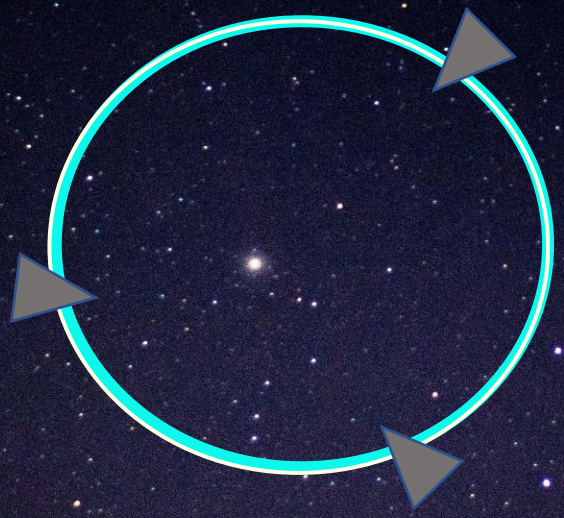
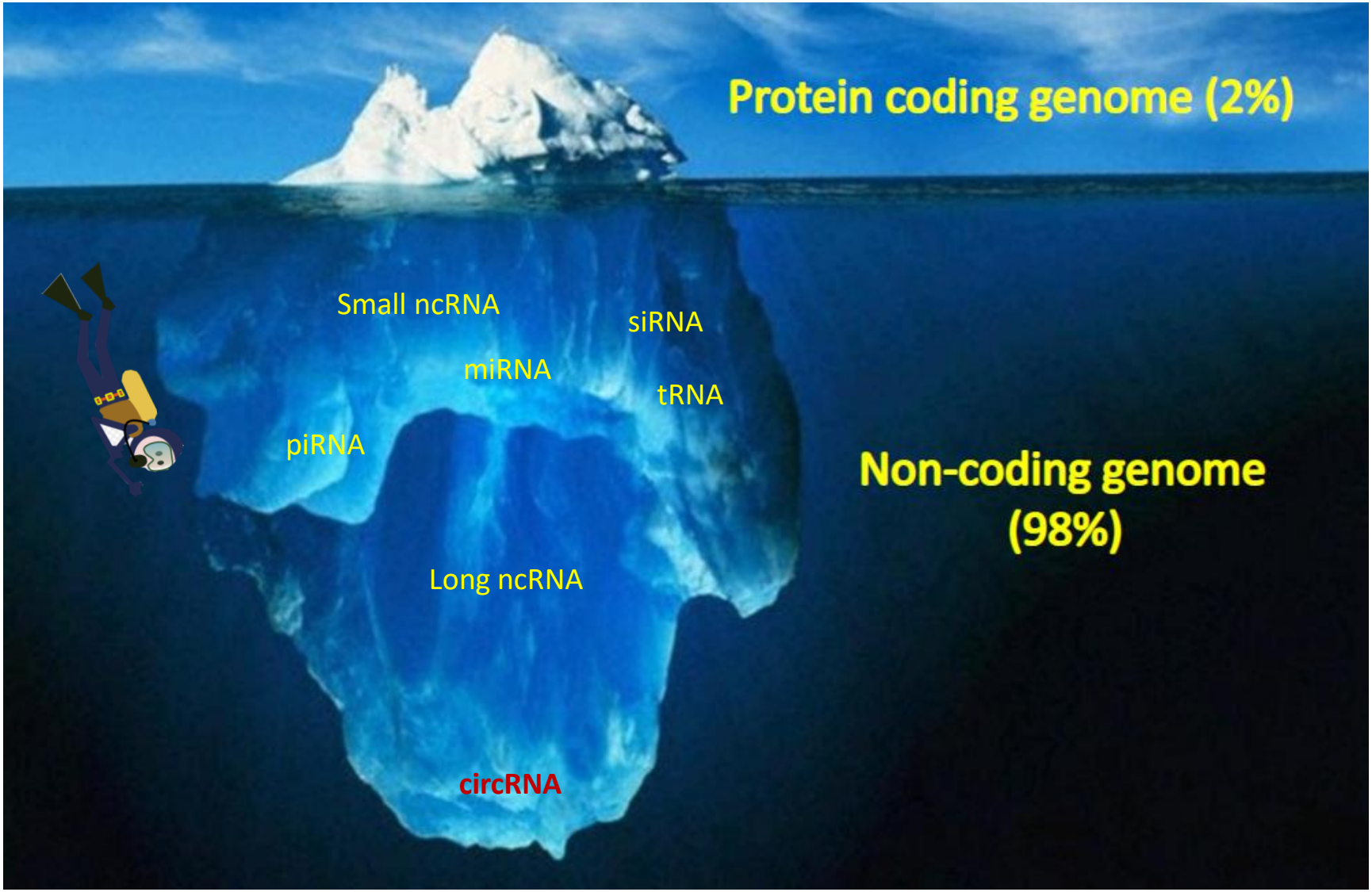
circRNAs

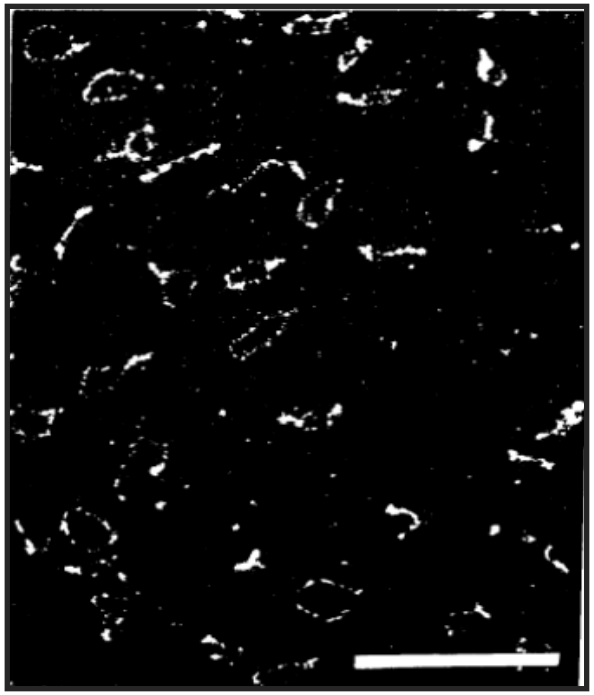
FRoM DARKNeSS To LiGHT



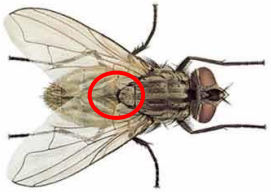

Protein coding genome (2%)
Small ncRNA
siRNA
miRNA
tRNA
piRNA
Non-coding genome
(98%)
Long ncRNA
circRNA

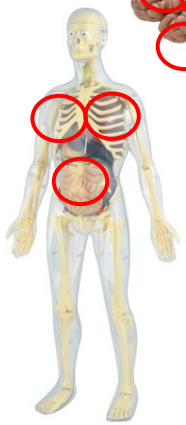
circRNA DISCOVERY

First viral circRNA reported in 1976

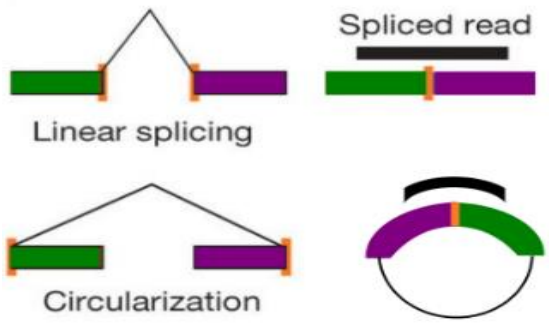


RNA sequencing in the 21st century

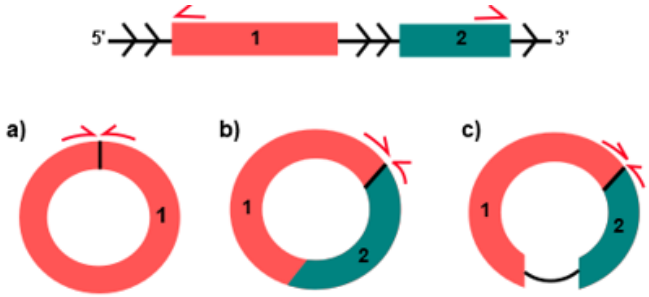




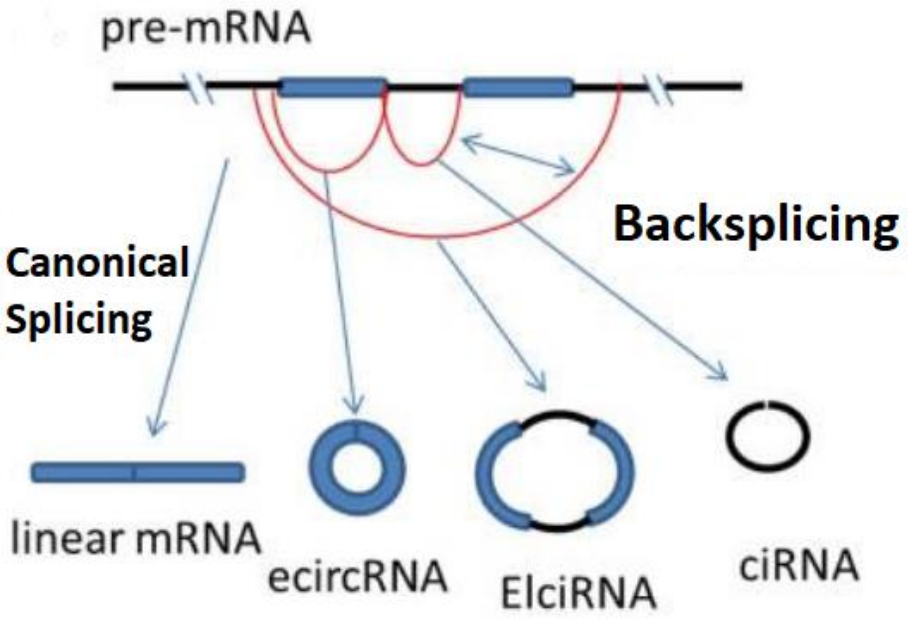
circRNA FORMATION



circRNAs can be detected by backspliced reads



circRNAs can arise from 1 or more Exons Are detected by divergent primers



Well stablished features

Less abudant than mRNAs

Predominantly cytoplasmic

Non polyadenylated

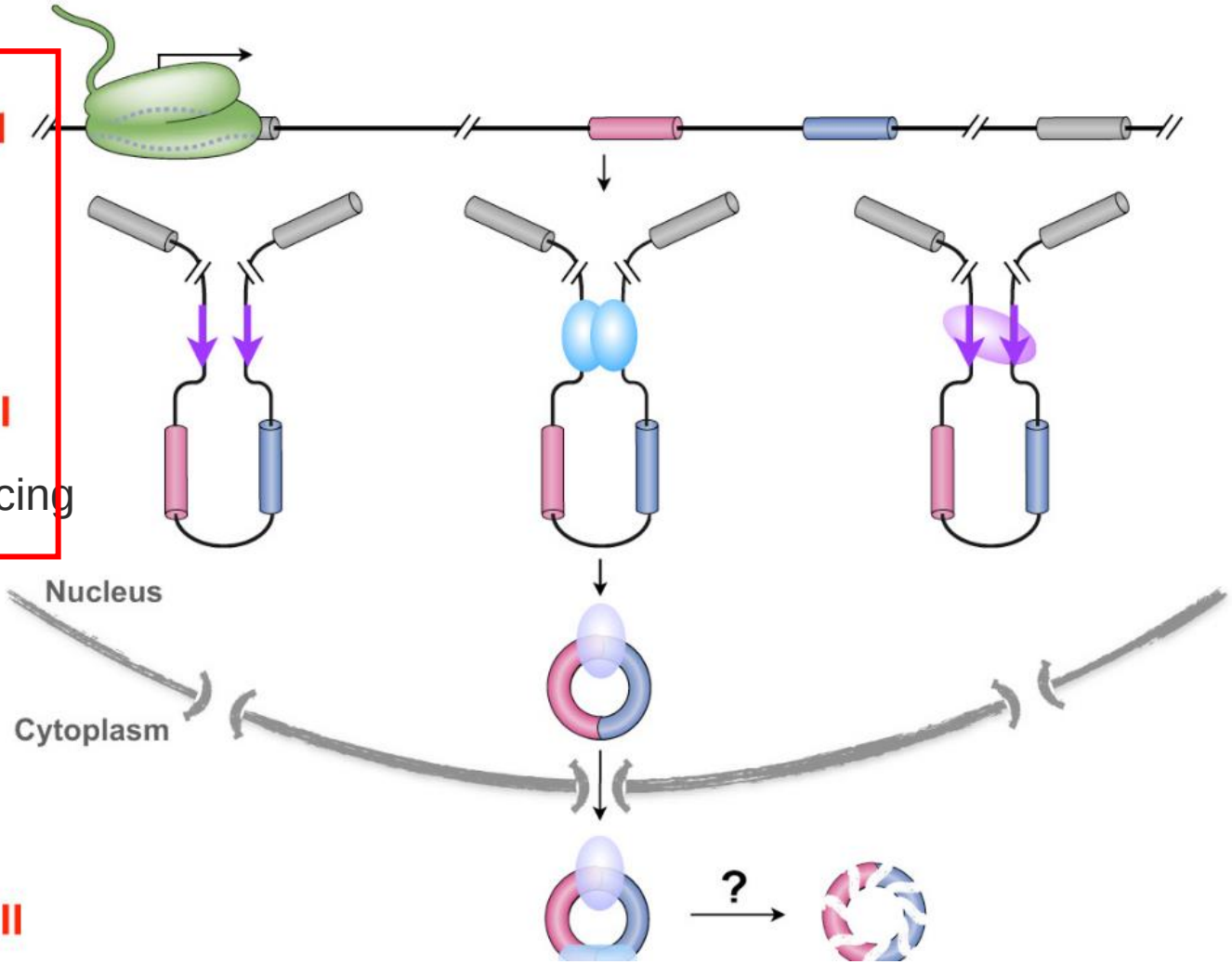
circRNA REGULATION

circRNA expression can be regulated at three levels

Transcription of circRNA-producing pre-mRNA by Pol II. I

cis and *trans* regulatory factors Influence backsplicing II

BACKSPLICING



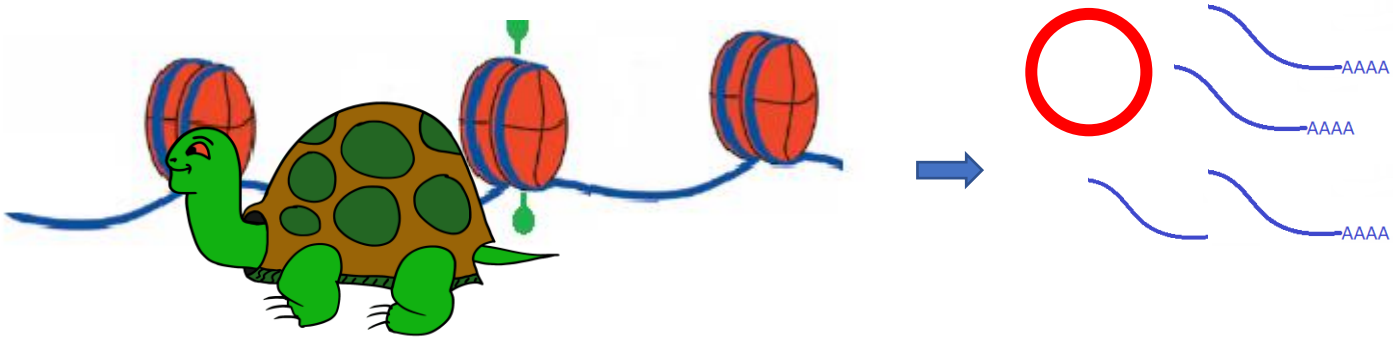
circRNA turnover III

BACKSPLICING

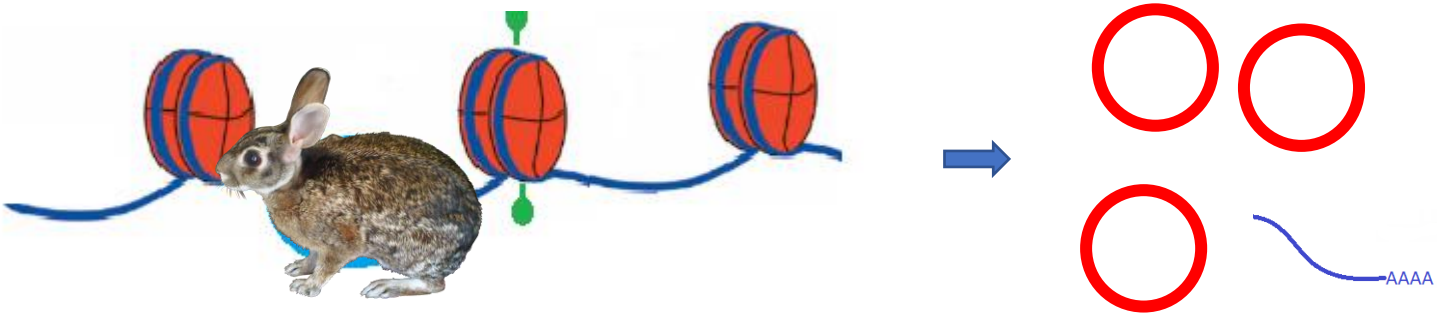
Backsplicing of circRNA can occur both co- and post-transcriptionally!!!!

Co-transcriptional regulation:

SLOW POLII

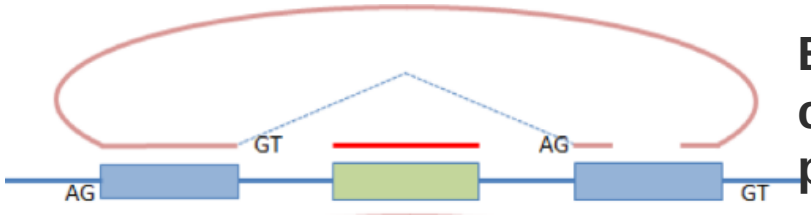


FAST POLII



Average Pol II TER of nascent circRNA host genes is higher

BACKSPLICING



Backsplicing efficiency depends on canonical ss bracketing circRNA-producing exons.

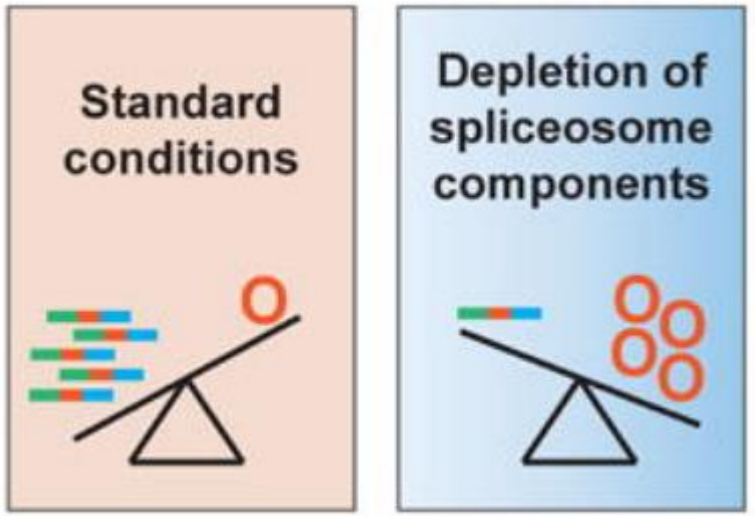
Efficiency of backsplicing is lower than that of canonical splicing

Loser

Vs

How do cells modulate the amounts of circular versus linear RNAs????

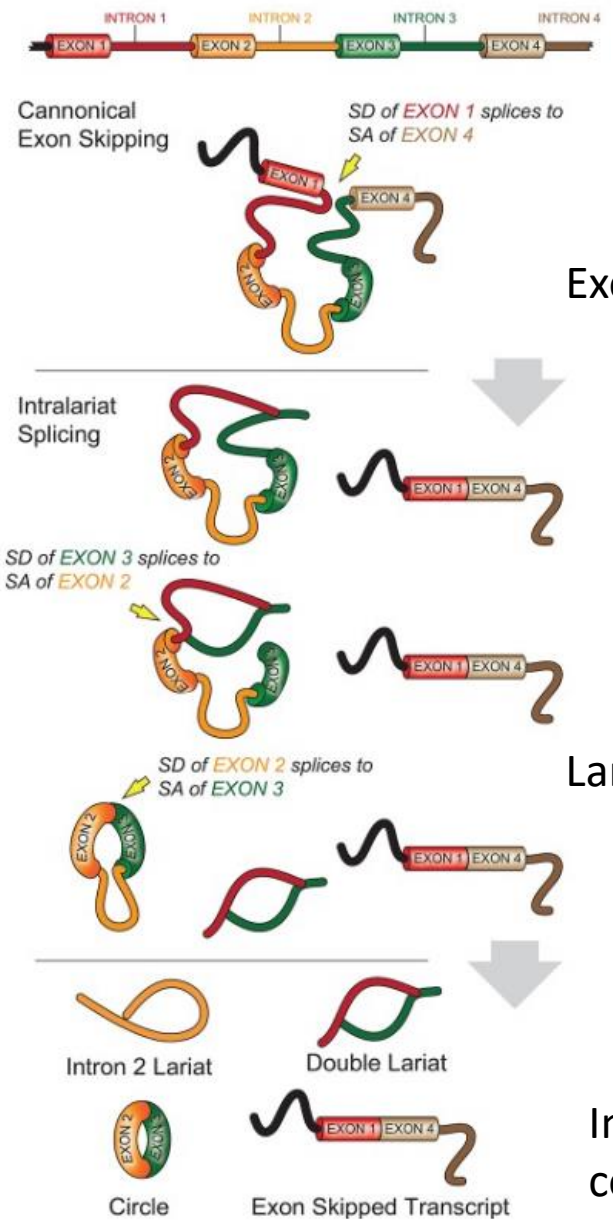
Shift from canonical splicing to back-splicing under spliceosome starving conditions by depleting spliceosomal factors: SF3b and SF3a



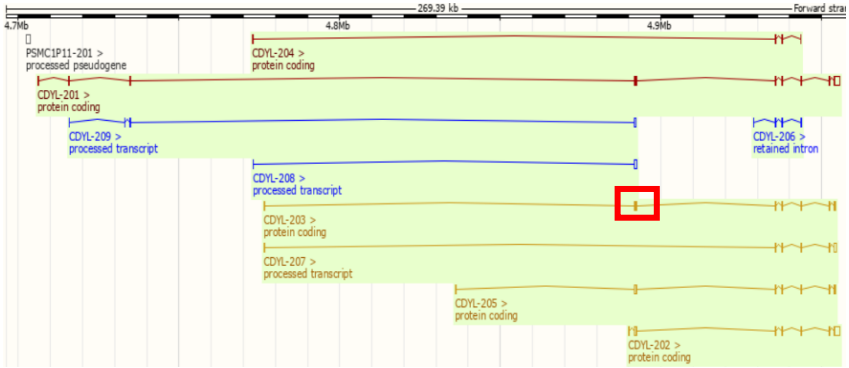
HOW the spliceosome is involved in backsplicing still remains unknown

BACKSPLICING

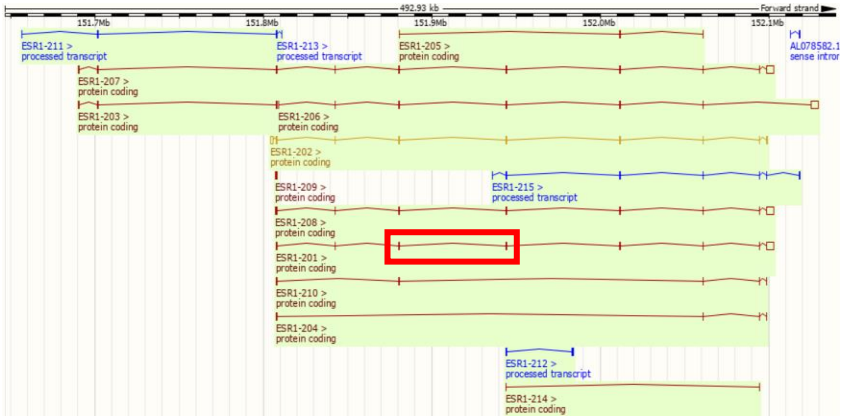
Circular RNAs can result from exon-skipping events



Exon skipping



Lariat formation



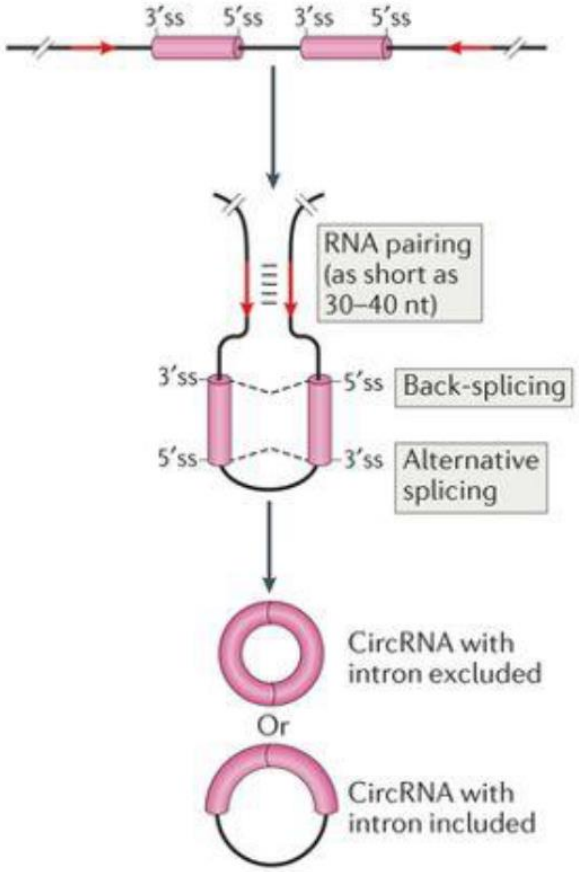
Internal splicing in the lariat containing exons

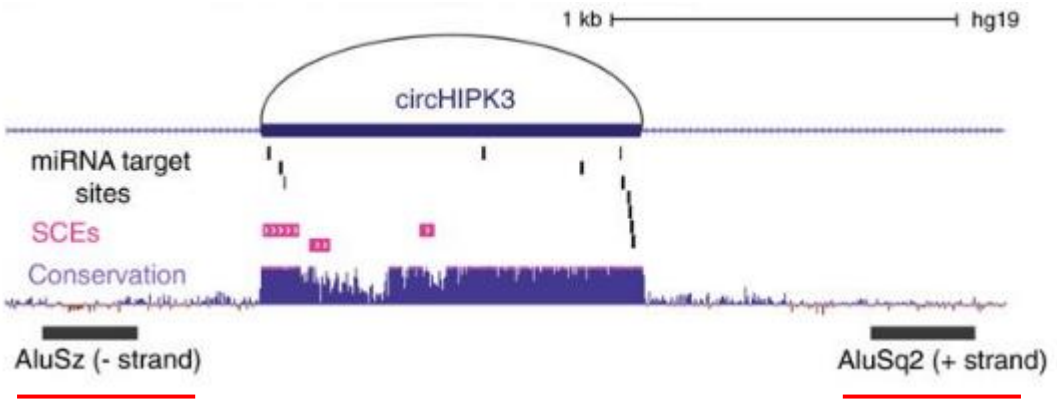
BACKSPLICING

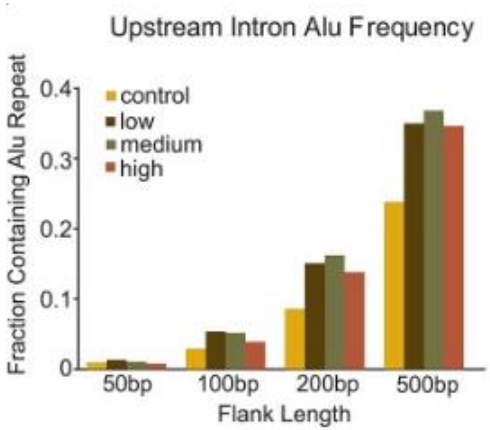
Regulation of circRNA Biogenesis by cis Elements

