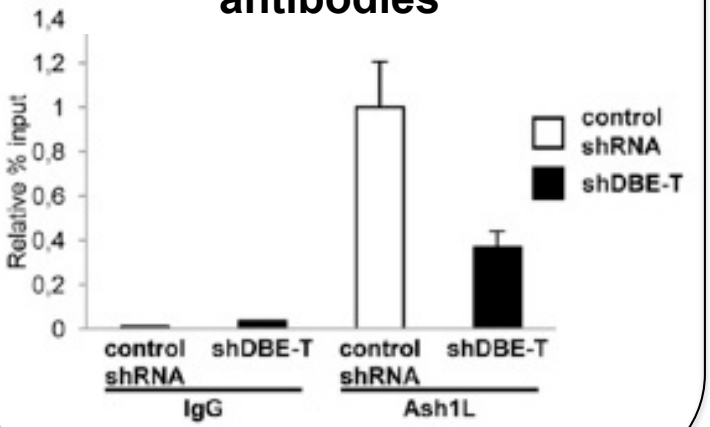
Ashl1 shRNA experiments

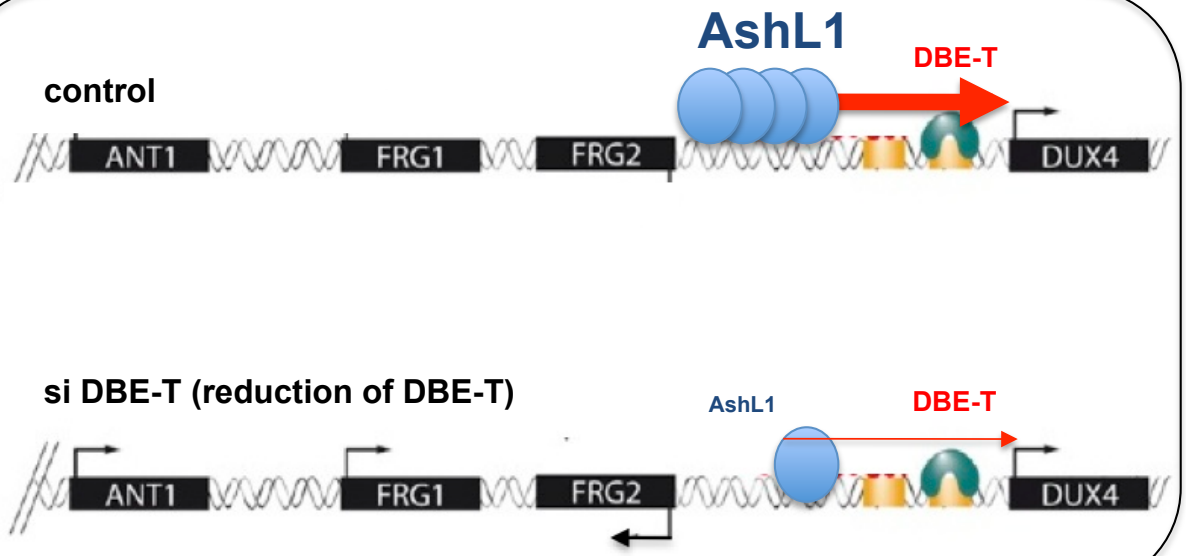
DBE-T INTERACTS WITH Ash1L



DBE-T BRINGS Ash1L TO D4Z4 REPEATS

ChIP using anti-Ash1L antibodies

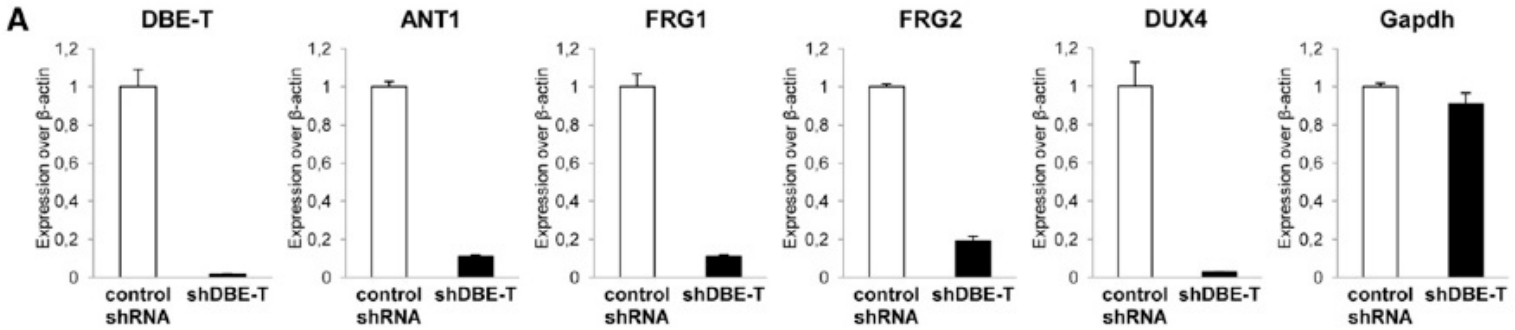




Loss of DBE-T results in increased expression of D4Z4 neighboring genes

Gene expression RT-PCR

control and si DBE-T (reduction of DBE-T)



A lincRNA IS USED TO TRANSMIT D4Z4 REPEAT NUMBER INTO A DISEAS RELEVANT MECHANISM