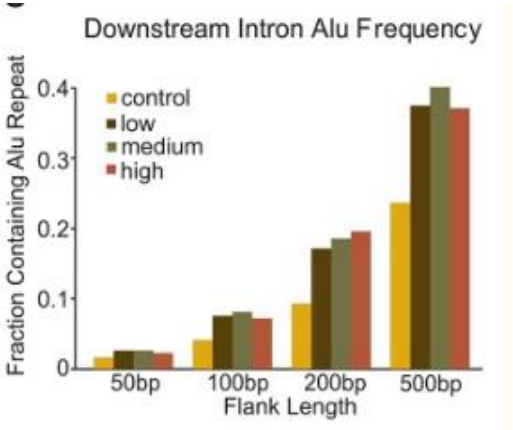
RNA pairing facilitates backsplicing

RNA pairs derive from repetitive elements: **ALU**







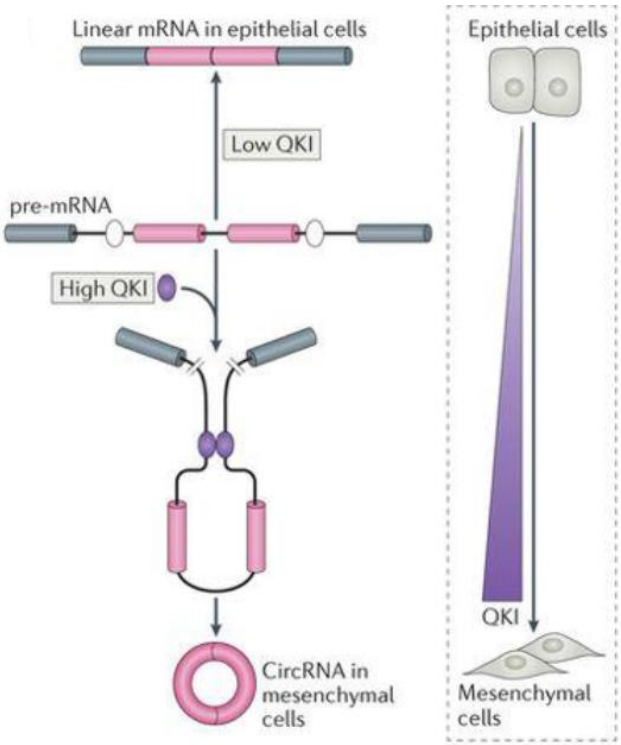


BACKSPLICING

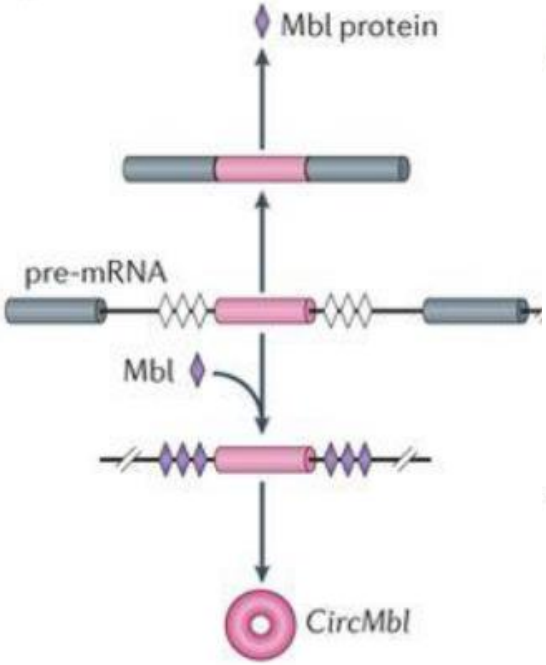
Regulation of circRNA Biogenesis by RBPs

RBPs can facilitate or inhibit backsplicing

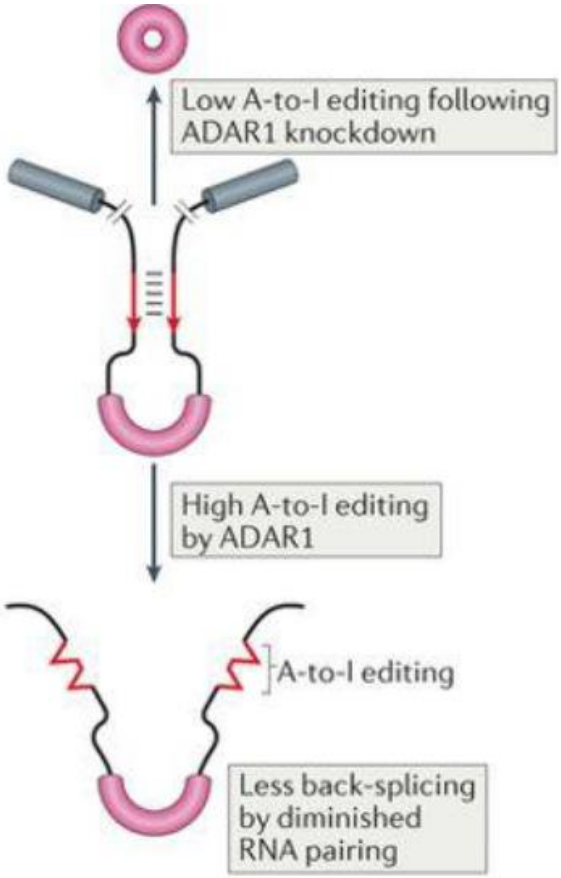
QKI, FUS,hnRNPL



MBL

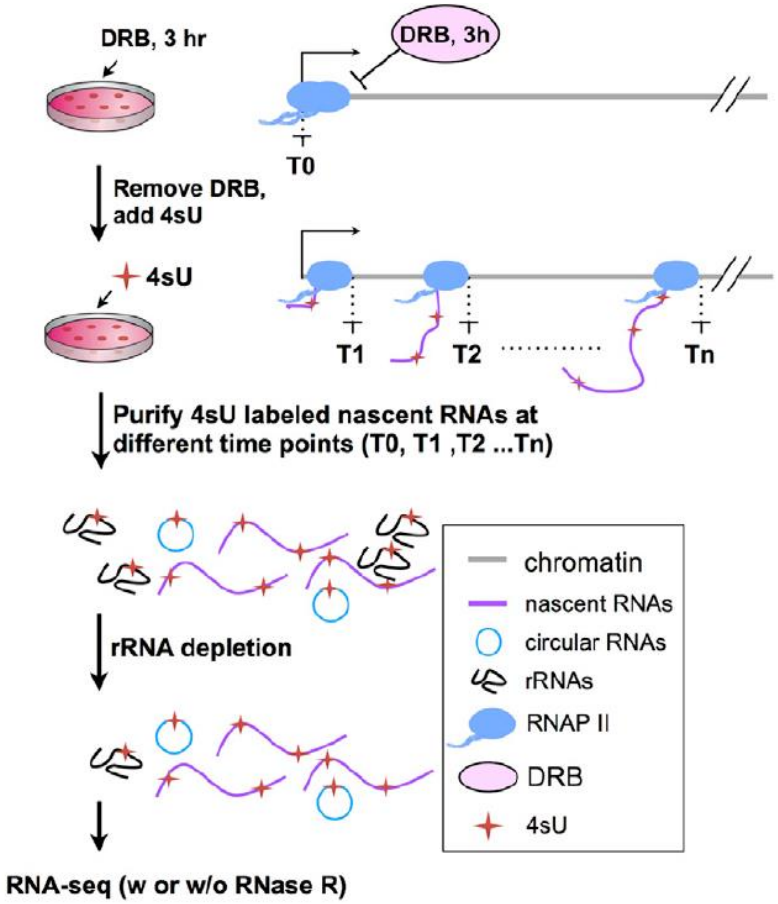


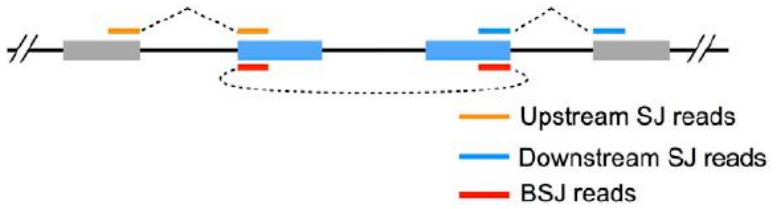
ADAR1



circRNA TURNOVER

Measuring nascent circRNA



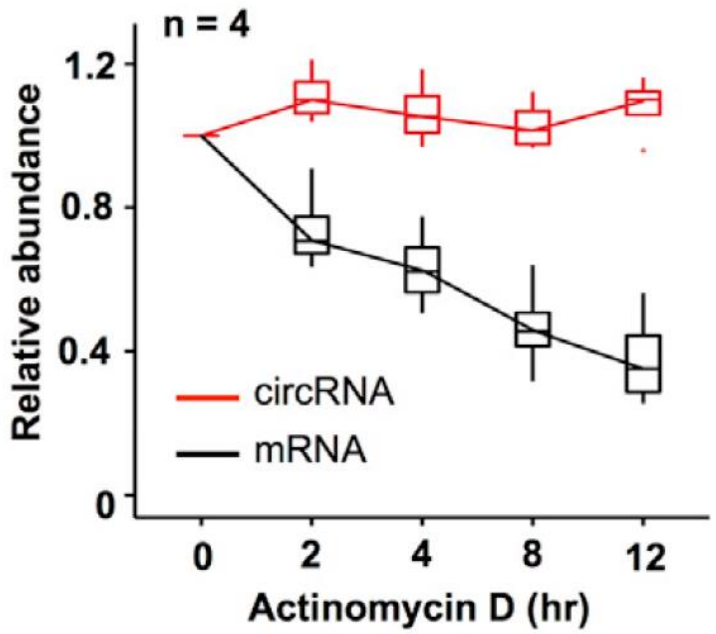


4sU tagging time	Total SJ reads	Total upstream SJ reads	Total BSJ reads	Total downstream SJ reads	4sU-labeled circRNAs	Host genes
0 min	N/A	N/A	N/A	N/A	0	0
10 min	471,029	253	22	331	11	11
15 min	519,319	368	15	294	10	10
30 min	1,265,638	2,064	130	2,267	106	101
60 min	4,354,945	16,938	319	20,353	255	239
120 min	11,606,719	111,865	913	120,368	641	534
4 hr	35,541,526	802,959	3,426	932,150	1,837	1,304
16 hr	61,414,247	3,182,197	19,023	3,590,635	7,156	3,141

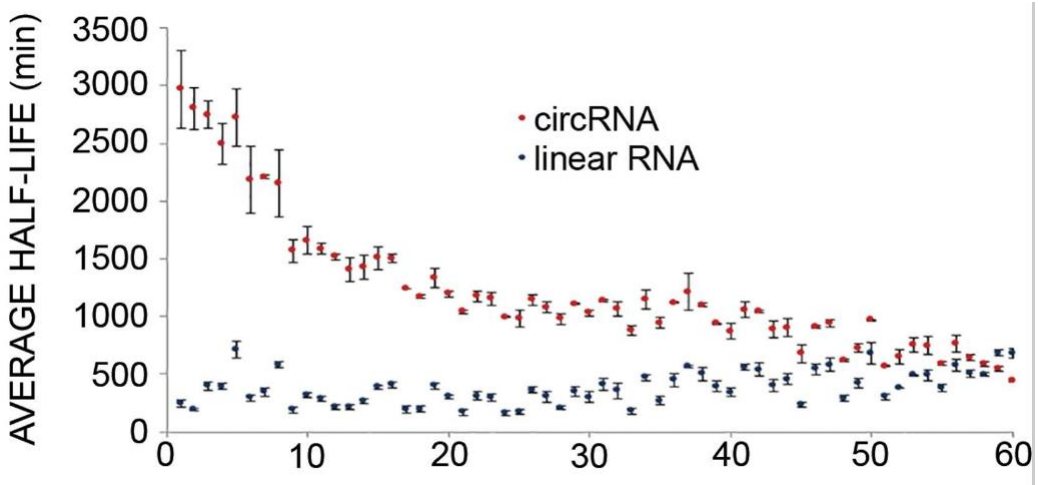
Backsplicing is low efficient and largely occurs post transcriptionally

circRNA TURNOVER

circRNAs are more stable than mRNAS



In cells with a slow division rate, like neurons, highly expressed circRNAs are the dominant RNA isoforms produced from some genes

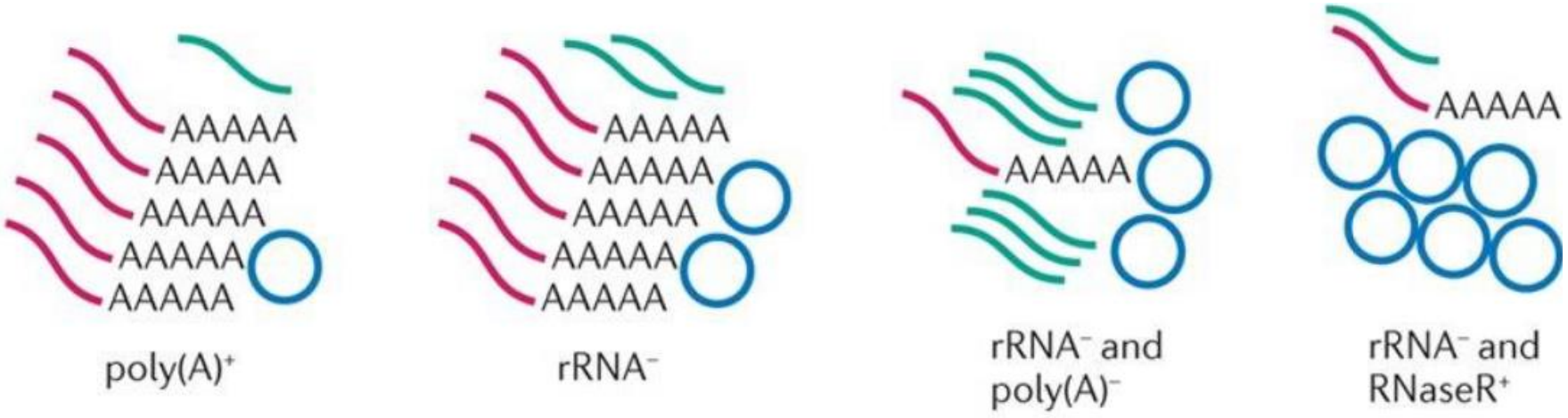


circRNA Half-life: 18.8-23.7h

mRNA Half-life: 4.0-7.4h

circRNA Detection

RNA sequencing



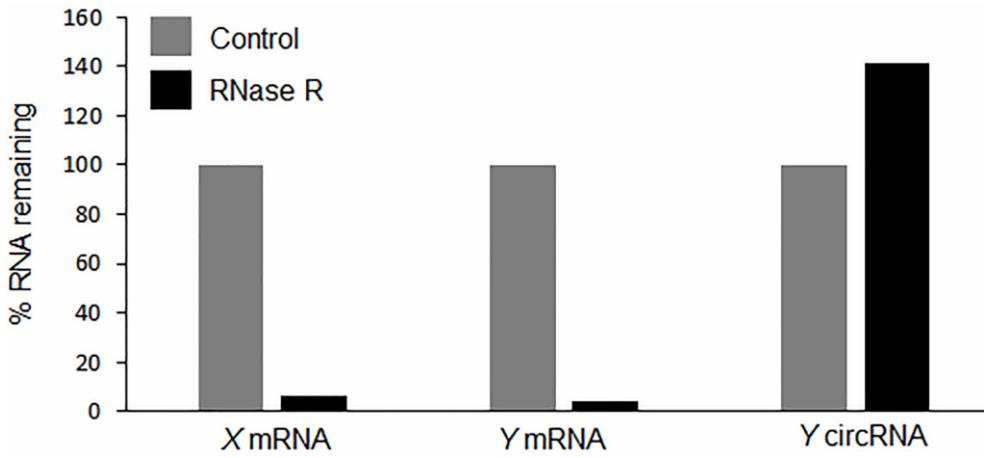
circRNA Detection

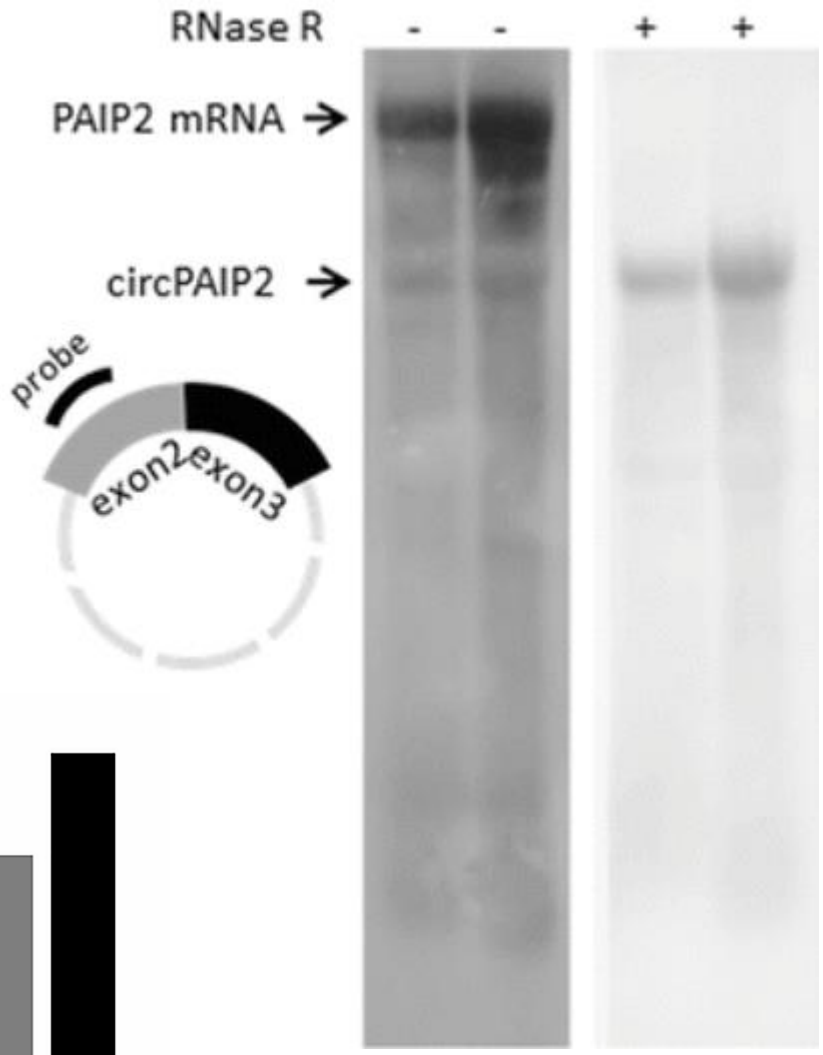
Bioinformatic tools for circRNA detection

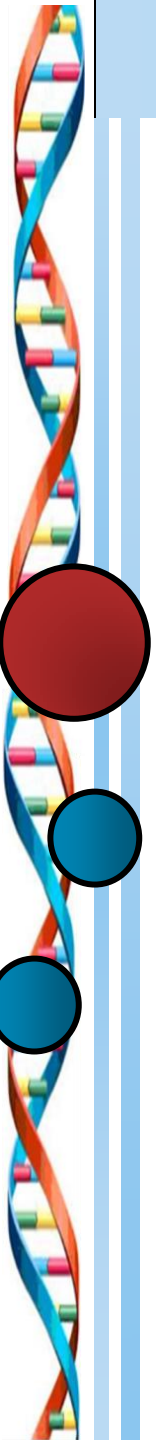
Tool	Version	Language	Mapper	*De novo*?	URL
ACFS	2.1	Perl	Bwa	Yes	https://github.com/arthuryxt/acfs
CIRCexplorer	1.0.6	Python	Tophat	No	https://github.com/YangLab/CIRCexplorer
CIRCexplorer2	2.0.1	Python	Tophat	Yes	https://github.com/YangLab/CIRCexplorer2
circRNA_finder	N/A	Perl	STAR	Default	https://github.com/orzechoj/circRNA_finder
CIRI	1.2	Perl	Bwa	Default	https://sourceforge.net/projects/ciri/files
CIRI2	2.0.6	Perl	Bwa	Default	https://sourceforge.net/projects/ciri/files/CIRI2/
DCC	0.4.4	Python	STAR	Default	https://github.com/dieterich-lab/DCC
Find_circ	N/A	Python	Bowtie2	Default	http://www.circbase.org/
KNIFE	1.4	Python/Perl/R	Bowtie2	Yes	https://github.com/lindaszabo/KNIFE
Mapsplice	2.1.8	Python	Tophat	No	http://www.netlab.uky.edu/p/bioinfo/MapSplice2
Uroborus	0.1.2	Perl	Tophat	No	https://github.com/WGLab/UROBORUS

circRNA Validation

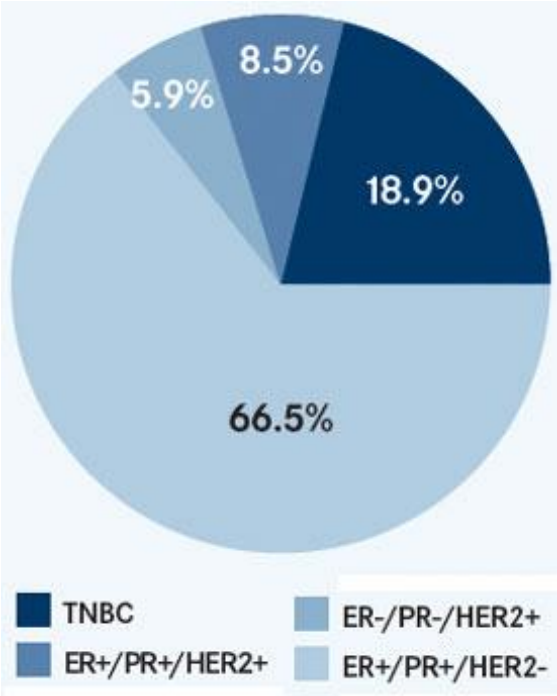
circRNAs can be detected by RNAseR treatment or Northern Blot







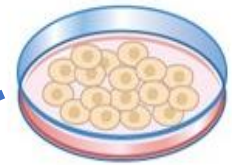
Breast Cancer



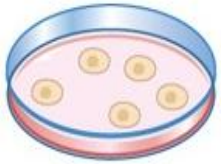
Deep characterization of circRNAs in the most spread subtype of Breast Cancer, the luminal subtype. Elucidate some aspects regarding detection, and biogenesis of circRNAs in cancer

EXPERIMENTAL DESIGN

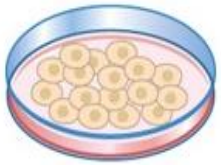
12 RNA-Seq PolyA- in MCF-7



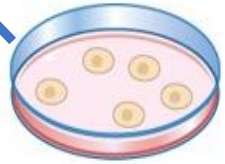
siRNA Ctr + Full Medium



siRNA Ctr + Hormone Deprived

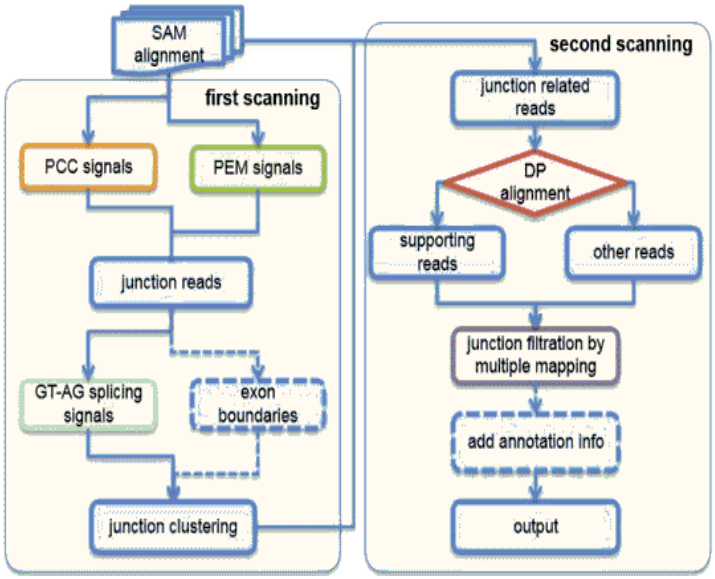


siRNA Ctr + E2 stimulation



siRNA ERα

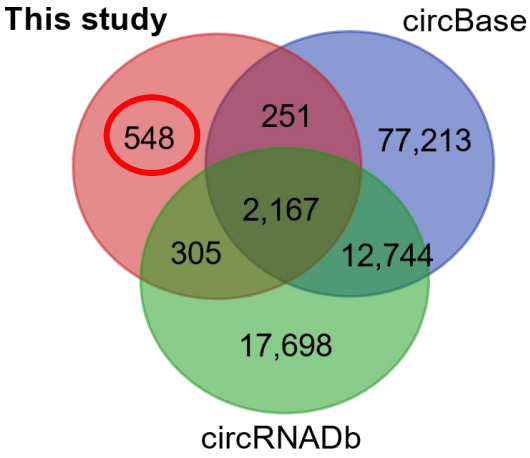
CIRI ALGORITHM



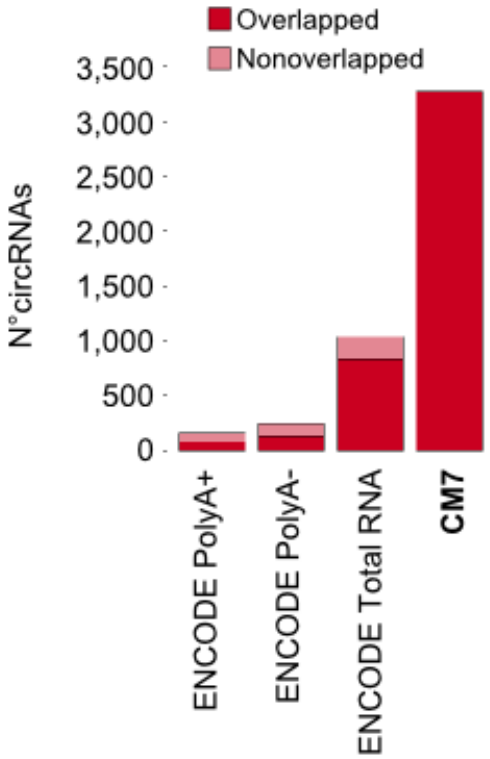
3,271 circRNAs CM7

DETECTION

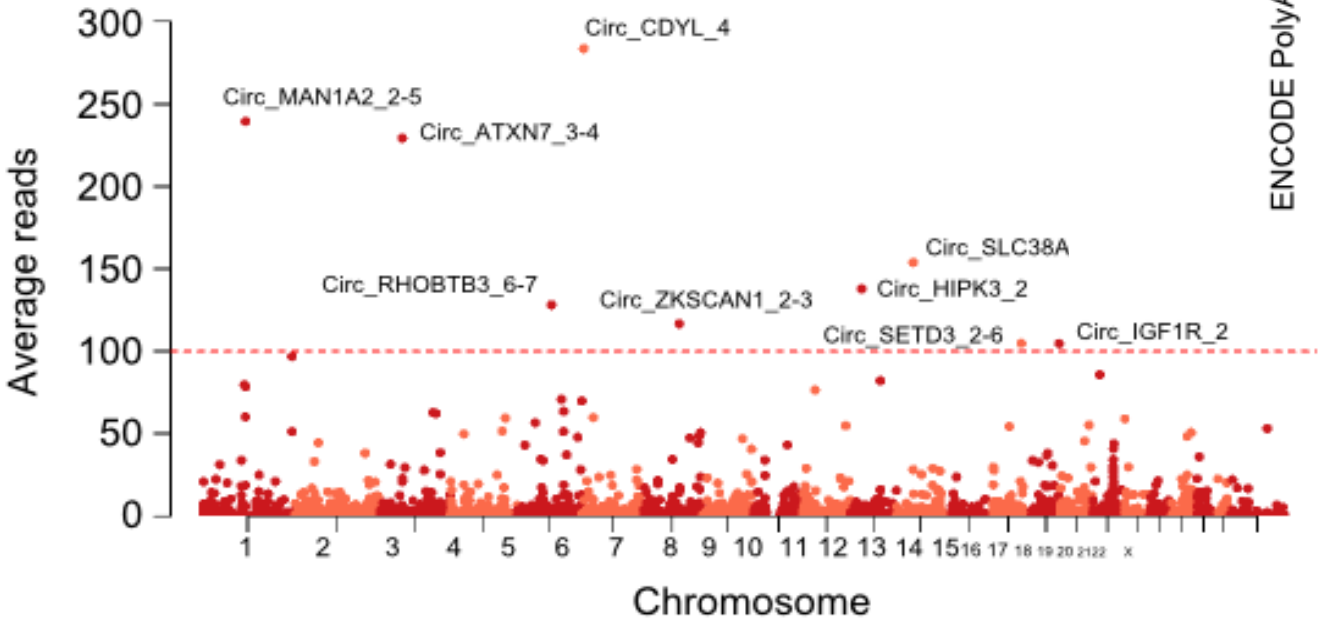
Overlap with public circRNA annotations



Overlap with public MCF-7 datasets

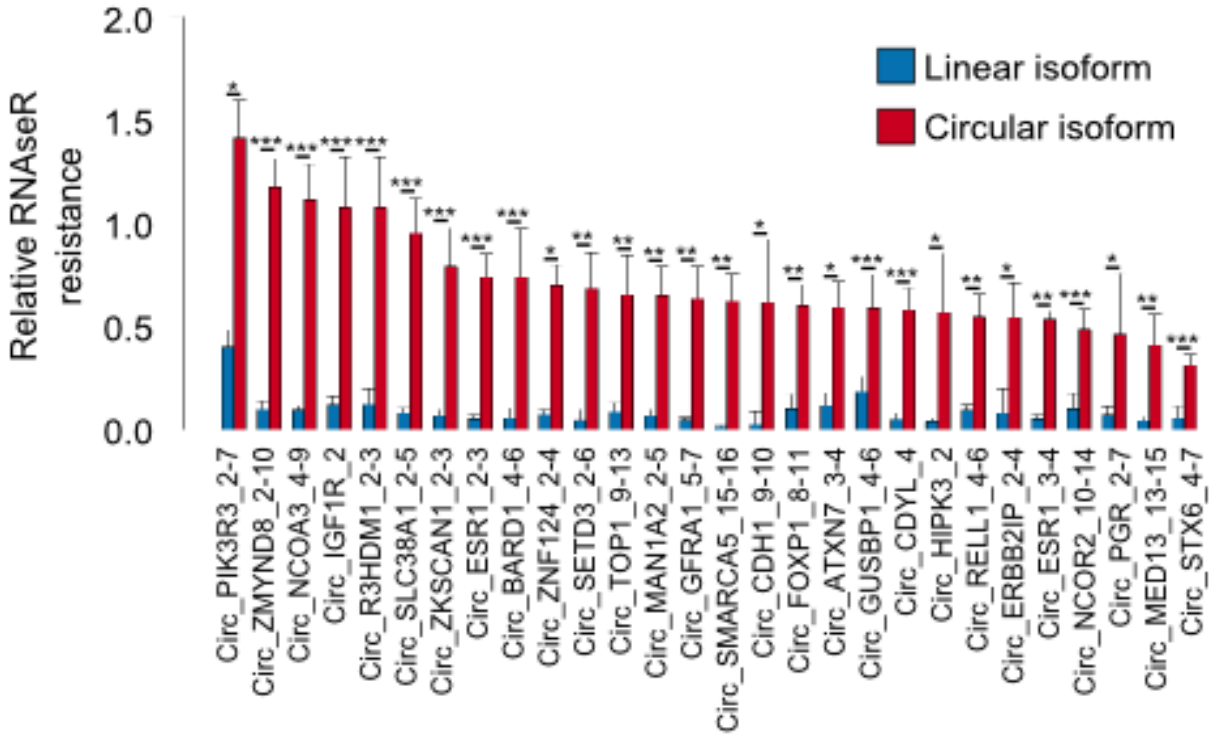


CM7 expression

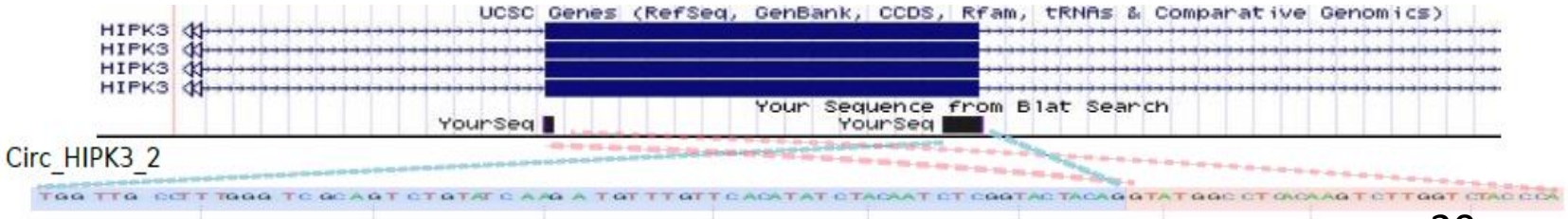


VALIDATION

RNAseR Treatment

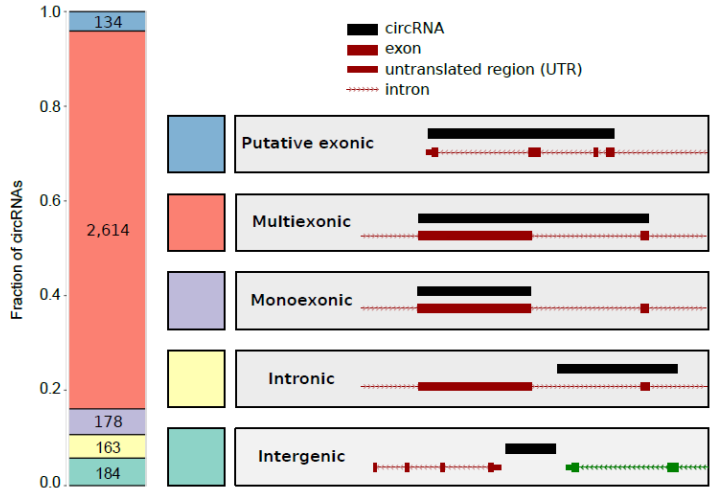


Sanger Sequencing

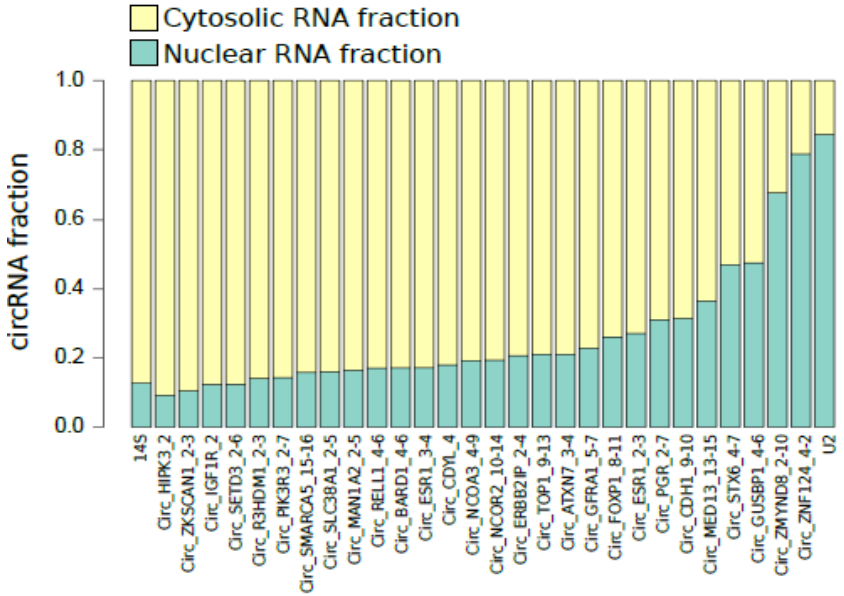


GENOMIC CHARACTERISATION

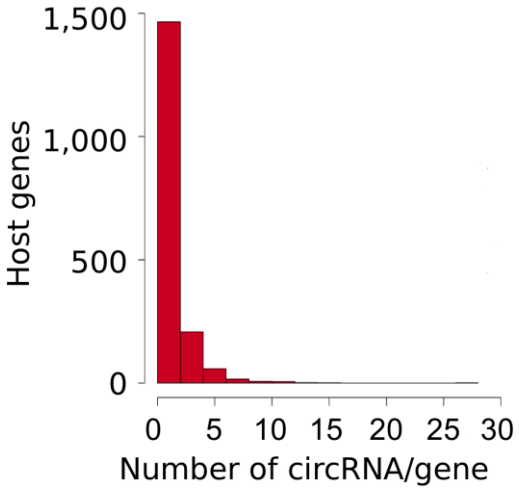
circRNA Classification



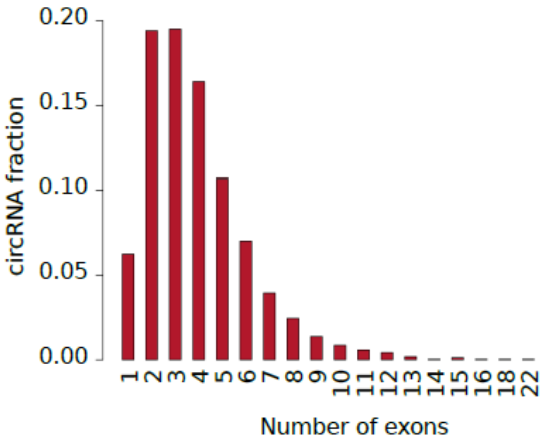
Cell Fractioning



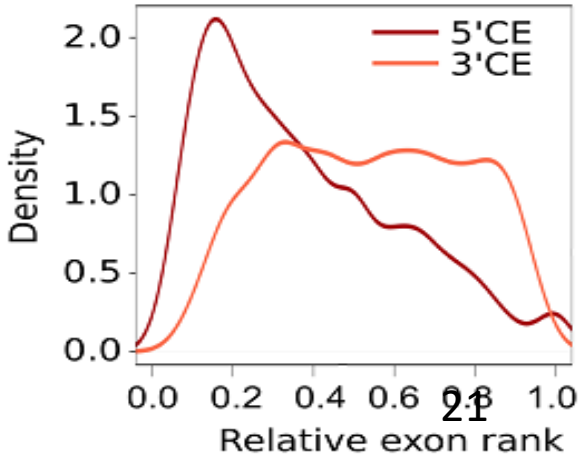
CircRNAs per Gene



Circularazing Exons

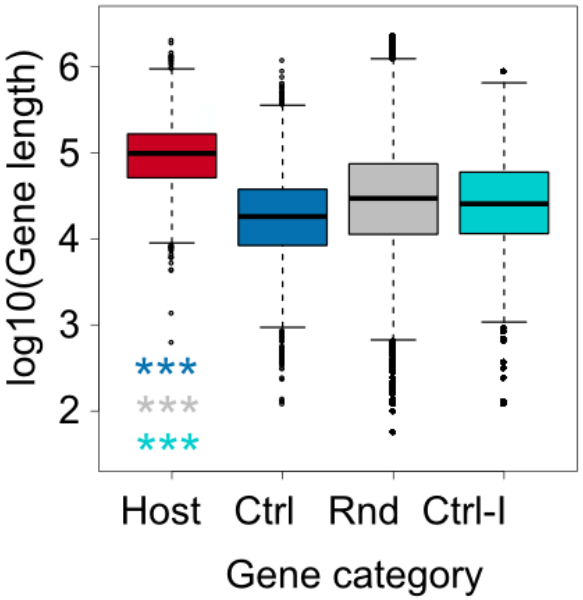


Exon distribution

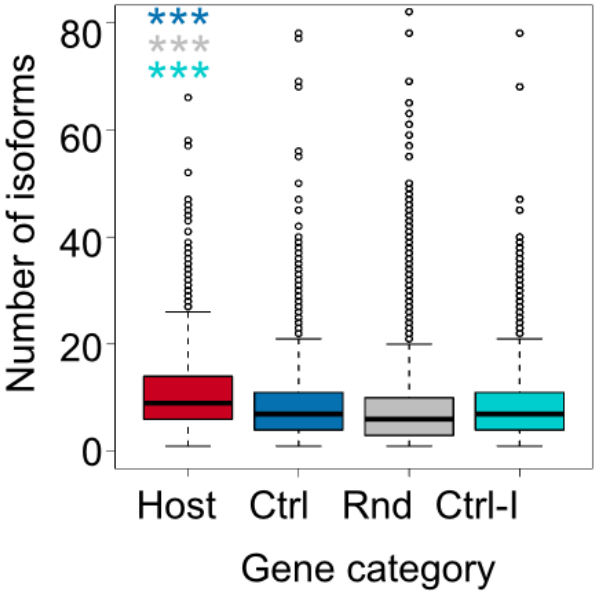


GENOMIC CHARACTERISATION

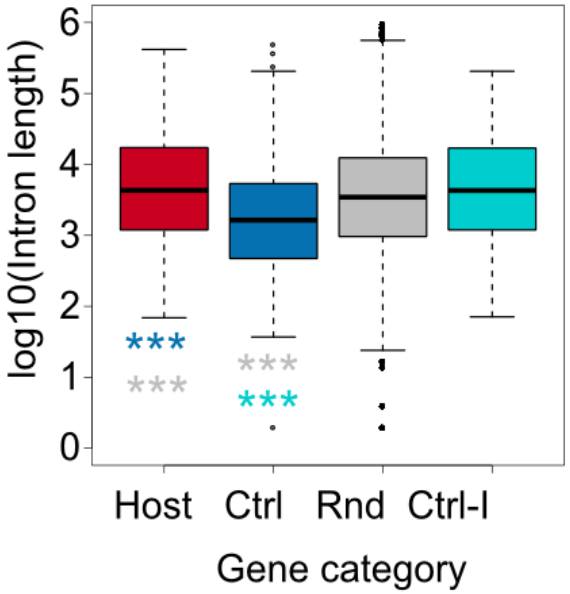
Gene length



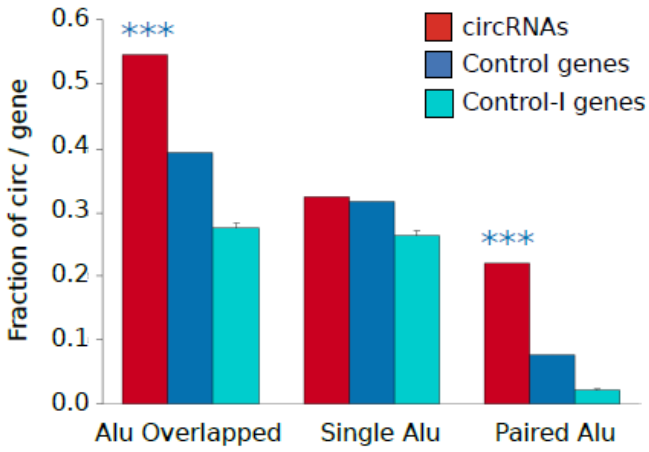
Nº of isoforms



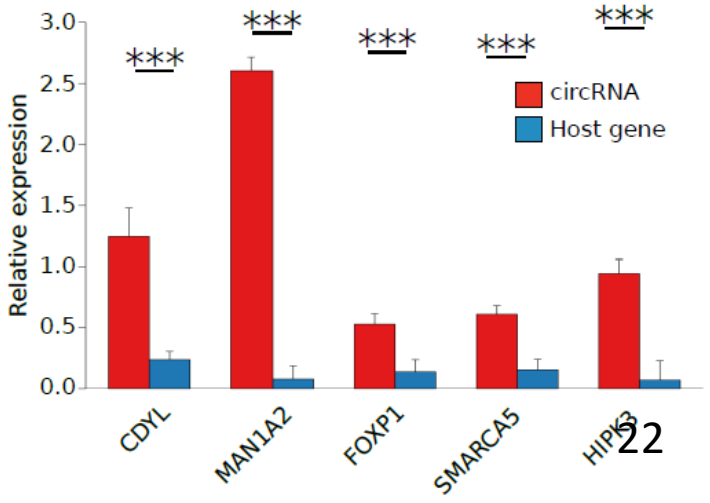
Intron length



ALU Repeats

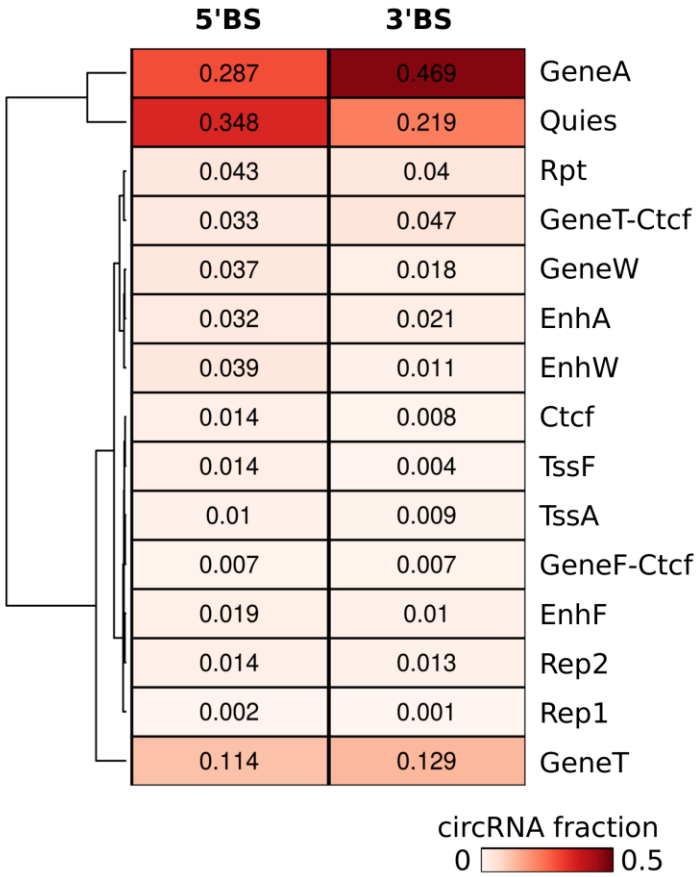


Top5 circRNA Expression

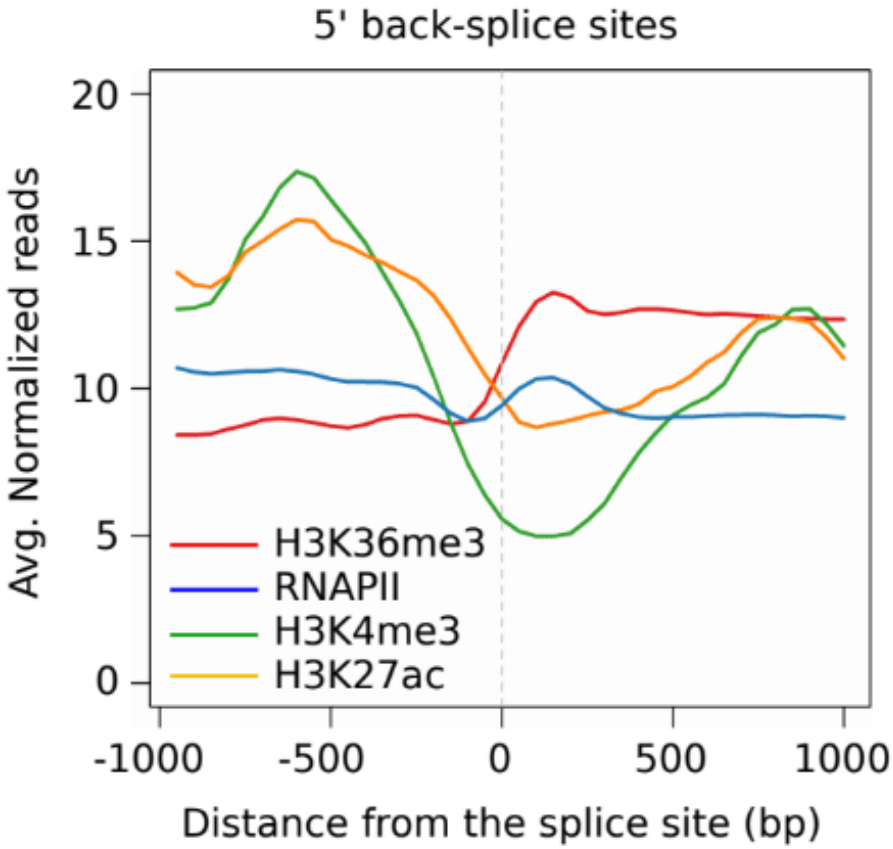


circRNA BIOGENESIS

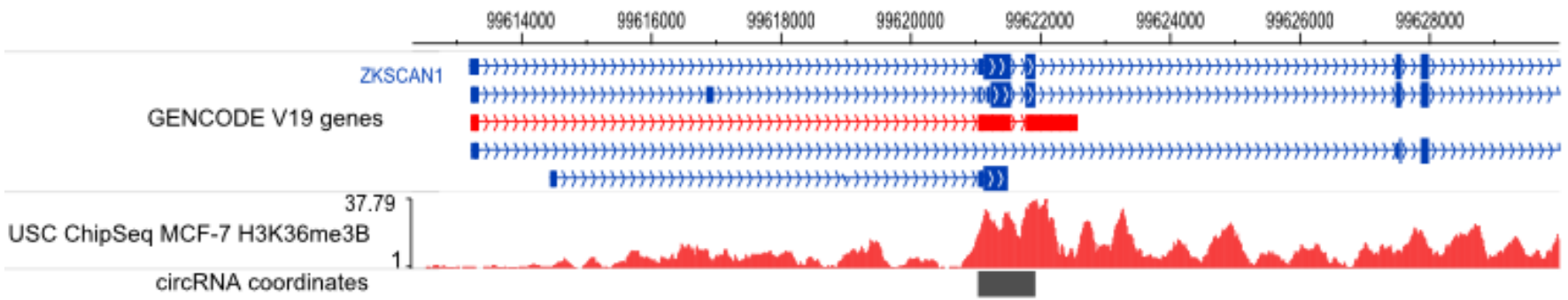
Chromatin States Overlap

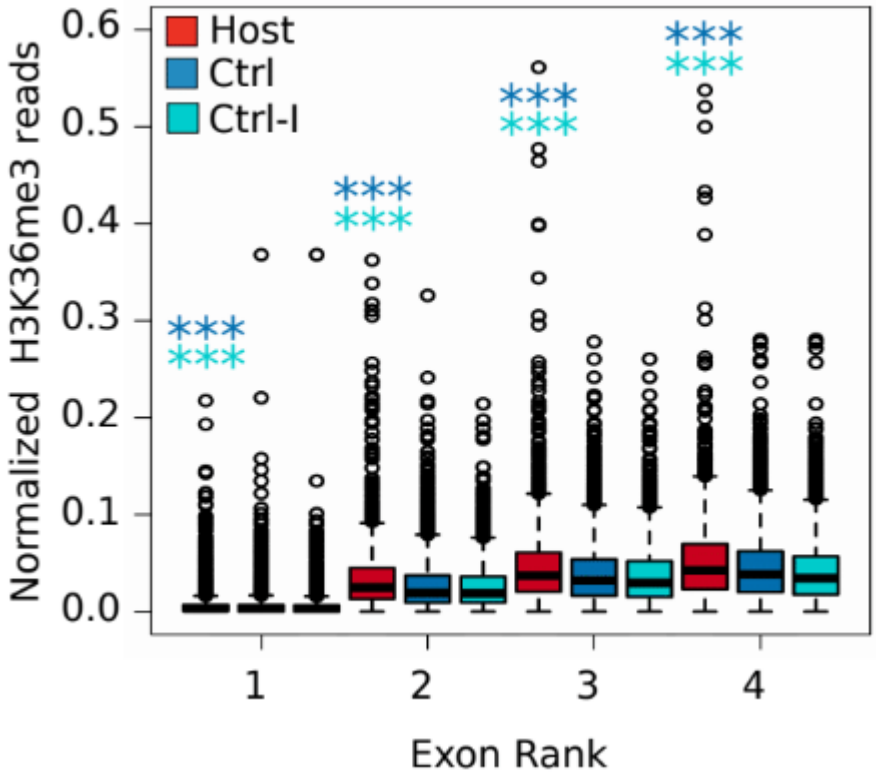


Histone Modification Analysis

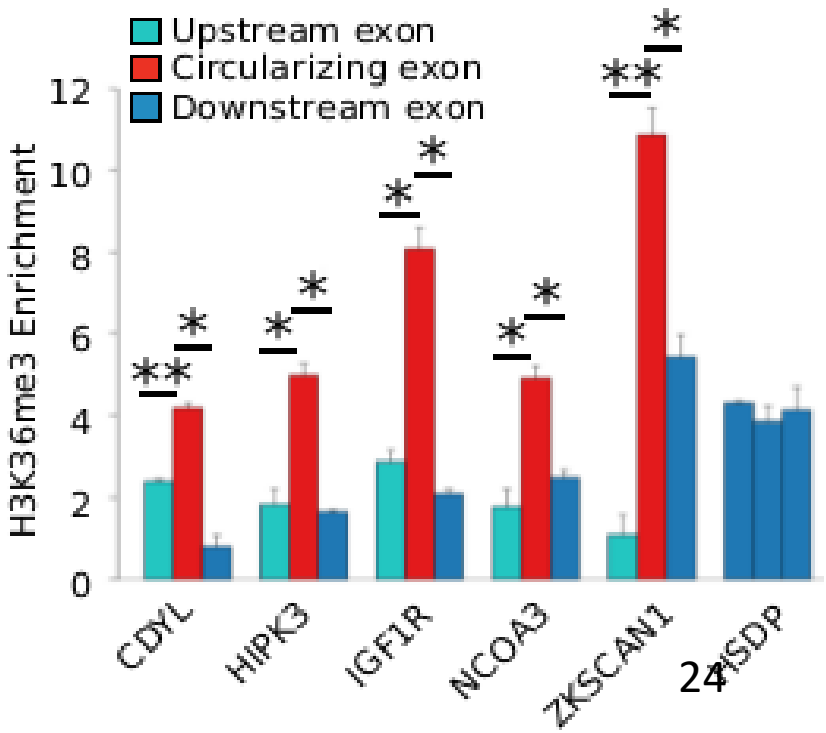


Circularizing exons have H3K36me3 enrichment





ChIP validation



Regulation of Alternative Splicing by Histone Modifications

Reini F. Luco,[1] Qun Pan,[2] Kaoru Tominaga,[3] Benjamin J. Blencowe,[2]
Olivia M. Pereira-Smith,[3] Tom Misteli[1]*

Alternative splicing of pre-mRNA is a prominent mechanism to generate protein diversity, yet its regulation is poorly understood. We demonstrated a direct role for histone modifications in alternative splicing. We found distinctive histone modification signatures that correlate with the splicing outcome in a set of human genes, and modulation of histone modifications causes splice site switching. Histone marks affect splicing outcome by influencing the recruitment of splicing regulators via a chromatin-binding protein. These results outline an adaptor system for the reading of histone marks by the pre-mRNA splicing machinery.

19 FEBRUARY 2010 VOL 327 **SCIENCE** www.sciencemag.org

Differential splicing of FGFR2 depends on the PTB regulator, which binds to a splicing silencer around exon IIIb and represses its inclusion

FGFR2 pre-mRNA tissue-specific exon IIIb / IIIc alternative splicing was studied in **PNT2** (prostate normal epithelium) and in **hMSC** (human mesenchymal stem) cells.

The level of H3K36 trimethylation was assessed by ChIP-qPCR along the gene in these cells. Cell-specific over-representation in hMSC was observed around exons/introns interested by alternative splicing:

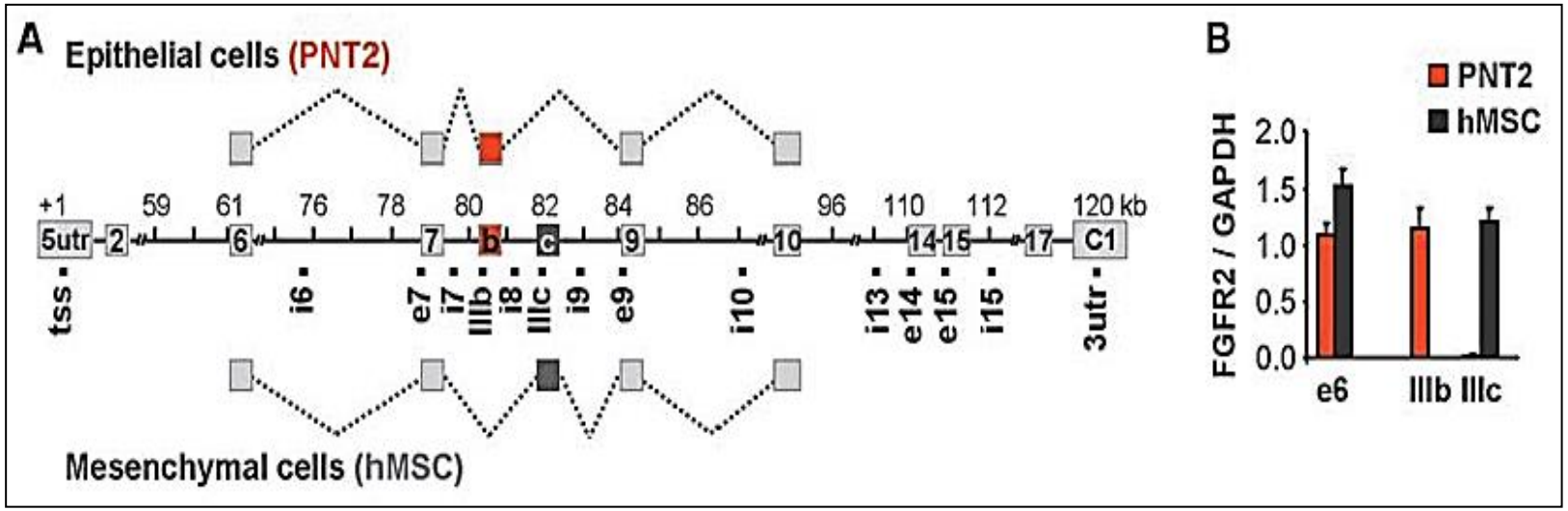


Fig. 1. Splicing-specific histone modifications. (A) Schematic representation of the human FGFR2 gene. Exon IIIb (red) is included in PNT2 epithelial cells, exon IIIc (black) is included in hMSCs. Square dots indicate oligonucleotide pairs used in analysis. (B) Levels of FGFR2 **exon inclusion** relative to GAPDH in PNT2 (red) or hMSCs (black) determined by quantitative polymerase chain reaction (PCR).

Is there any special histone PTMs at these exons ?

- Chromatin Immunoprecipitation using Antibodies against PTMs

- PCR analysis of single sites as in Figure 1

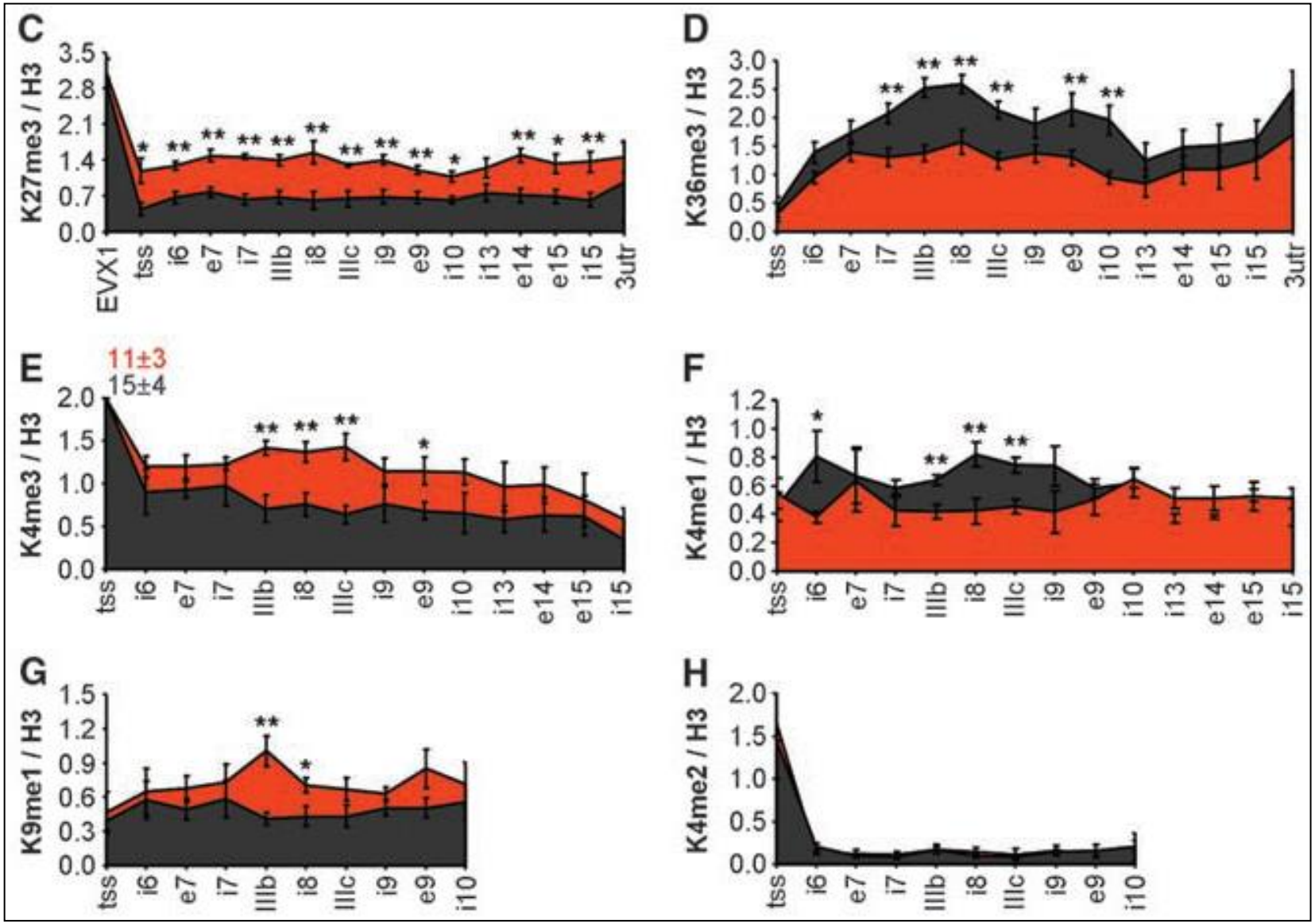
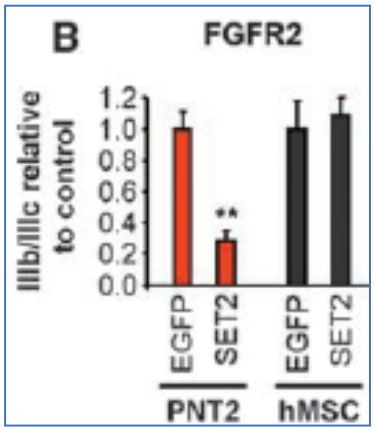


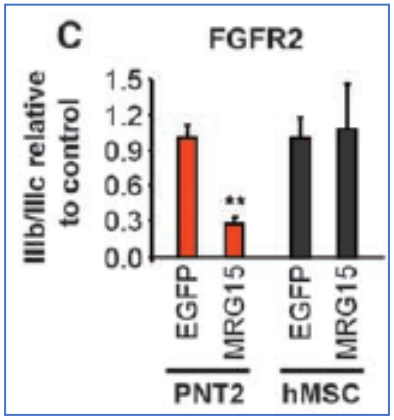
Fig. 1 - (C to H) Mapping of H3-K27me3 (C), H3-K36me3 (D), H3-K4me3 (E), H3-K4me1 (F), H3-K9me1 (G), and H3-K4me2 (H) in FGFR2 in **PNT2 (red)** and **hMSC (black)** cells by **quantitative ChIP.** The percentage of input was normalized to unmodified H3. Values represent means ± SEM from four to six independent experiments. *P <0.05, **P < 0.01, Student's t test.

The HMT specific to H3K36 is SET2.
When SET2 is overexpressed in epithelial cells, IIIb/IIIc ratio falls by 75%:



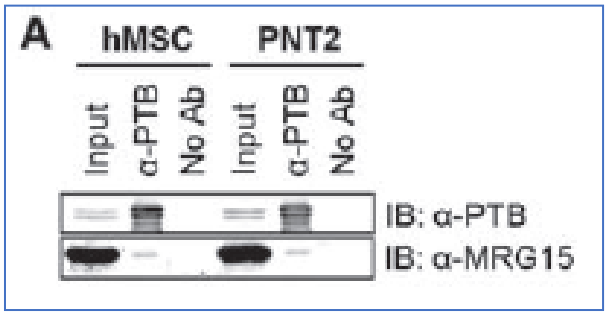
SET2 overexpression

H3K36(me3) is recognized by the bromodomain protein **MRG15 (reader)**
When MRG15 is overexpressed in epithelial cells, IIIb/IIIc raio falls by 75%:

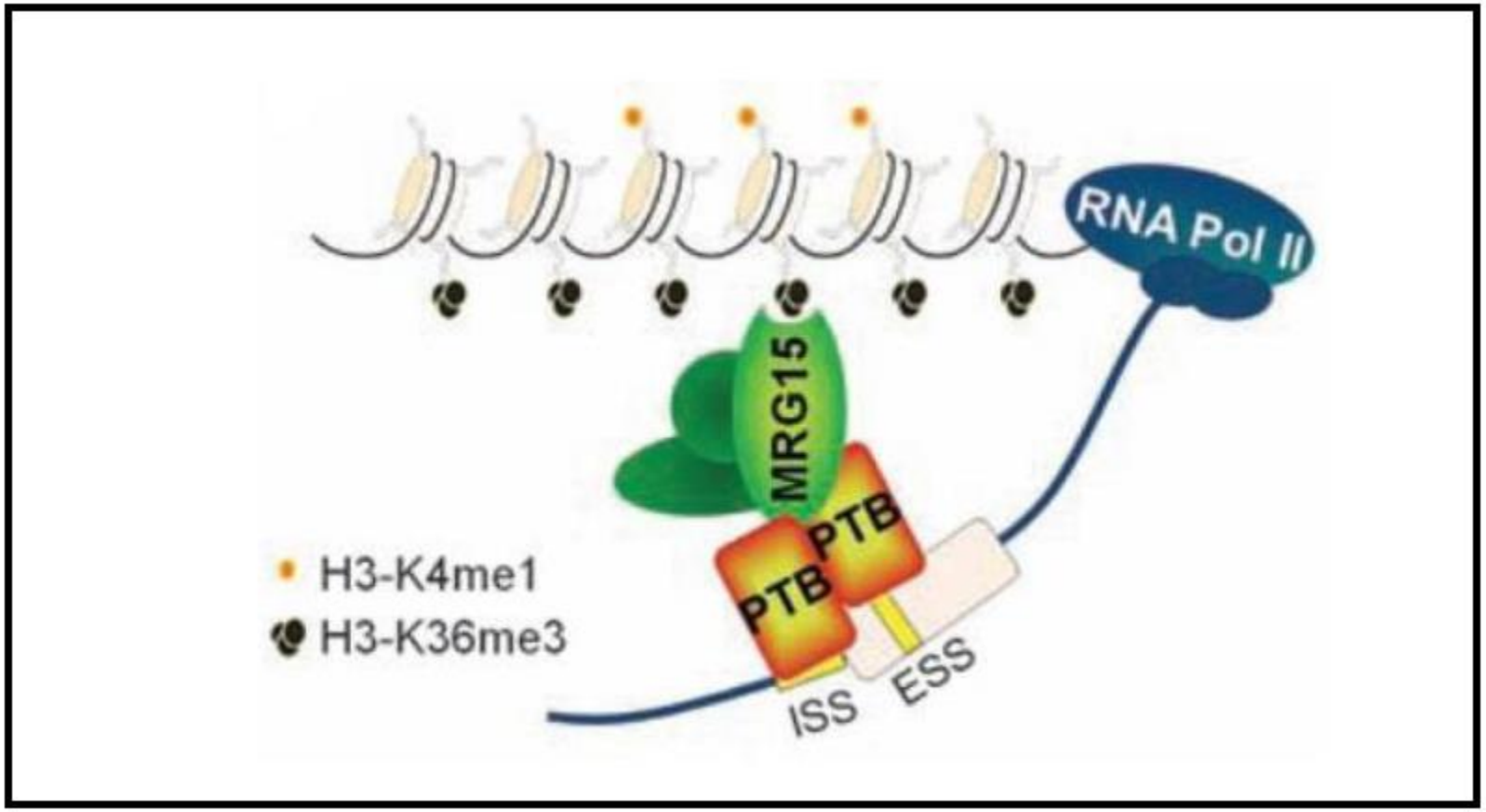


MRG15 overexpression

Exon IIIb has weak site for **PTB**.
MRG15 co-immunoprecipitates with the RNA binding protein PTB →



Co-IP



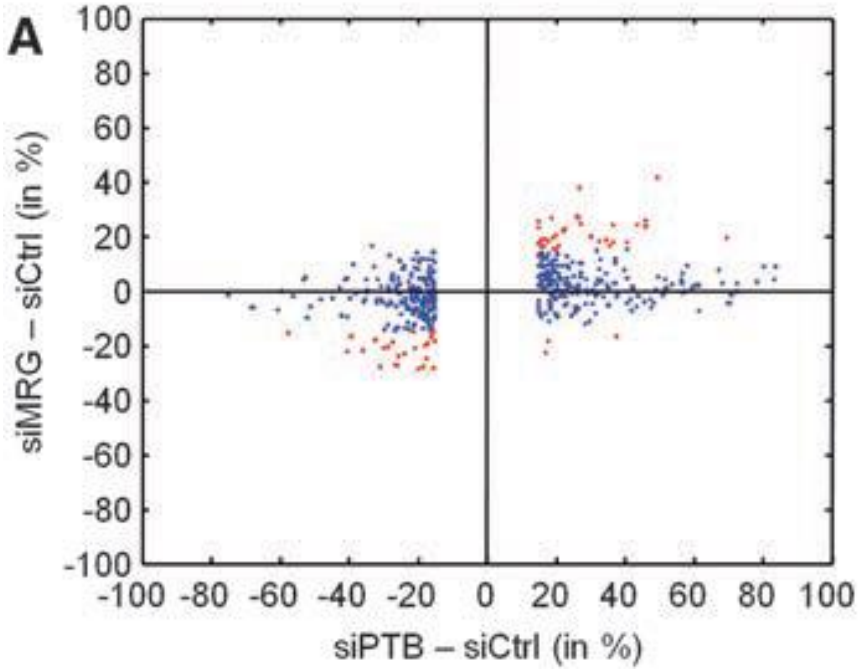
An adaptor system for reading histone marks by the splicing machinery, consisting of a histone mark signature, a chromatin-binding protein (MRG15), and a splicing regulator (PTB).

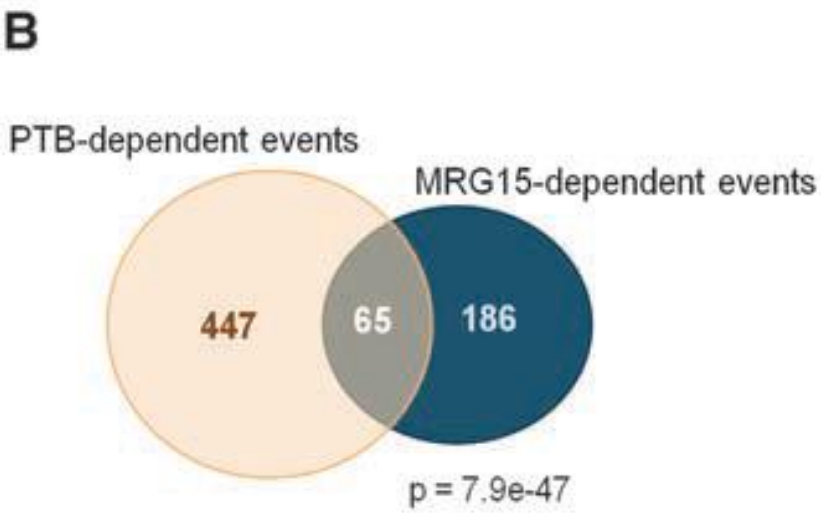
Luco et al, 2011

Are PTB and MRG15 effects limited to FGFR2 exons ?

siRNA-mediated down-regulation of either PTB or MRG

RNA-seq → splicing read mapped and quantitated for each AS event





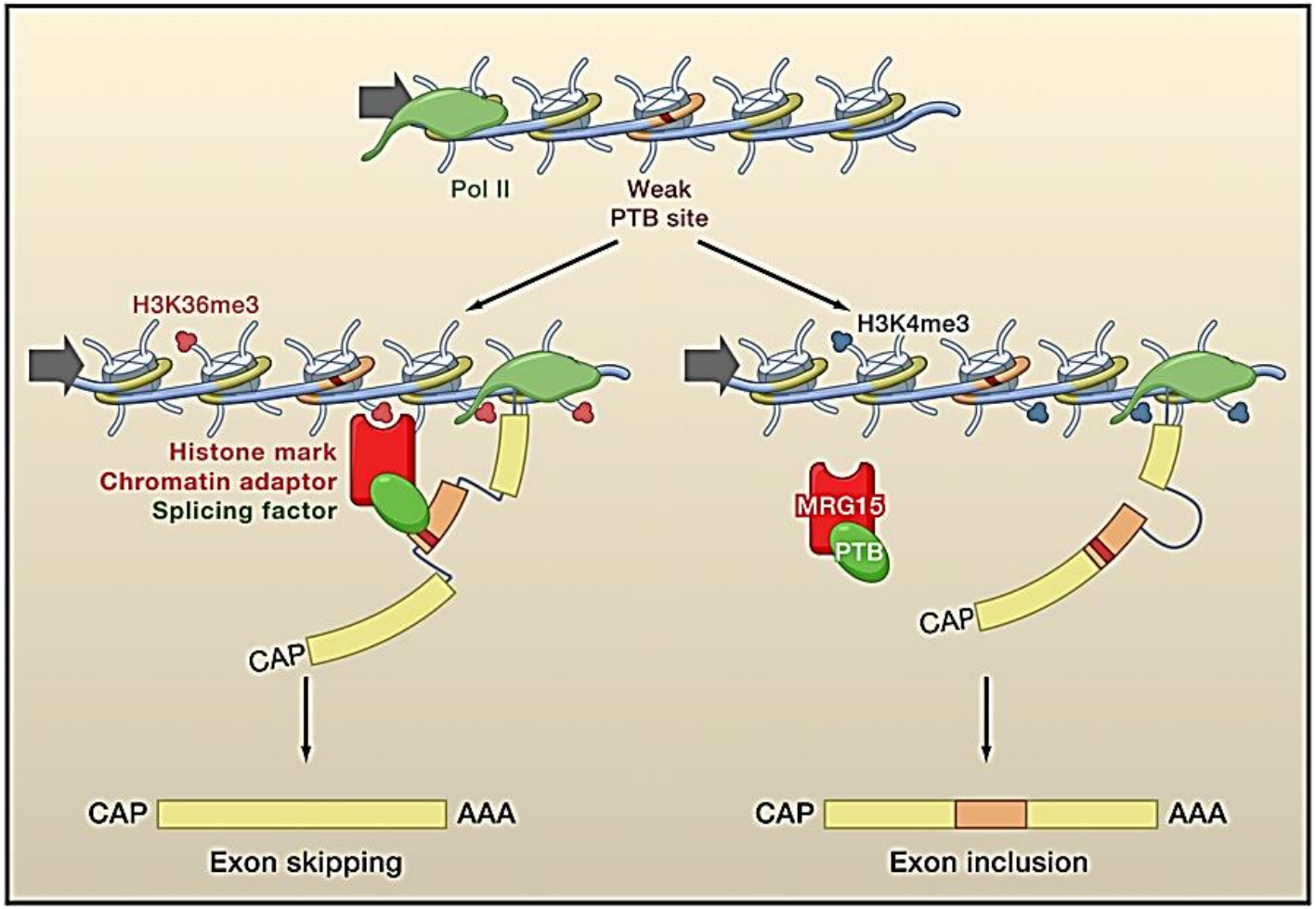


Figure 3. The Chromatin-Adaptor Model of Alternative Splicing. Histone modifications along the gene determine the binding of an adaptor protein that reads specific histone marks and in turn recruits splicing factors. In the case of exons whose alternative splicing is dependent on poly-pyrimidine tractbinding protein (PTB) splicing factor, high levels of trimethylated histone 3 lysine 36 (H3K36me3, red) attract the chromatin-binding factor MRG15 that acts as an adaptor protein and by protein-protein interaction helps to recruit PTB to its weaker binding site inducing exon skipping. If the PTBdependent gene is hypermethylated in H3K4me3 (blue), MRG15 does not accumulate along the gene, and PTB is not recruited to its target premRNA, thus favoring exon inclusion.

This is the first demonstration of a mechanistic link between chromatin and alternative splicing. Other protein-protein interaction between chromatin-competent proteins and RNA binding proteins is present in the literature, however no direct demonstration of a mechanism was given to date. Nonetheless, interactions suggest a possible functional role that should be worked out in the future.

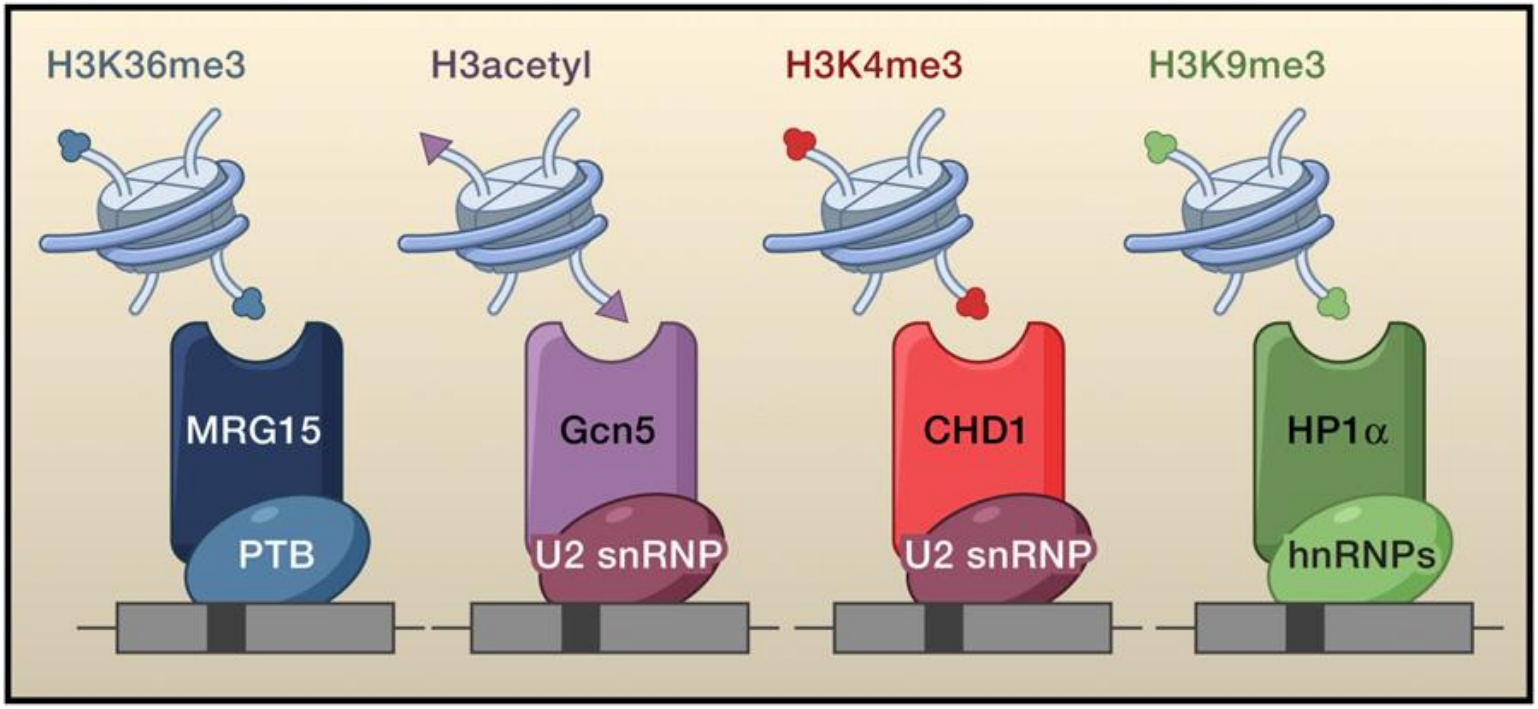


Figure 4. Chromatin-Adaptor Complexes
Several histone modification-binding chromatin proteins interact with splicing factors (Luco et al., 2010; Sims et al., 2007; Gunderson and Johnson, 2009; Piacentini et al., 2009; Loomis et al., 2009).

A model of epigenetic “memory” of alternative splicing in the cells

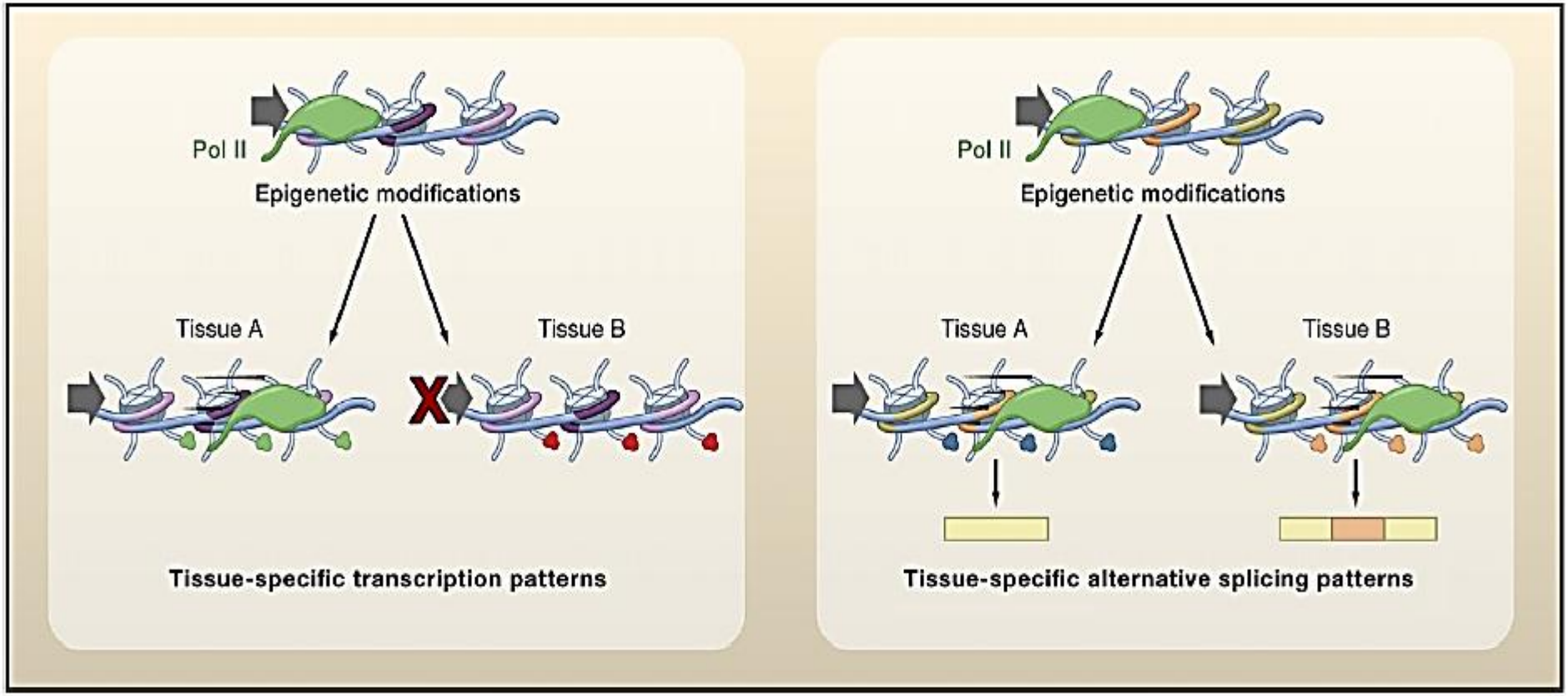
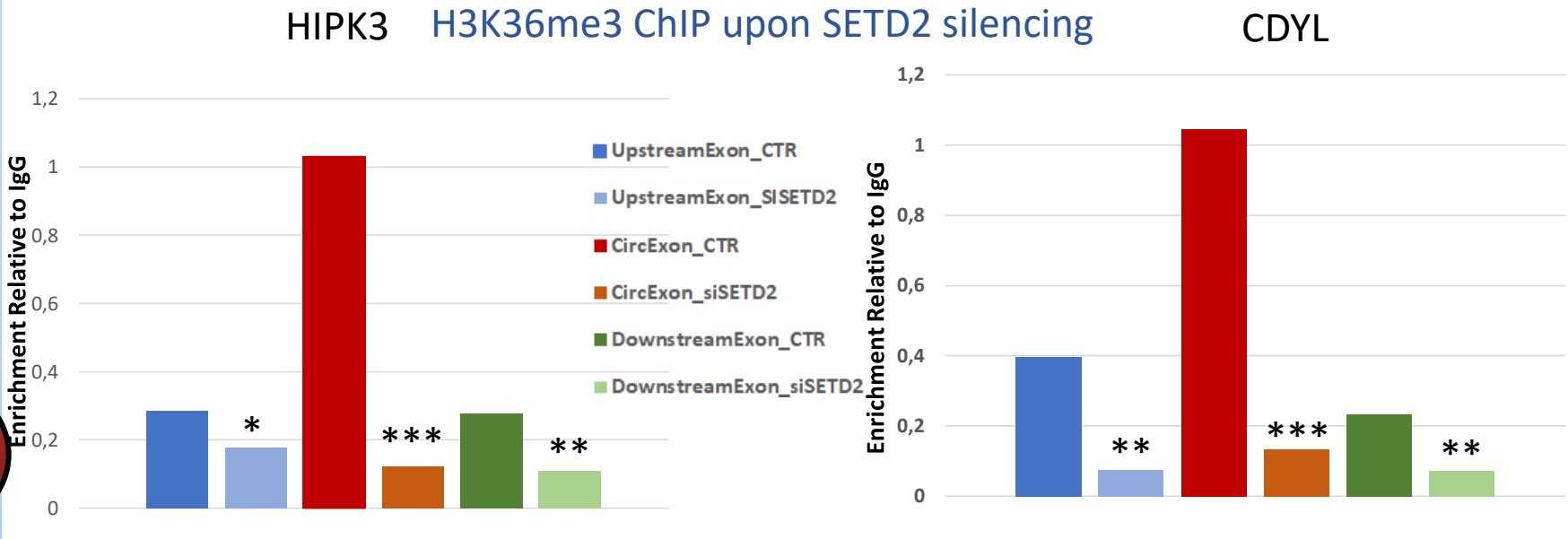
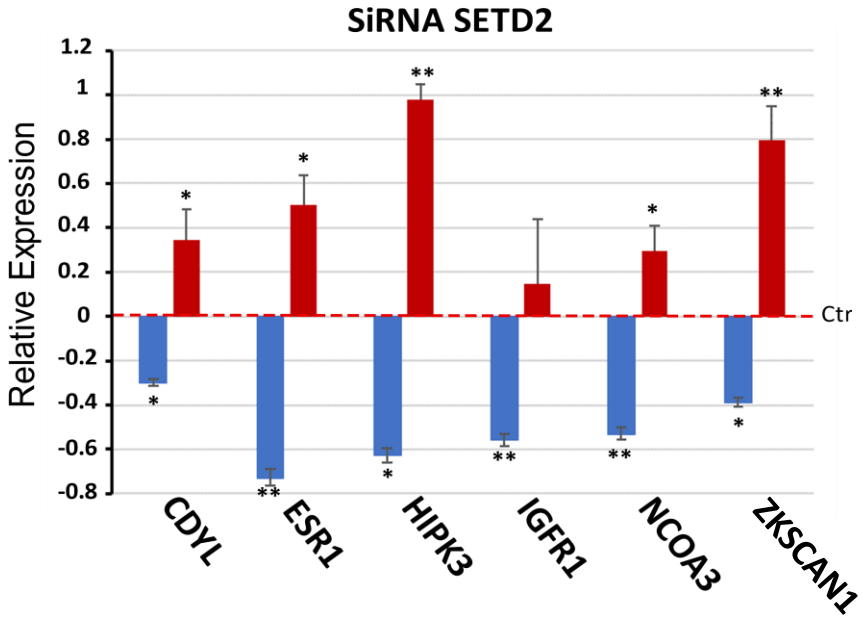


Figure 6. The Epigenetics of Alternative Splicing
The combination of histone modifications along a gene establishes and maintains tissue-specific transcription patterns (left panel), as well as heritable tissue-specific alternative splicing patterns (right panel).

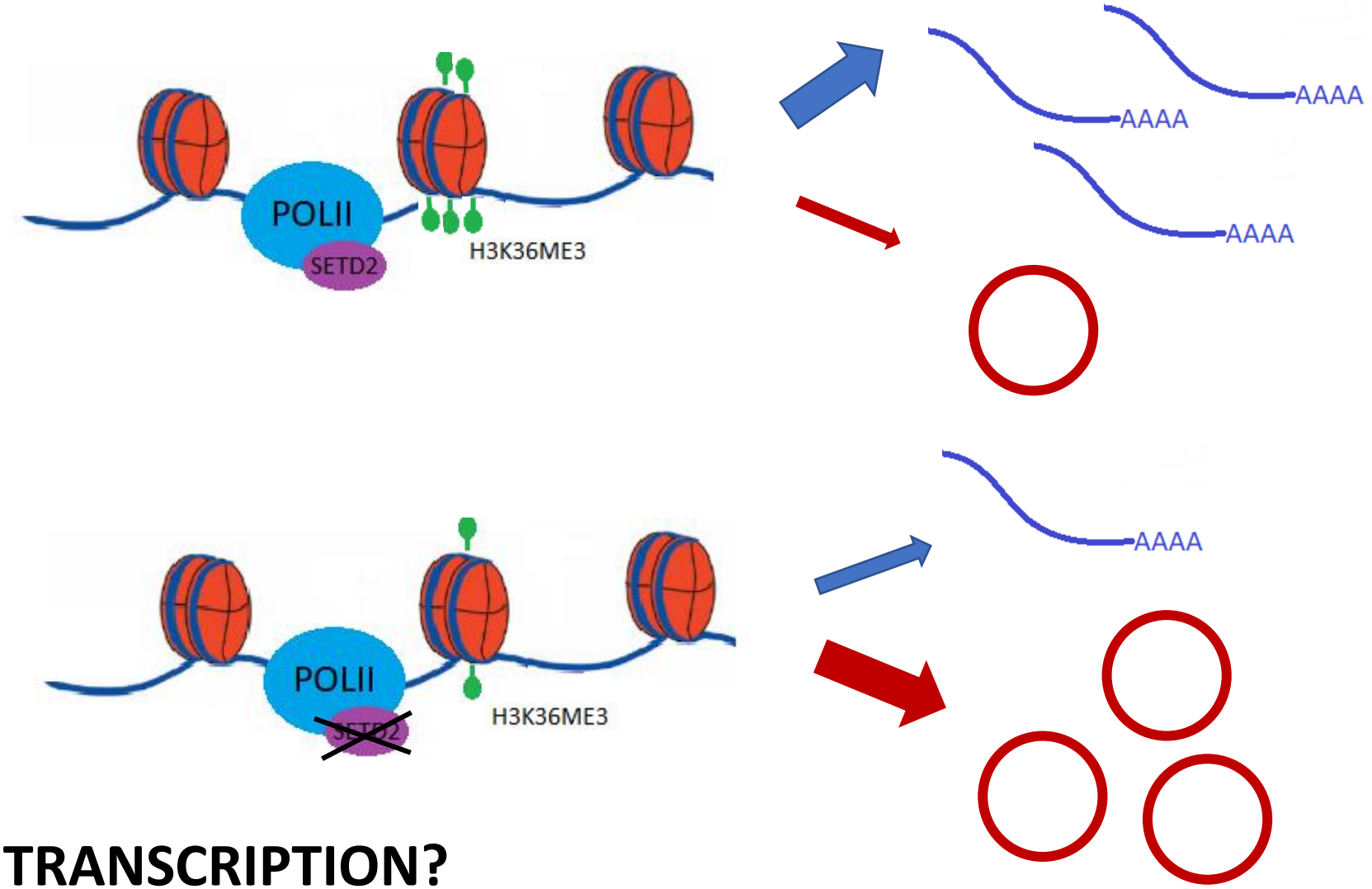
SETD2 knockdown reduces H3k36me3 levels and increases circRNA expression





circRNAs
Linear transcripts

H3K36ME3 involved in circRNA biogenesis



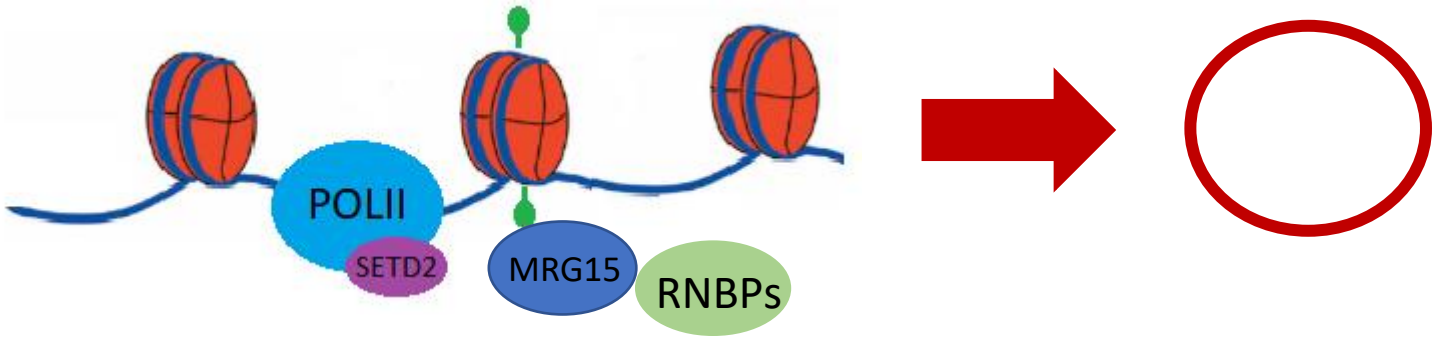
TRANSCRIPTION?

SPLICING?

STABILITY?

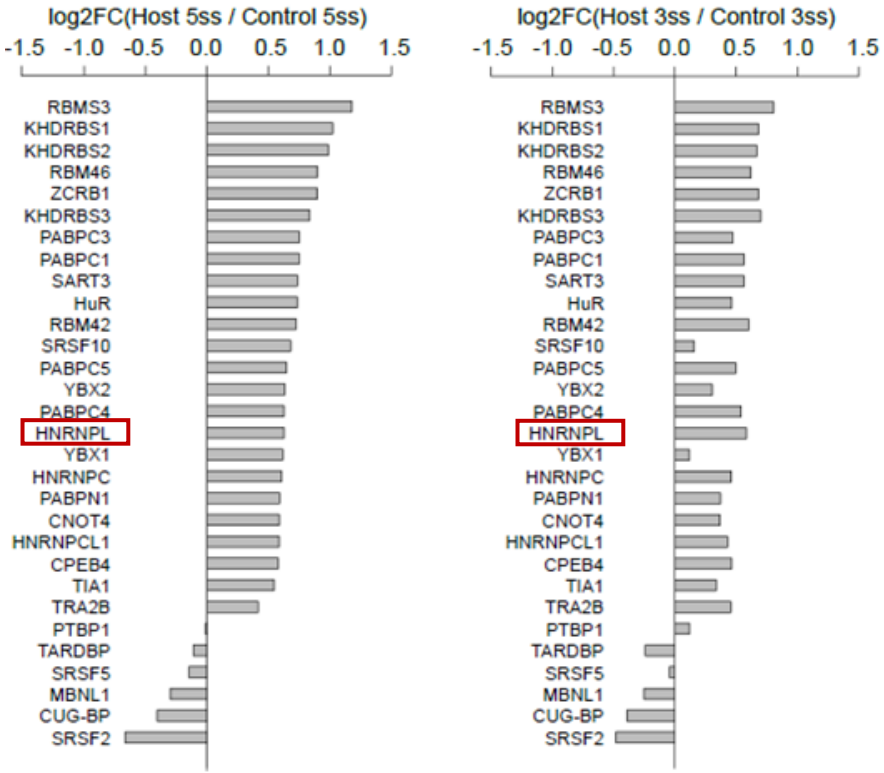
SPLICING REGULATION

H3K36ME3 serves as signal for **the recruitment of Splicing factors**

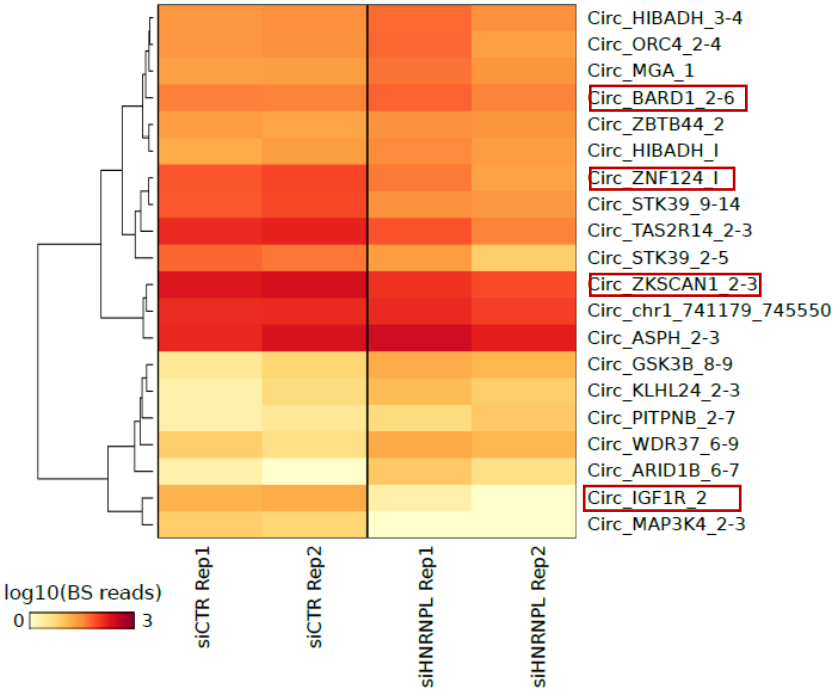


RBPs MOTIF ENRICHMENT

Host VS Control RBP enrichment



DE expression analysis of circRNA in hnRNPL knockdown



circRNA IN TUMORS

Direct search of backsplicing junction sequences in RNA-Seq datasets

HASH-CIRC

Reconstructed backsplice junction

GTCGTGCACG
GTCG
TCGT
CGTG

70bp

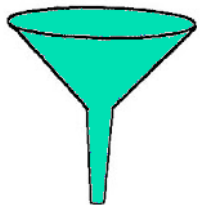
GTCGTGCAAG

RNA-Seq Reads

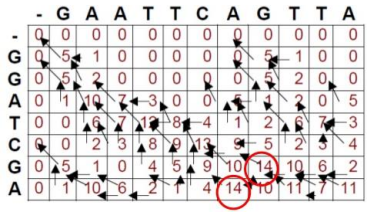
GGGTGATGGCCG

CGTACGCTAC
CGTA
GTAC
TACG

HASH function



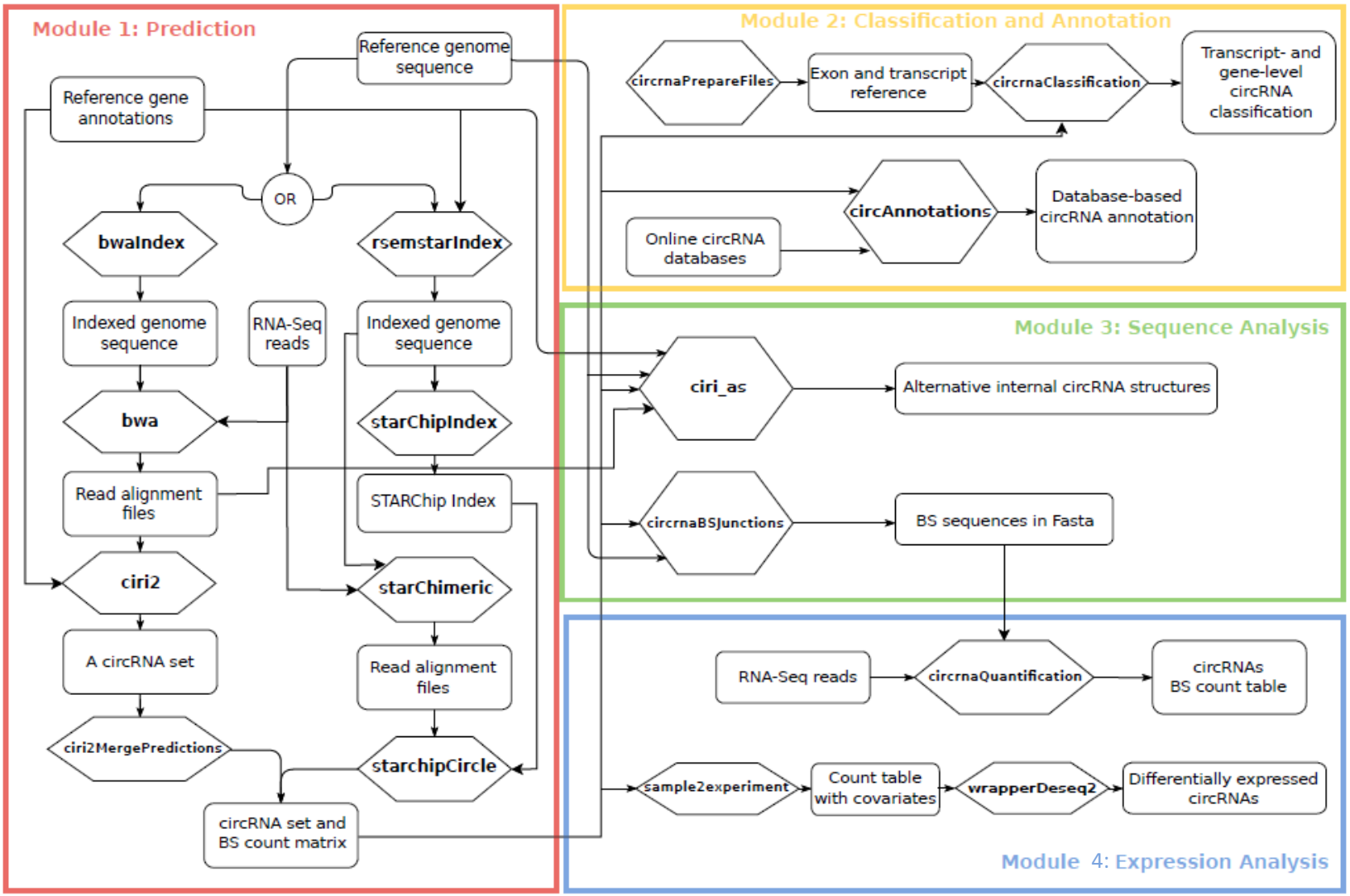
Smith-Waterman alignment



ACCGTGCACG
ACCGTGCACG

OUTPUT: Read counts in the reconstructed backsplicing junction

Docker4Circ work-flow

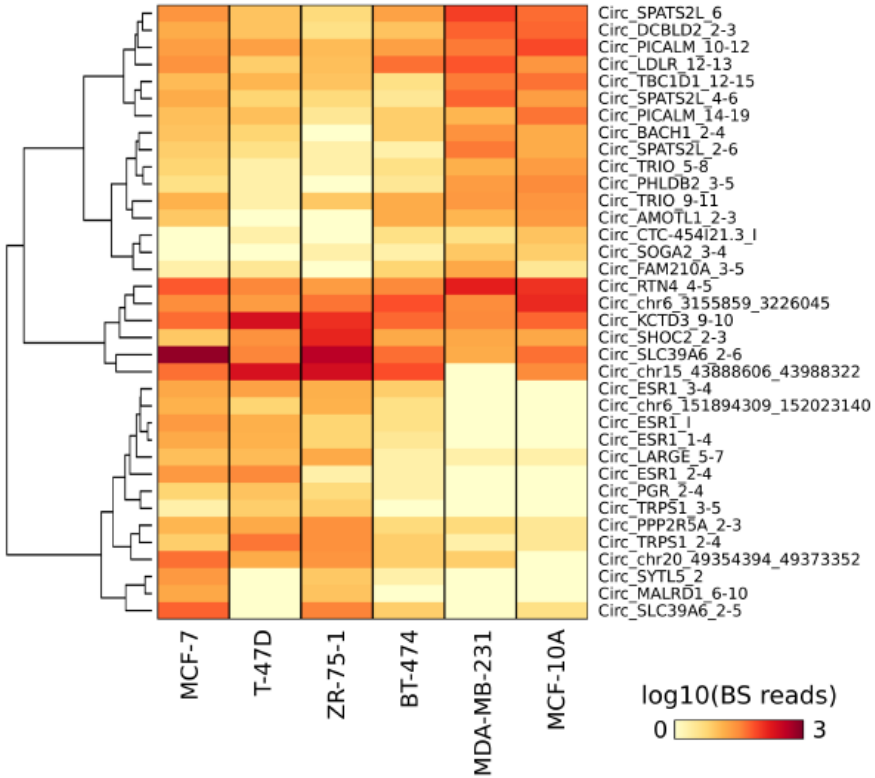


Ferrero et al. Submitted to GigaScience

circRNA IN TUMORS

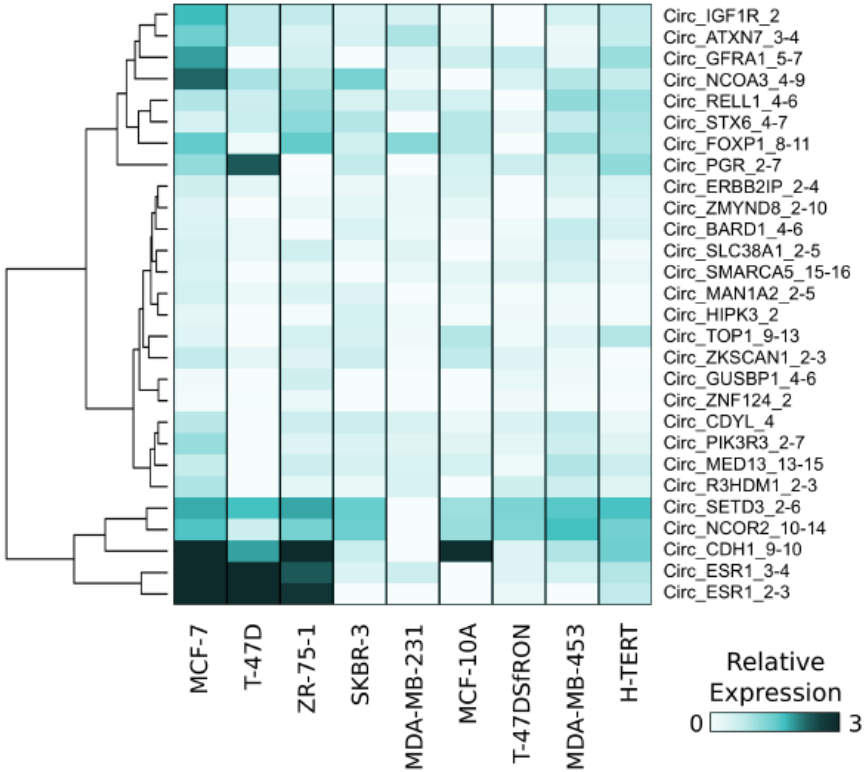
Differential expression (DE) circRNA analysis on BC cell lines

HASH prediction on BC cell lines

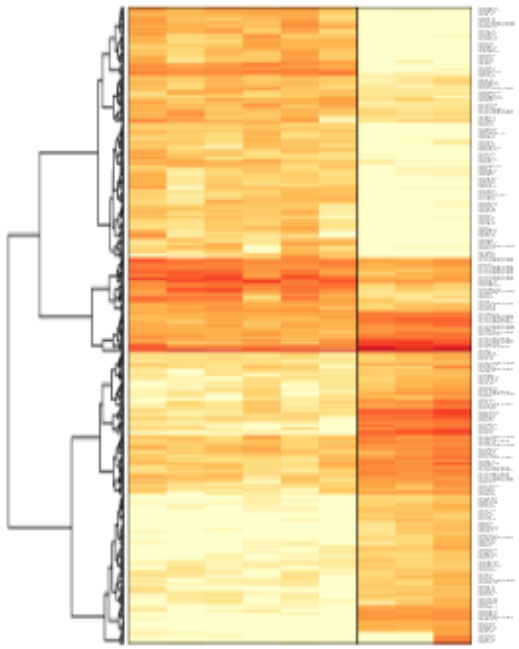


38 DE

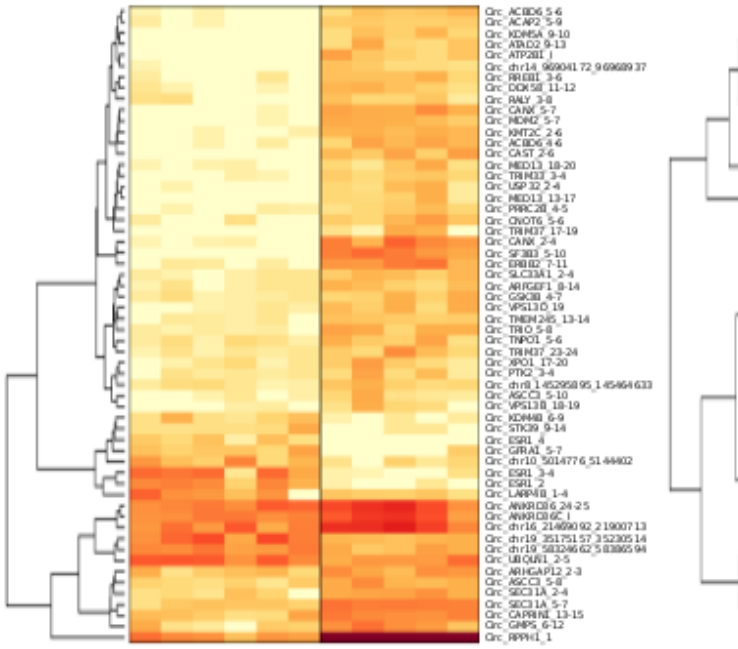
RT-PCR on Breast cell lines



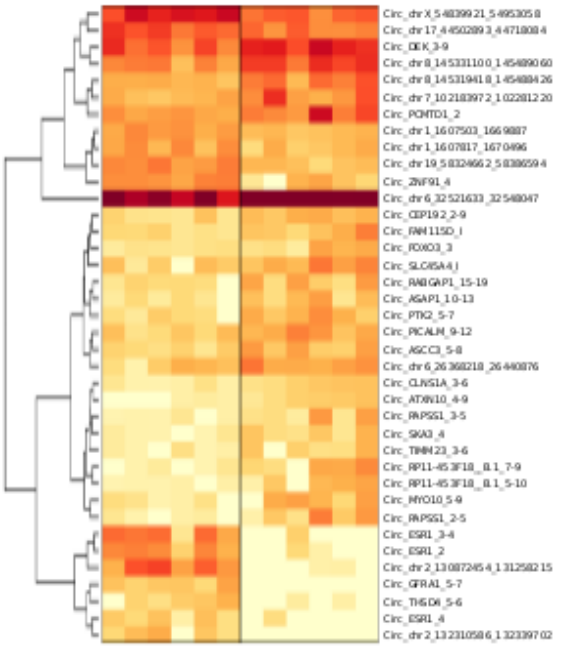
Differential expression (DE) circRNA analysis on BC samples



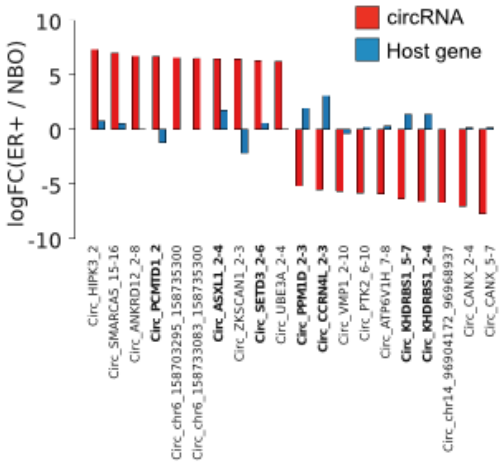
622 DE

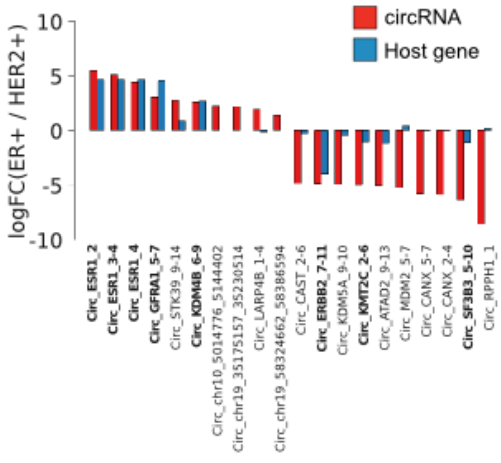


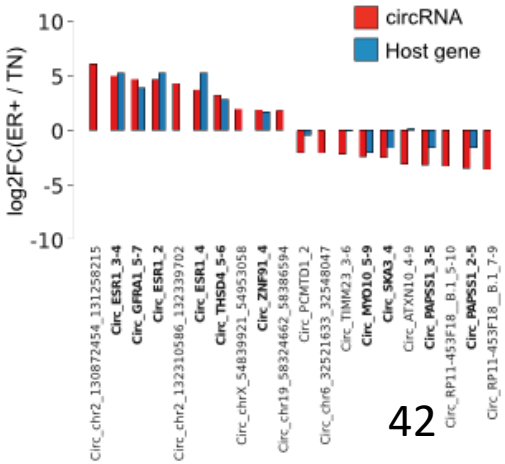
58 DE



38 DE

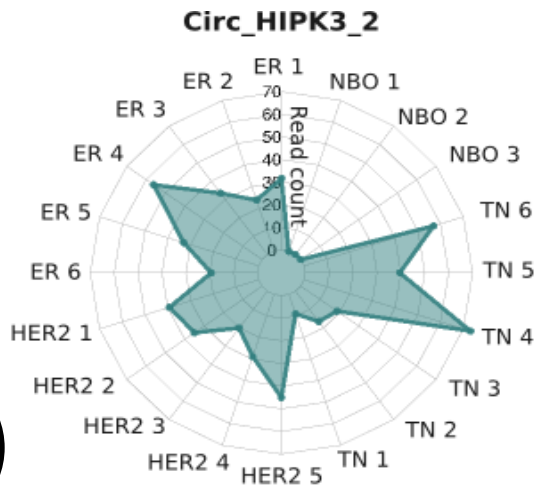




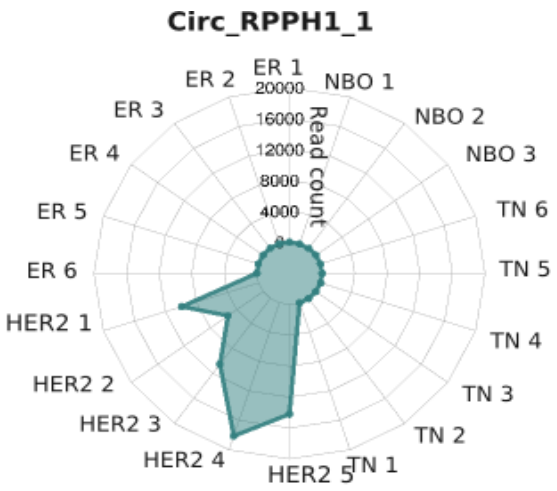


DE circRNA candidates

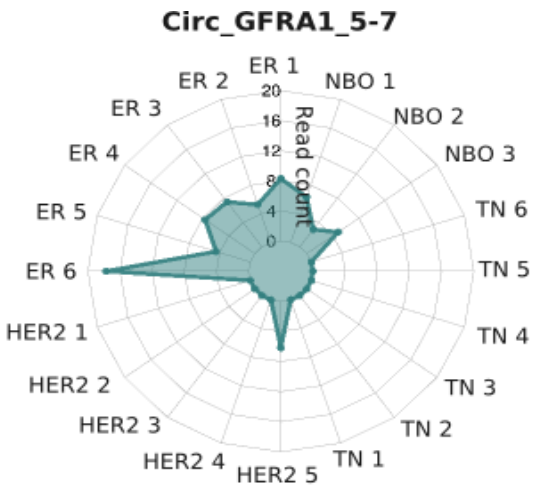
ER+/NBO



ER+/HER2

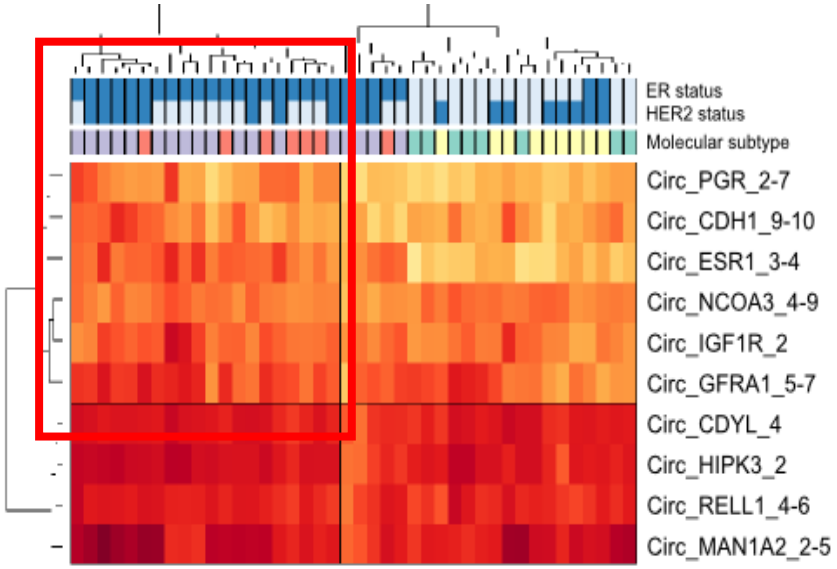


ER+/TN

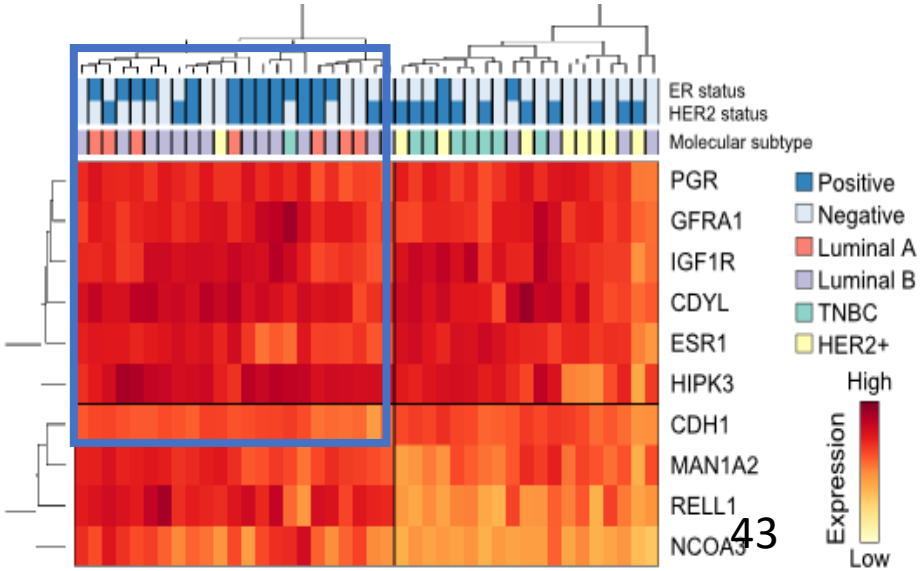


qRT-PCR in 42 BC samples

circRNAs



Host genes

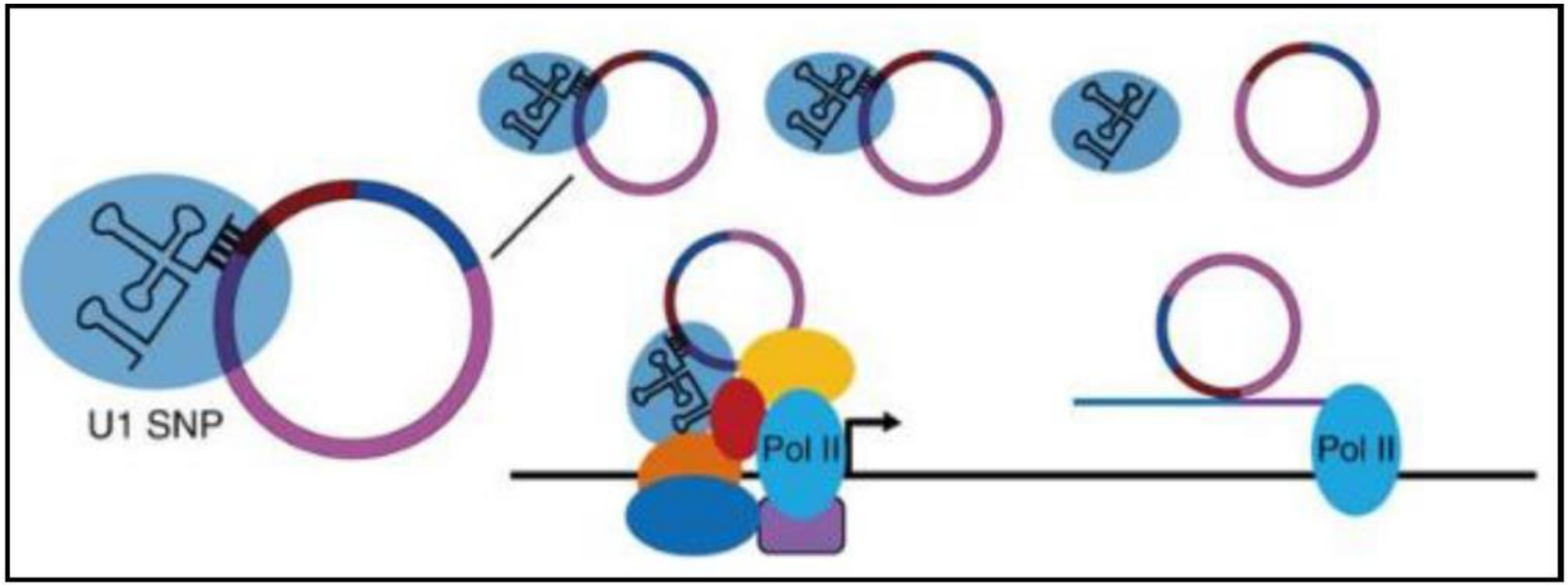


circRNA FUNCTIONS

- **nuclear circRNAs enhance host gene transcription**
- **circRNAs can act as miRNA sponges**
- **circRNAs can be translated into proteins**
- **circRNAs as potential biomarkers**

EIcircRNAs ENHANCE TRANSCRIPTION

Nuclear EIcircRNA acts in complex with POL2 in a RNA-RNA interaction with U1SNP



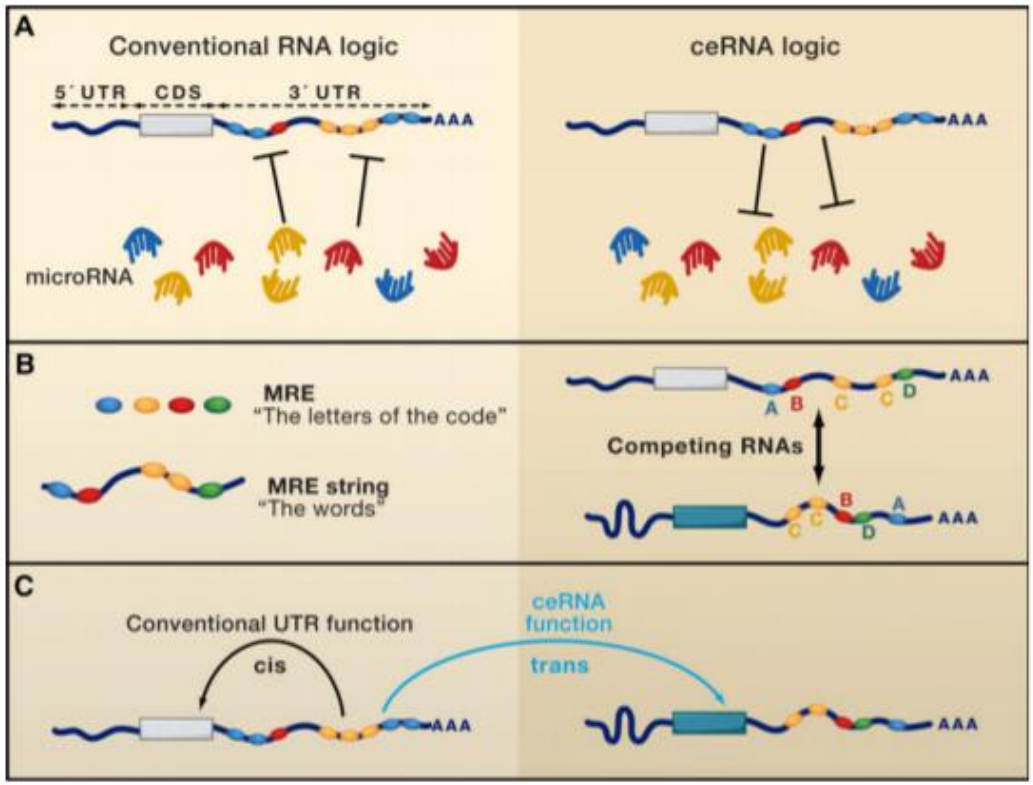
Mechanism to enhance host gene's transcription, generating a positive feedback

miRNA SPONGES

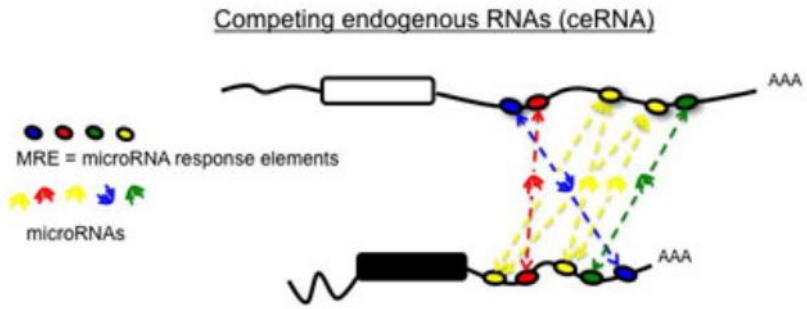
A *ceRNA* Hypothesis: The Rosetta Stone of a Hidden RNA Language?

Leonardo Salmena,[1] Laura Poliseno,[1,2] Yvonne Tay,[1] Lev Kats,[1] and Pier Paolo Pandolfi[1,*]
[1]Cancer Genetics Program, Beth Israel Deaconess Cancer Center, Departments of Medicine and Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA
[2]Present address: Department of Dermatology, New York University School of Medicine, New York, NY 10016, USA
*Correspondence: ppandolf@bidmc.harvard.edu
DOI 10.1016/j.cell.2011.07.014

Here, we present a unifying hypothesis about how messenger RNAs, transcribed pseudogenes, and long noncoding RNAs "talk" to each other using microRNA response elements (MREs) as letters of a new language. We propose that this "competing endogenous RNA" (ceRNA) activity forms a large-scale regulatory network across the transcriptome, greatly expanding the functional genetic information in the human genome and playing important roles in pathological conditions, such as cancer.



miRNA SPONGES



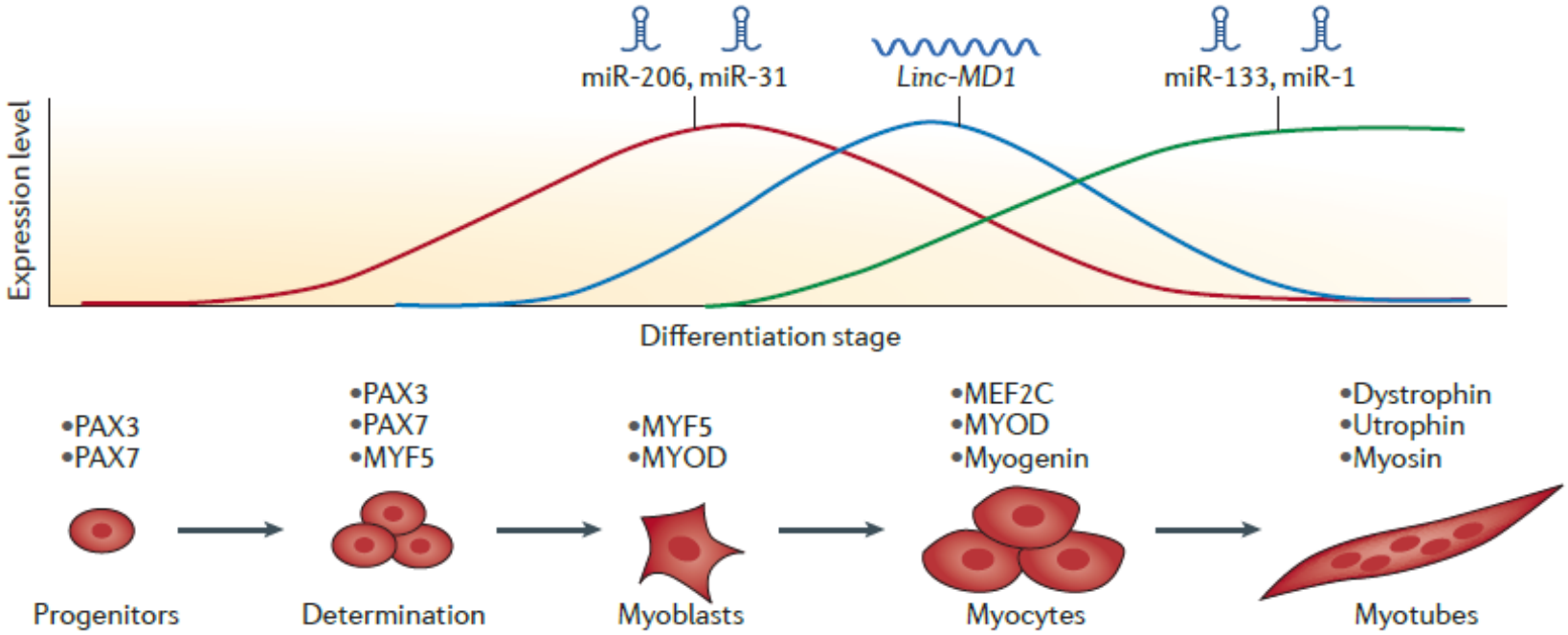
Another layer of regulation

A Long Noncoding RNA Controls Muscle Differentiation by Functioning as a Competing Endogenous RNA

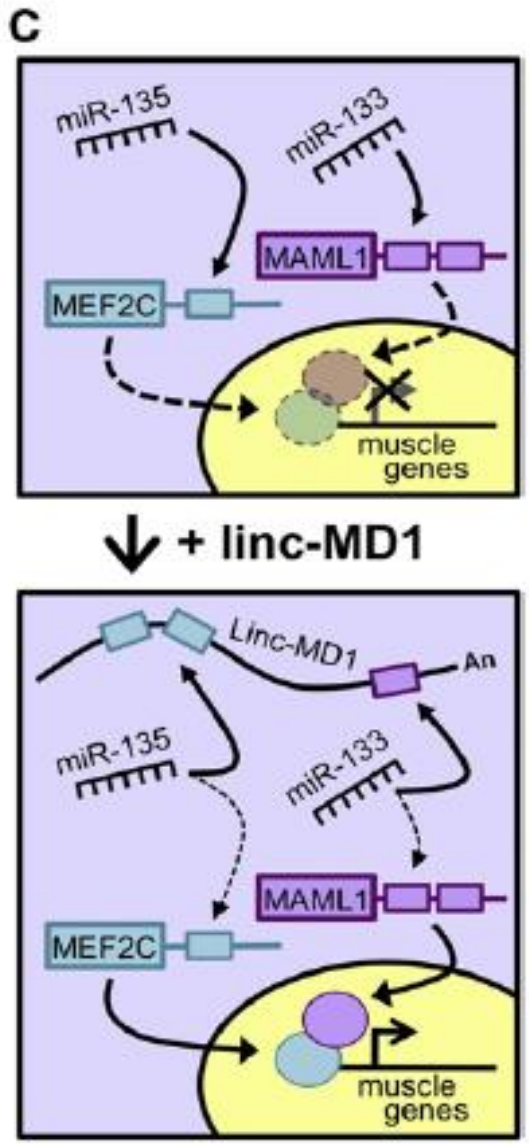
Marcella Cesana,[1,6] Davide Cacchiarelli,[1,6] Ivano Legnini,[1] Tiziana Santini,[1] Olga Sthandier,[1] Mauro Chinappi,[2] Anna Tramontano,[2,3,4] and Irene Bozzoni[1,3,4,5,*]
[1]Department of Biology and Biotechnology "Charles Darwin"
[2]Department of Physics
[3]Institut Pasteur Fondazione Cenci-Bolognetti
[4]Center for Life Nano Science @Sapienza, Istituto Italiano di Tecnologia
[5]IBPM of Consiglio Nazionale delle Ricerche (CNR)
Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy
[6]These authors contributed equally to the work
*Correspondence: irene.bozzoni@uniroma1.it

Recently, a new regulatory circuitry has been identified in which RNAs can crosstalk with each other by competing for shared microRNAs. Such competing endogenous RNAs (ceRNAs) regulate the distribution of miRNA molecules on their targets and thereby impose an additional level of post-transcriptional regulation. Here we identify a muscle-specific long noncoding RNA, linc-MD1, which governs the time of muscle differentiation by acting as a ceRNA in mouse and human myoblasts. Downregulation or overexpression of linc-MD1 correlate with retardation or anticipation of the muscle differentiation program, respectively. We show that linc-MD1 "sponges" miR-133 and miR-135 to regulate the expression of MAML1 and MEF2C, transcription factors that activate muscle-specific gene expression. Finally, we demonstrate that linc-MD1 exerts the same control over differentiation timing in human myoblasts, and that its levels are strongly reduced in Duchenne muscle cells. We conclude that the ceRNA network plays an important role in muscle differentiation.

miRNA SPONGES

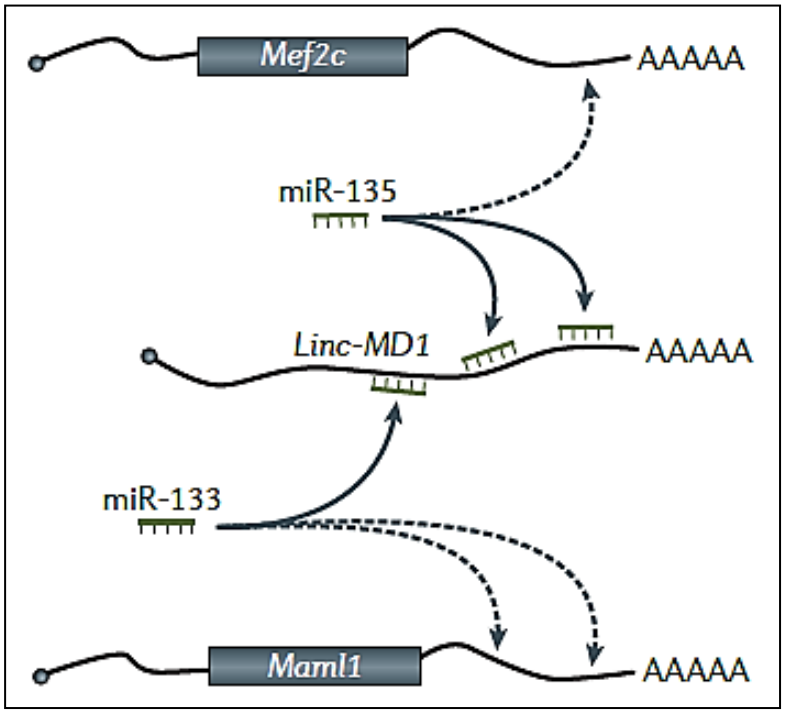


miRNA SPONGES

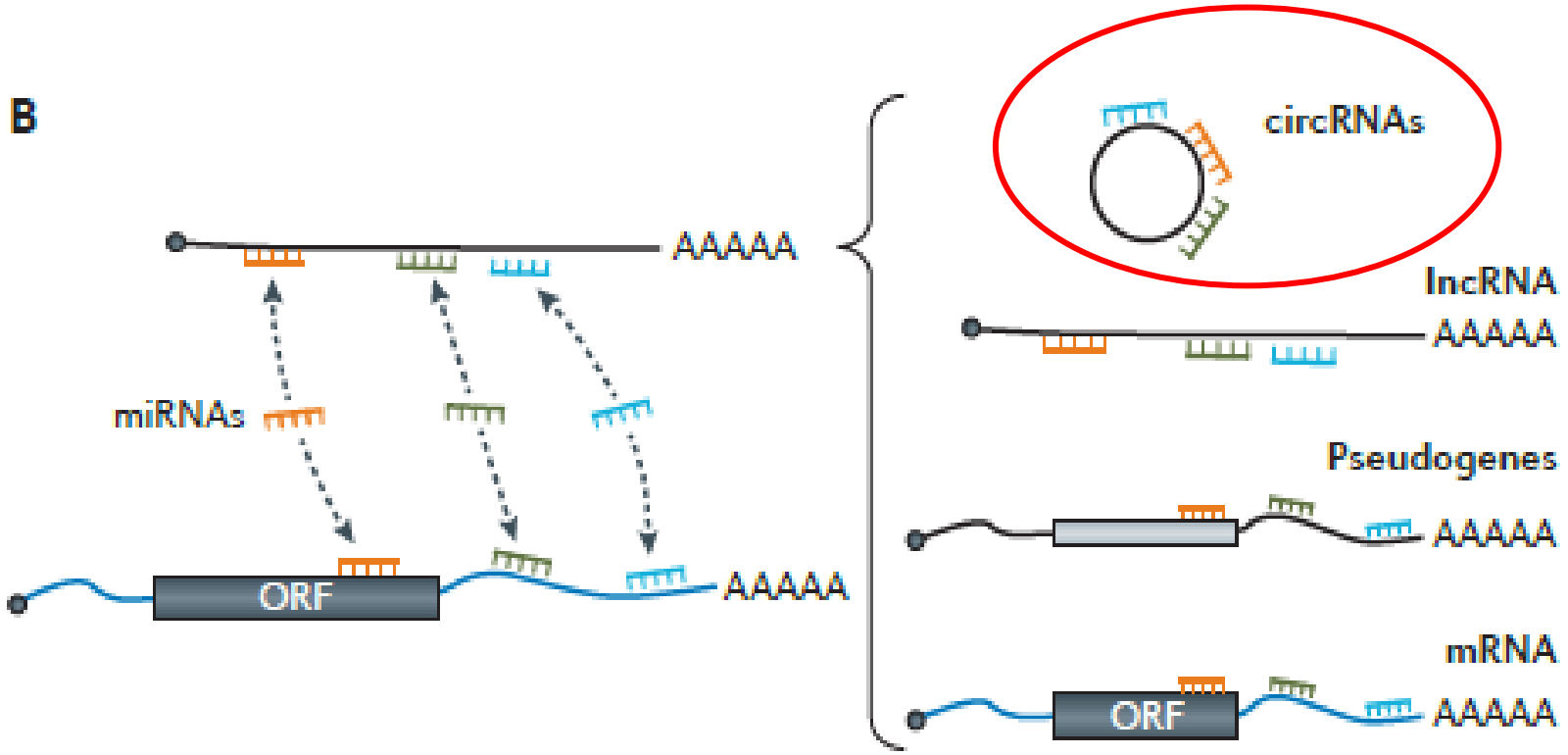


linc-MD1 Is Conserved in Humans, and It Improves Differentiation of Duchenne Myoblasts

(C) Schematic representation of the circuitry linking linc-MD1, miR-135, miR-133, and muscle differentiation. (Cesana et al., 2011)

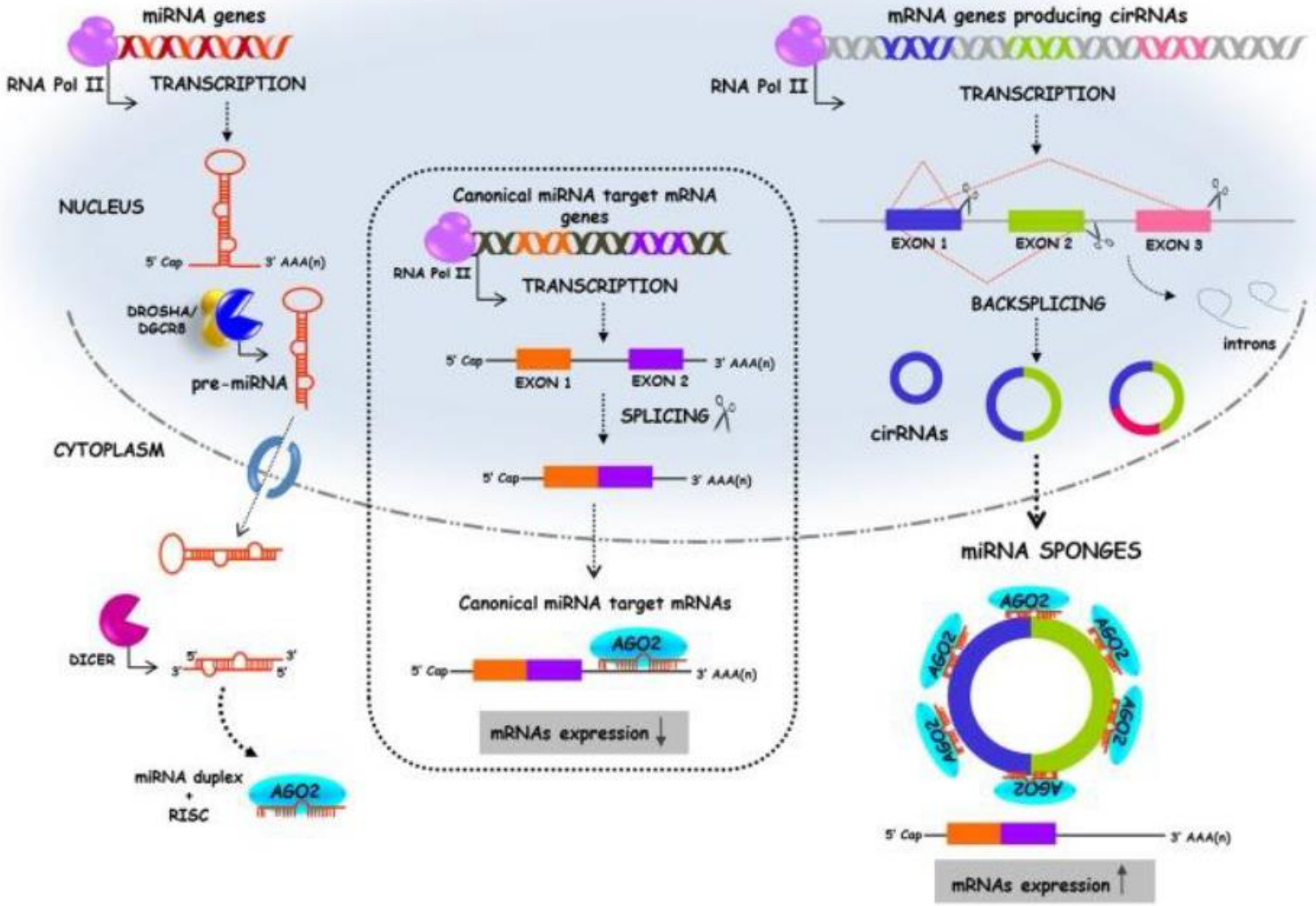


circRNA CAN ACT AS miRNA SPONGES



Base pairing is the mode of action of competing endogenous RNAs. In this case, however, the complementarity is between microRNAs (miRNAs) and different targets (circRNAs, lncRNAs, pseudogene transcripts and mRNAs).

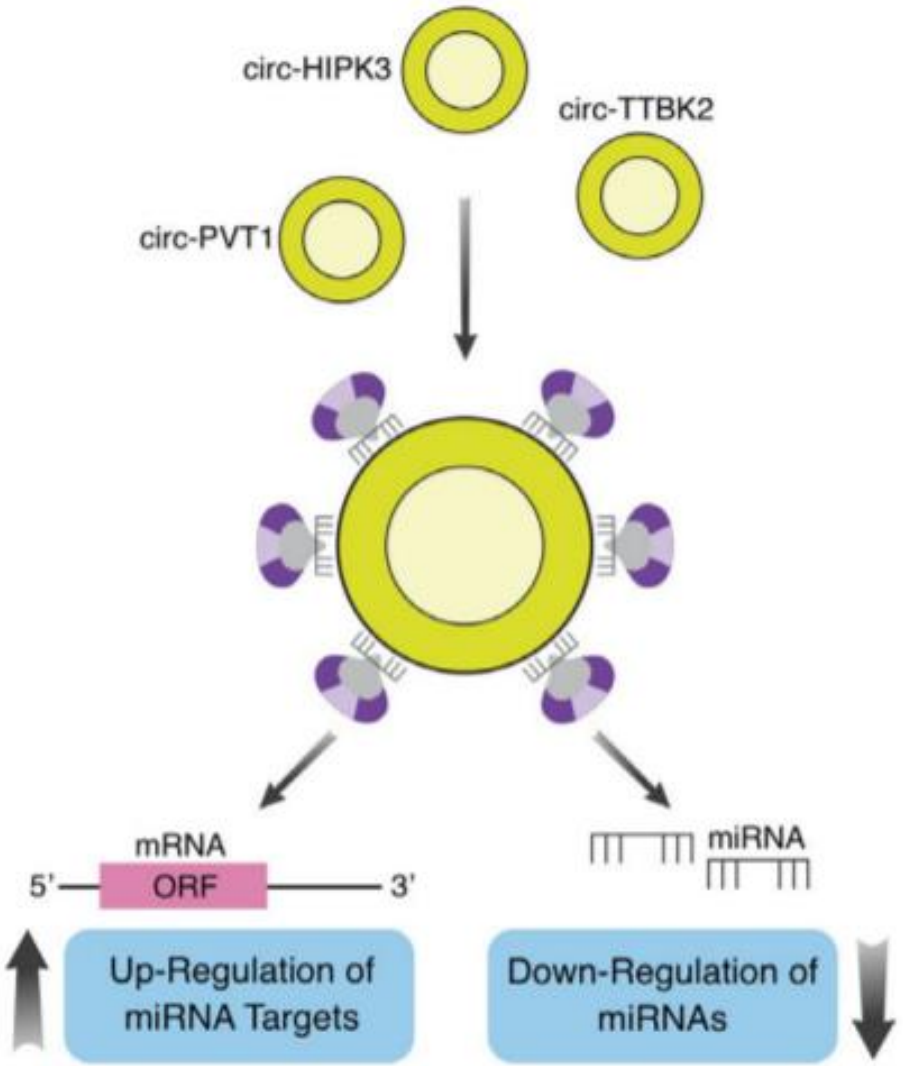
circRNA CAN ACT AS miRNA SPONGES



Biogenesis of miRNAs and circRNAs controling gene expression

circRNA CAN ACT AS miRNA SPONGES

Sponge consequences

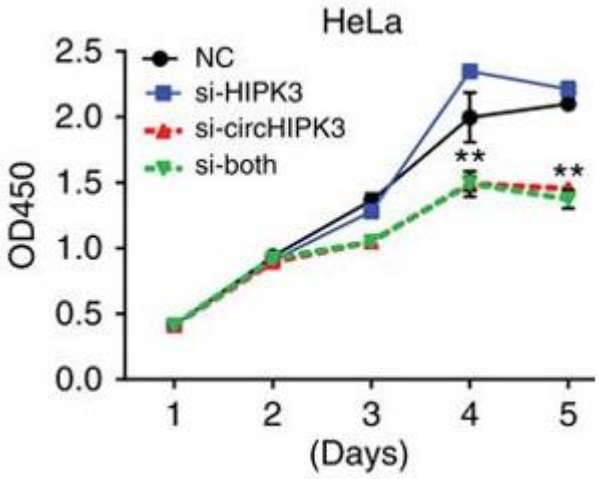


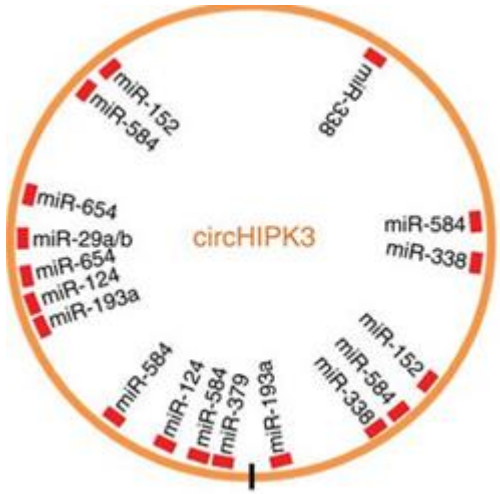
circRNA CAN ACT AS miRNA SPONGES

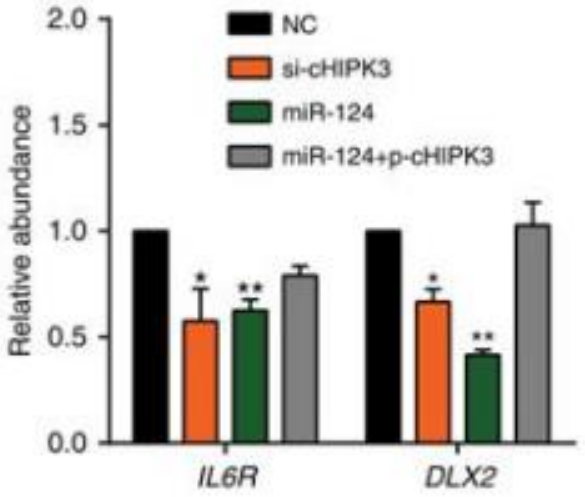
Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs

Qiupeng Zheng, Chunyang Bao, Weijie Guo, Shuyi Li, Jie Chen, Bing Chen, Yanting Luo, Dongbin Lyu, Yan Li, Guohai Shi, Linhui Liang, Jianren Gu, Xianghuo He & Shenglin Huang

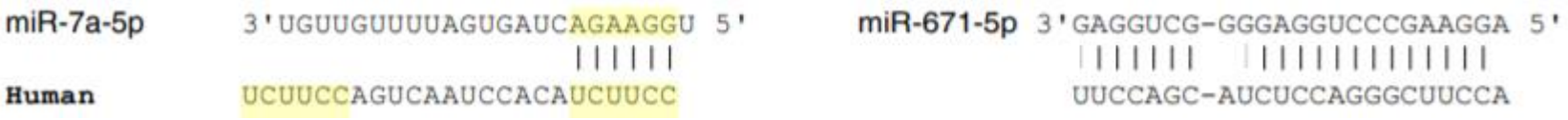
Nature Communications **7**, Article number: 11215 (2016) | Download Citation

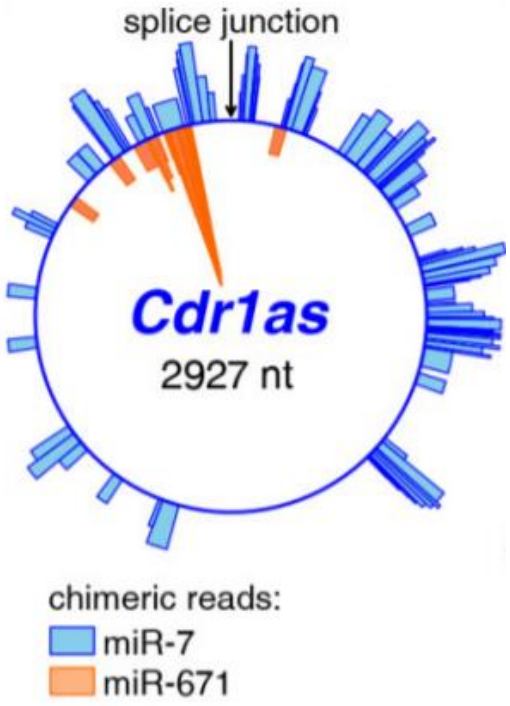




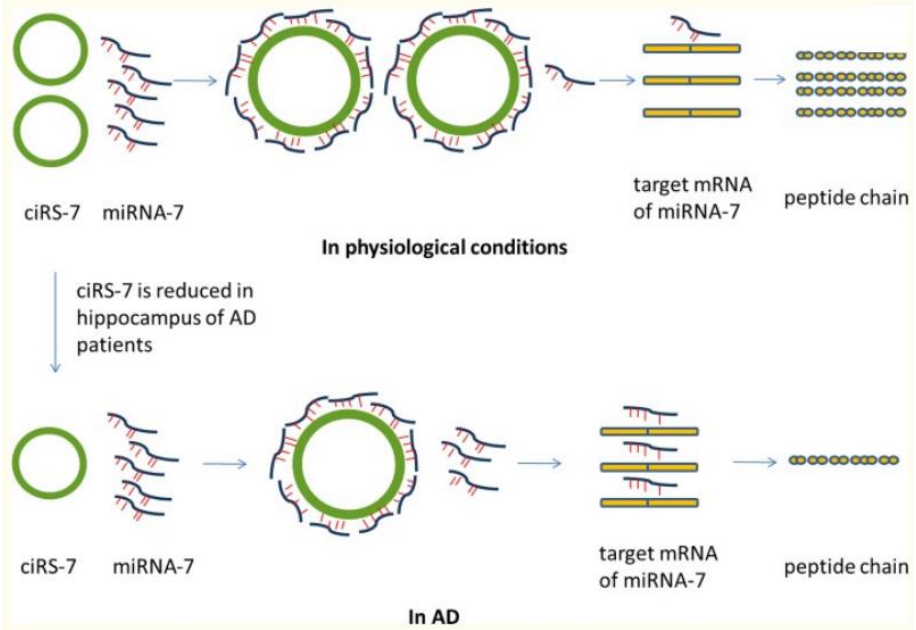


circRNAs CAN ACT AS miRNA SPONGES





- > 70 binding sites for miR- 7
- 1 binding site for miR-671
- miR-671 slices Cdr1as



circRNAs CAN ACT AS miRNA SPONGES?

The quantitative relationship between a miRNA and its endogenous target sites is important!!!!

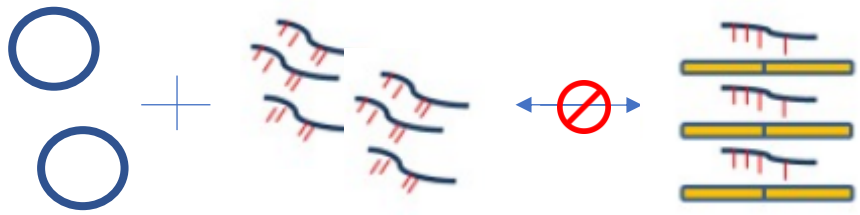
Mol Cell. Author manuscript; available in PMC 2015 Jun 5.
Published in final edited form as:
Mol Cell. 2014 Jun 5; 54(5): 766–776.
Published online 2014 May 1. doi: 10.1016/j.molcel.2014.03.045

PMCID: PMC4267251
NIHMSID: NIHMS641864
PMID: 24793693

Assessing the ceRNA hypothesis with quantitative measurements of miRNA and target abundance

Rémy Denzler,[1,2] Vikram Agarwal,[3,4,5] Joanna Stefano,[3,4] David P Bartel,[3,4,6] and Markus Stoffel[1,2,6]

For miR-122 in hepatocytes, derepression began to be observed at a threshold of 1.5×10^5 added sites per cell, a value exceeding the physiological levels of any endogenous target



Modulation of miRNA target abundance is unlikely to cause significant effects on gene expression and metabolism through a ceRNA effect

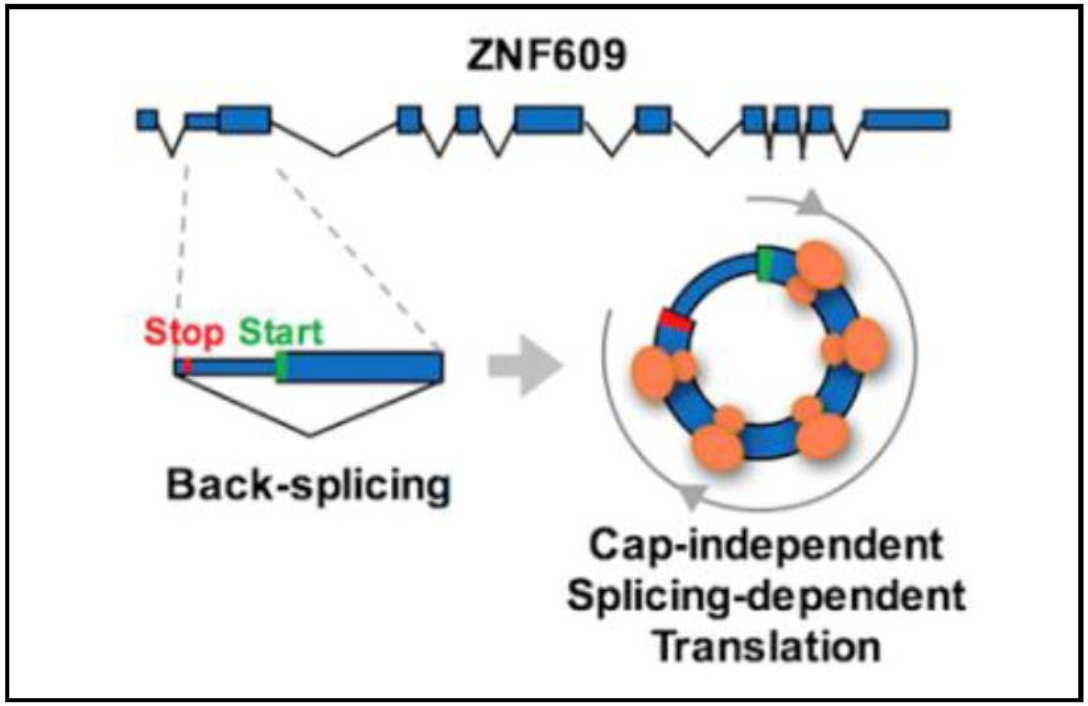
NOT ALL circRNAs ACT AS miRNA SPONGES

List of CircRNAs descrived to act as miRNA sponges

Name of circRNAs	Type of cancer	Expression	Targets	References
CDR1	Hepatocellular carcinoma	Up	miR-7	Yu *et al.* (2016)
Sry	Anaplastic thyroid carcinoma	Up	miR-138	Hansen *et al.* (2013)
circ-ITCH	Esophageal squamous cell carcinoma	Down	miR-17, miR-214, miR-7	Li et al. (2015a)
circHIPK3	Liver cancer	Up	miR-124	Zheng et al. (2016)
circ-000984	Colorectal cancer	Up	miR-106b	Xu et al. (2017)
circ-TTBK2	Human malignant glioma	Up	miR-217	Zheng *et al.* (2017)
circPVT1	Head and neck squamous cell carcinoma	Up	miR-497-5p	Verduci *et al.* (2017)
	Gastric cancer	Up	miR-125 family	Chen *et al.* (2017)
circ-PABPN1	Cervical carcinoma	Down	RNA-binding protein HuR	Abdelmohsen *et al.* (2017)
circ-Foxo3	Different cancer cell lines	Down	p21-CDK2	Du *et al.* (2016)
circ-Amotl1	Breast cancer	Up	c-myc	Yang *et al.* (2017)
circ_002059	Gastric cancer	Down	Unknown	Li *et al.* (2015b)

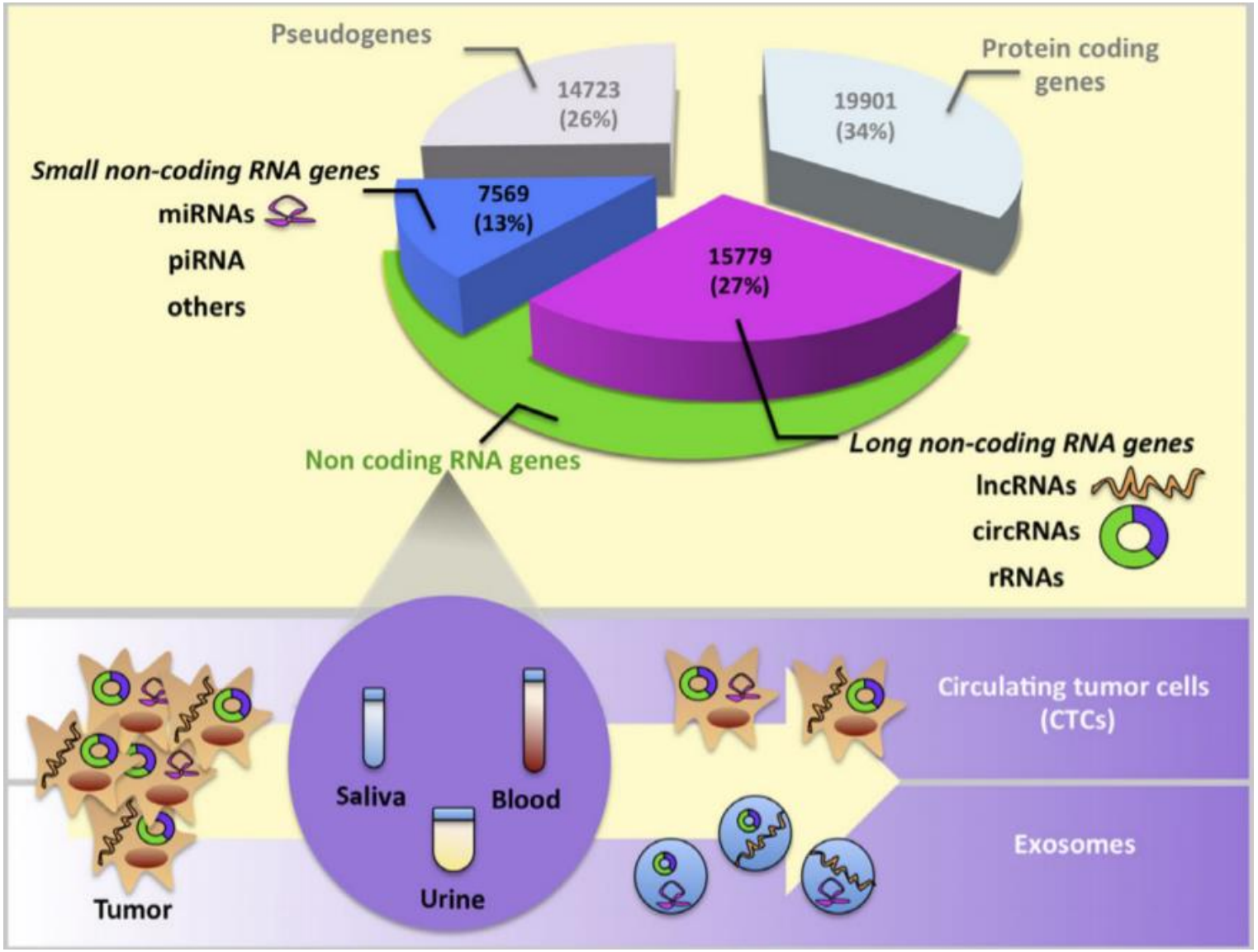
> 230 Papers of circRNAs as miRNA sponges!!!!!!!!

circRNAs CAN BE TRANSLATED

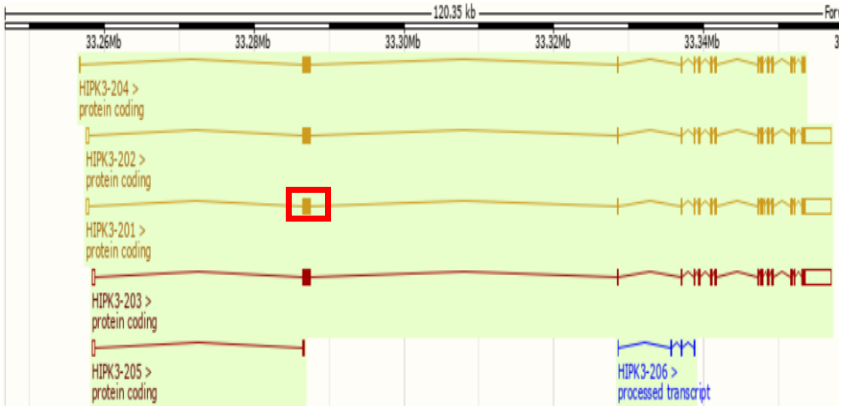


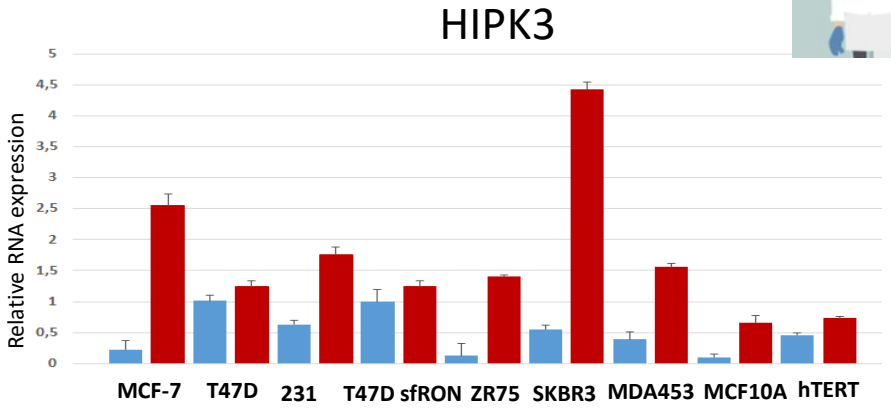
CircRNAs lack 5′ end 7-methylguanosine (m7G) cap structure and a 3′ poly(A) tail, often required for linear mRNA translation and thus translation of circRNAs occurs in a cap-independent manner.

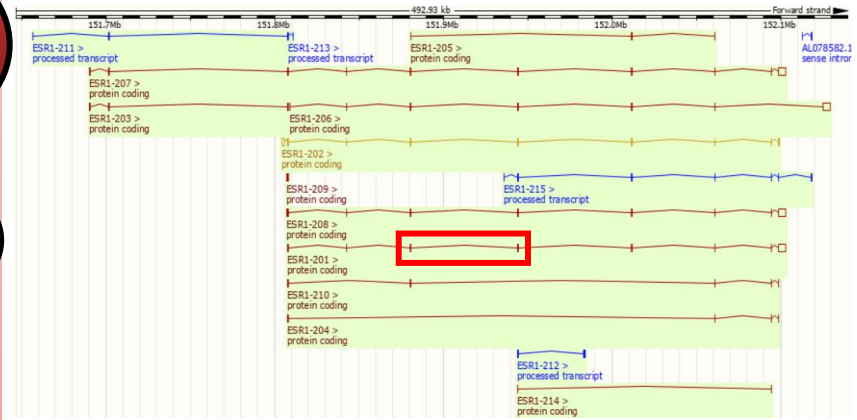
BIOMARKERS

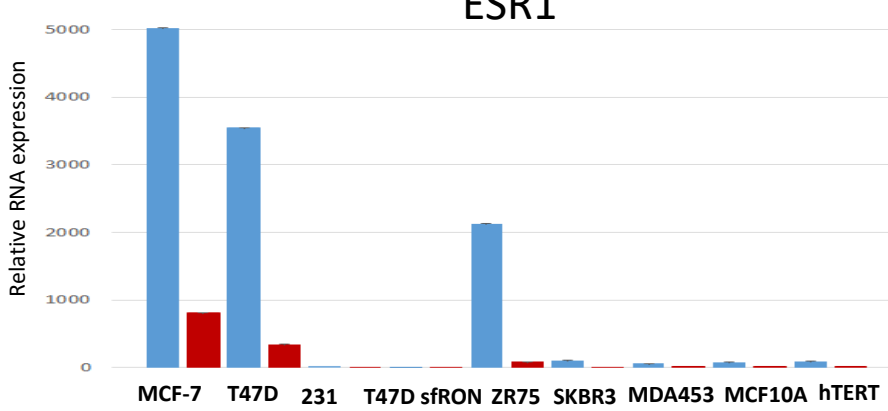

Pseudogenes
14723
(26%)
Protein coding
genes
19901
(34%)
Small non-coding RNA genes
miRNAs
piRNA
others
7569
(13%)
15779
(27%)
Non coding RNA genes
Long non-coding RNA genes
lncRNAs
circRNAs
rRNAs
Saliva
Blood
Urine
Tumor
Circulating tumor cells
(CTCs)
Exosomes

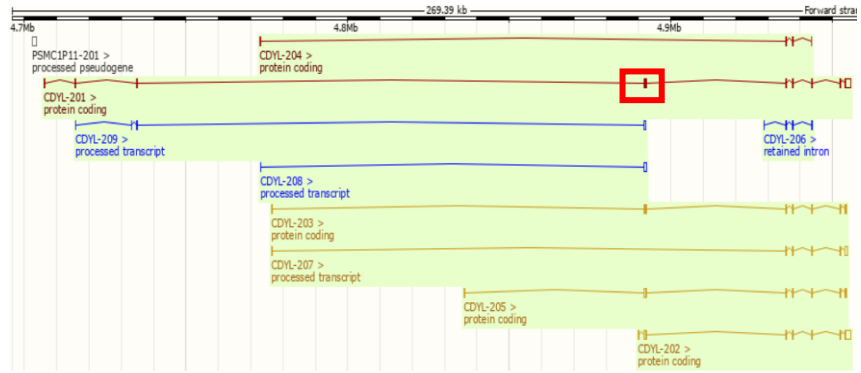
circRNA FUNCTION

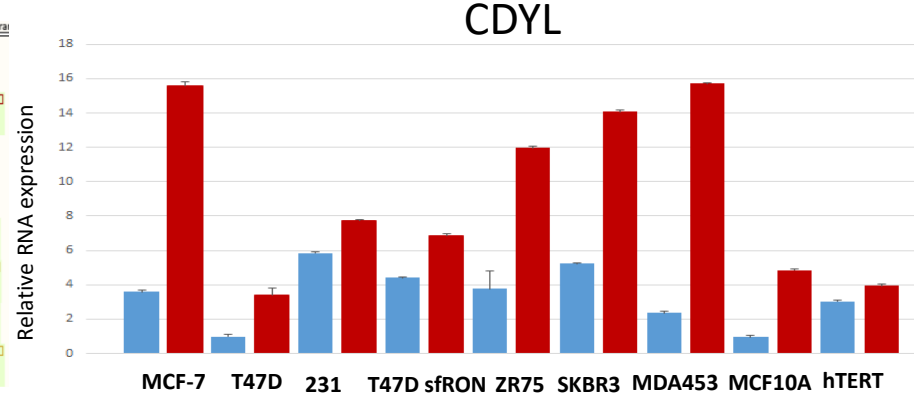






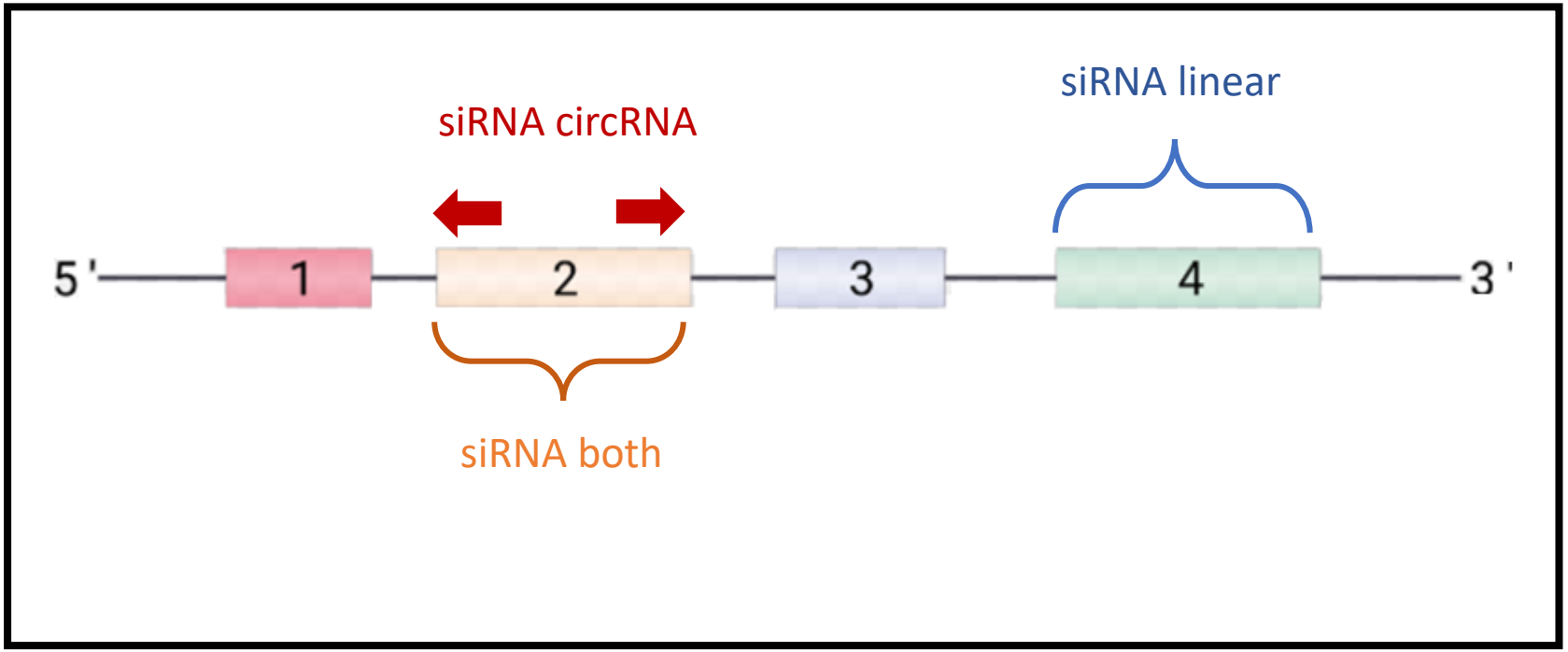






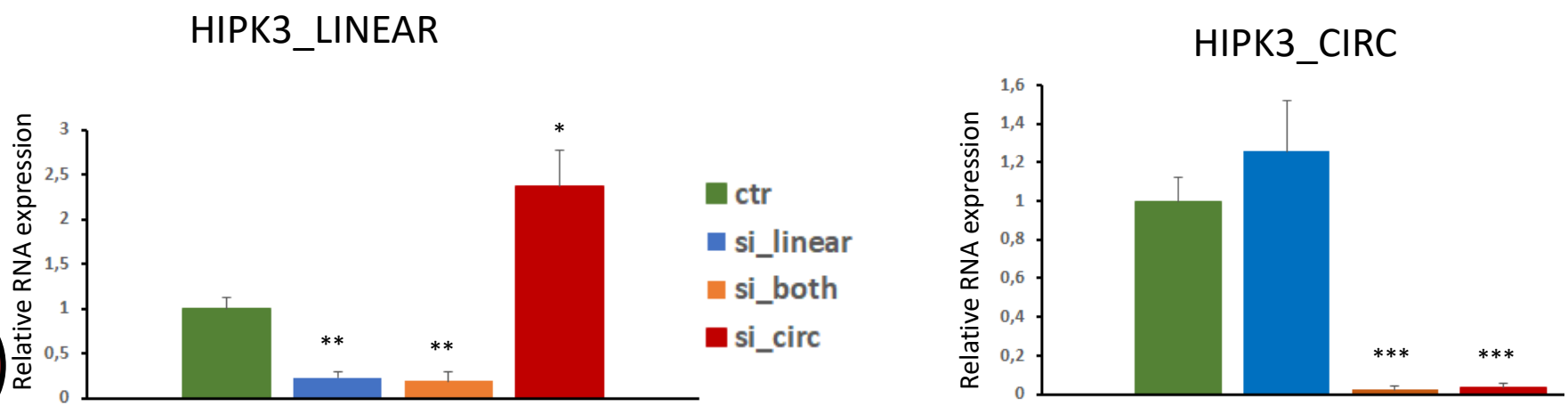
circRNA knockdown

siRNA design

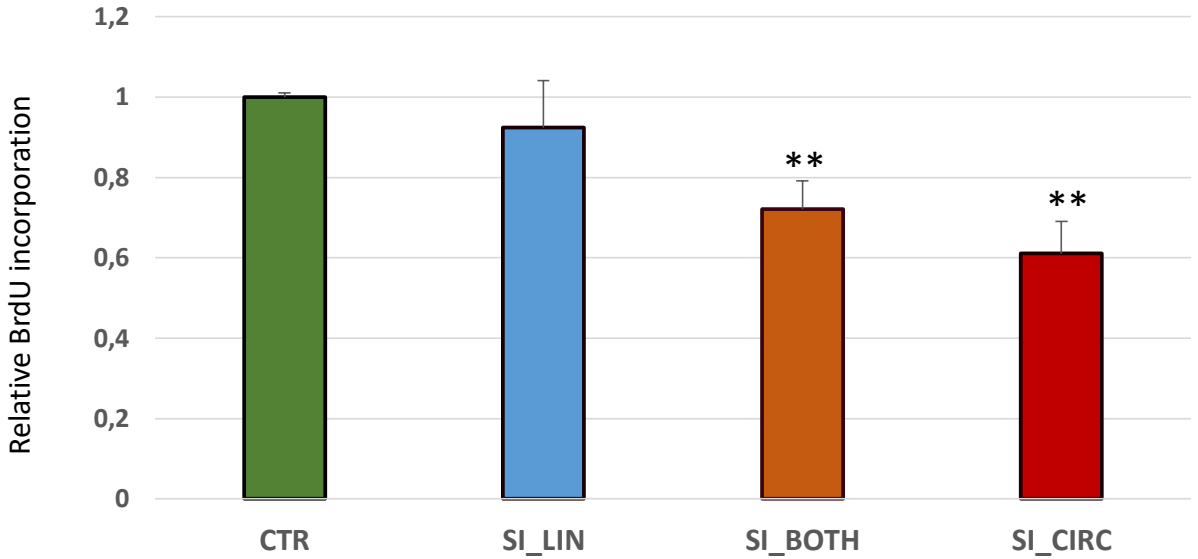


circHIPK3 KNOCKDOWN IMPAIRS PROLIFERATION

HIPK3 KNOCKDOWN



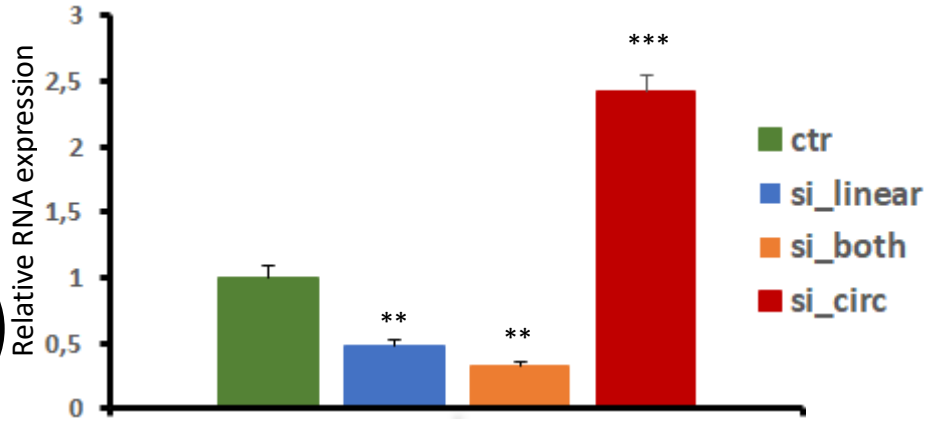
HIPK3 PROLIFERATION MCF-7 48H



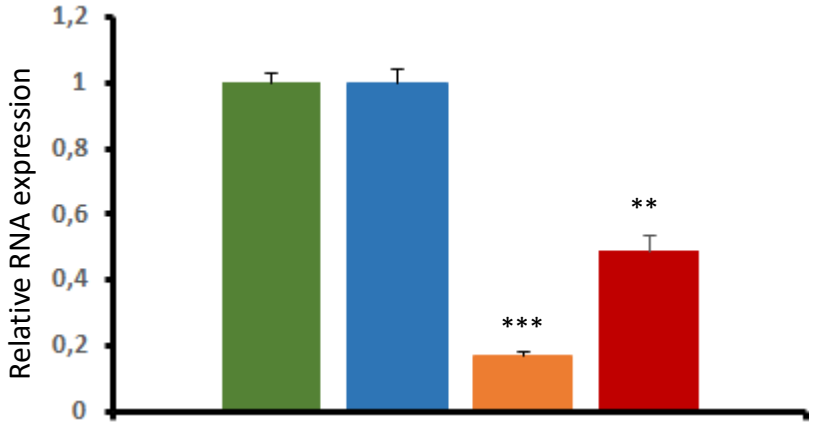
ESR1 KNOCKDOWN IMPAIRS PROLIFERATION

ESR1 KNOCKDOWN

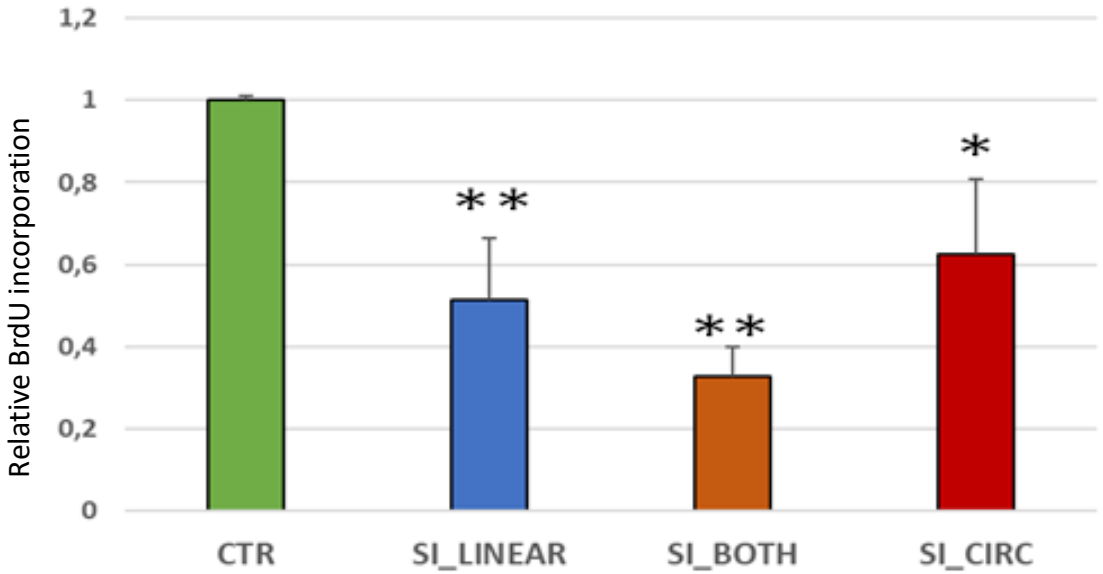
ESR1_LINEAR



ESR1_CIRC

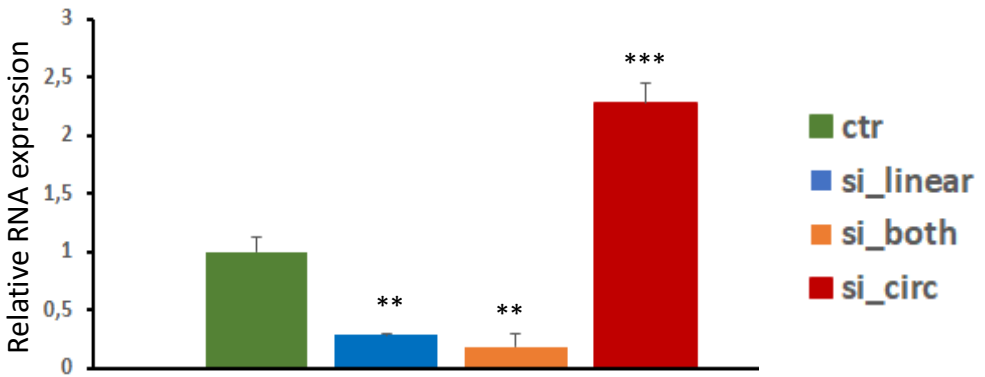


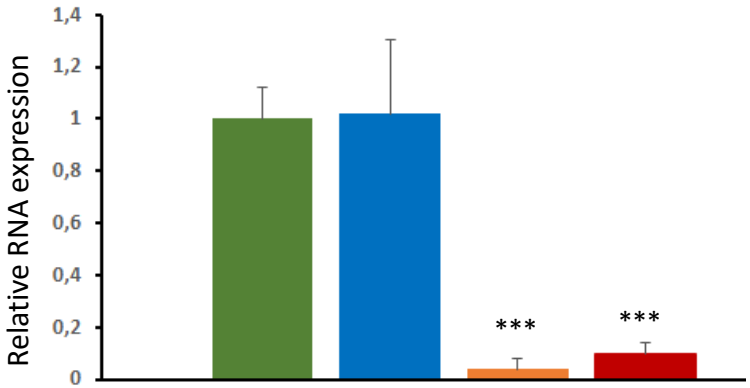
ESR1 PROLIFERATION MCF-7 48H

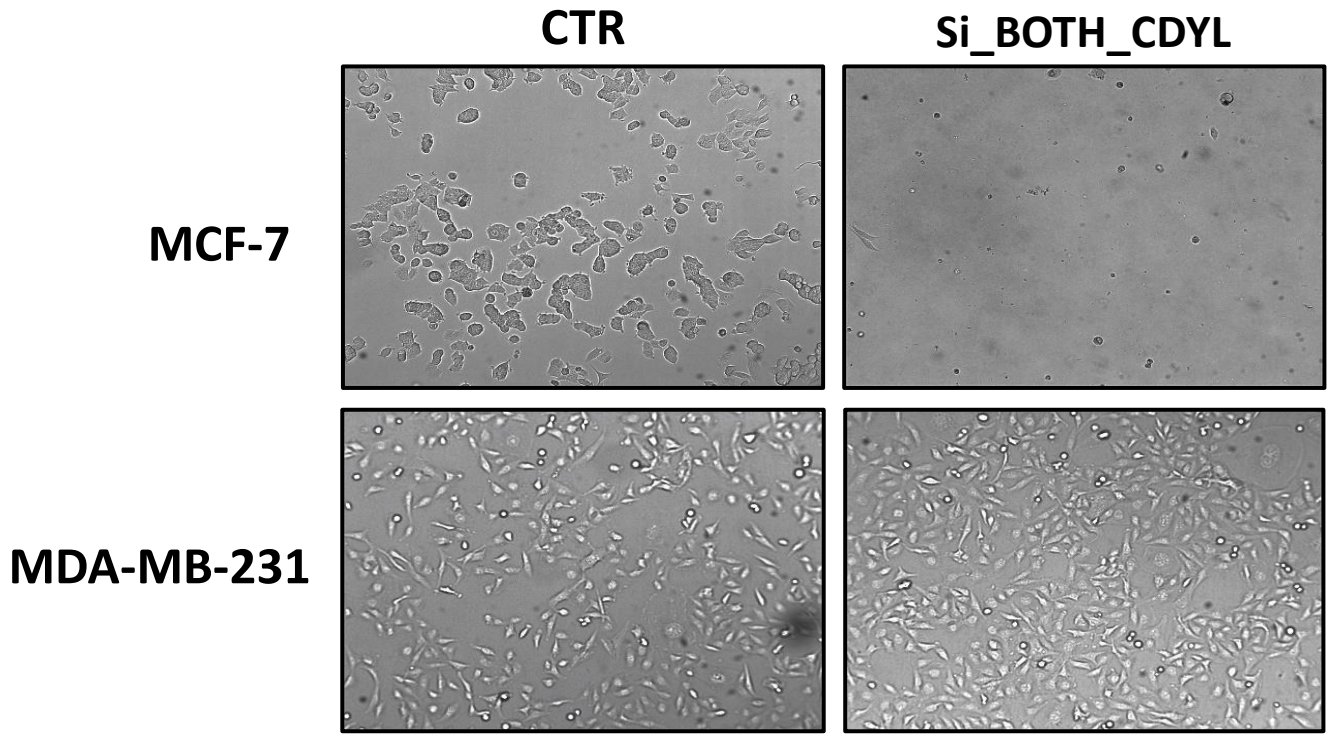


CDYL circularizing exon could determine the survival of luminal breast cancer cells

CDYL KNOCKDOWN

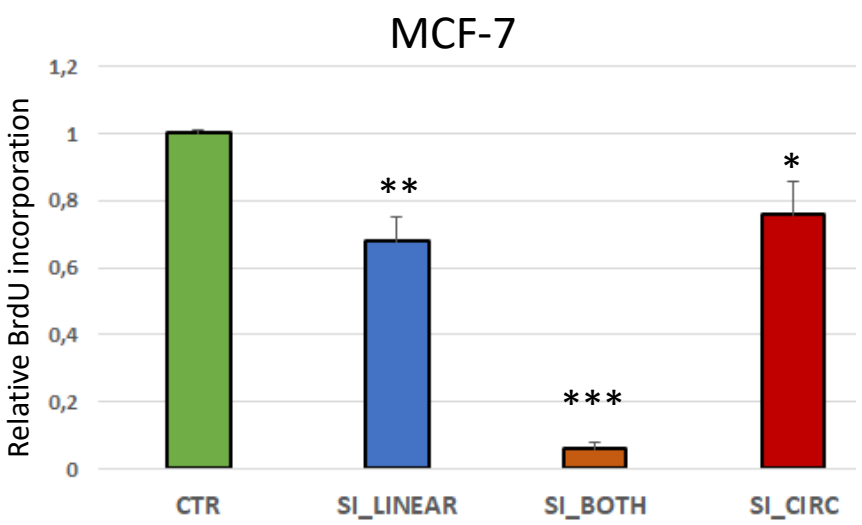


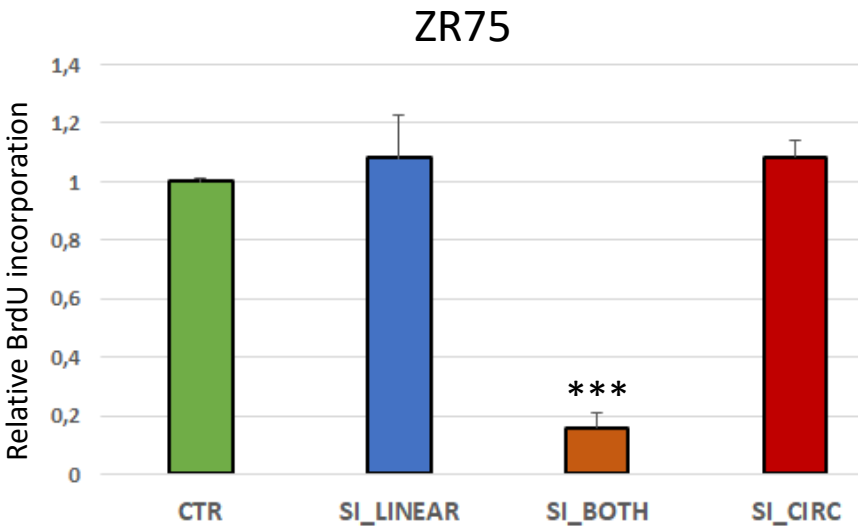


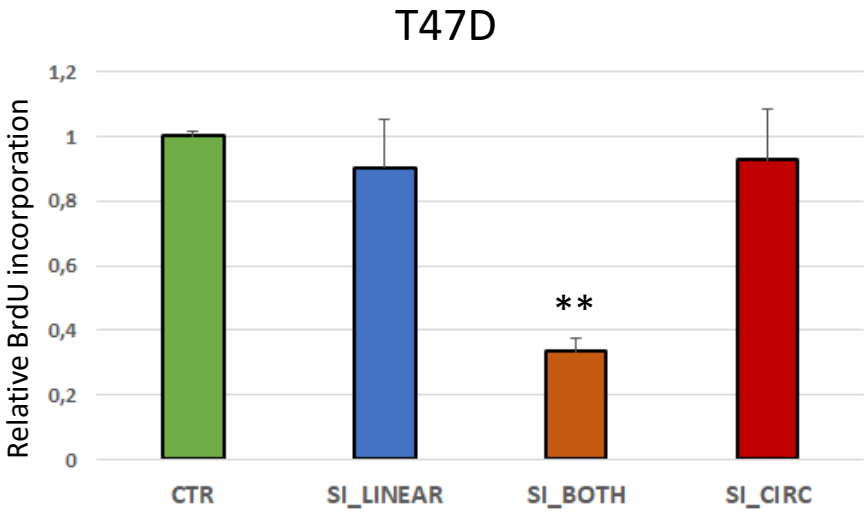


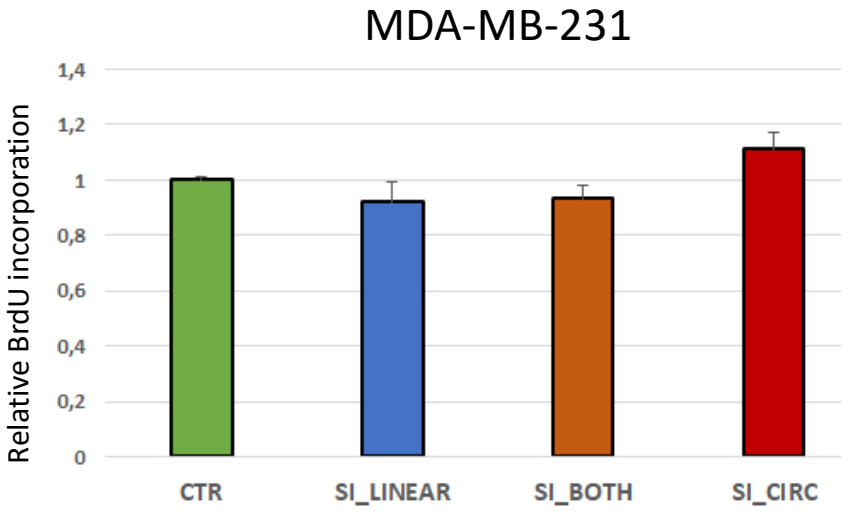
CDYL circularizing exon could determine the survival of luminal breast cancer cells

CDYL PROLIFERATION 48H

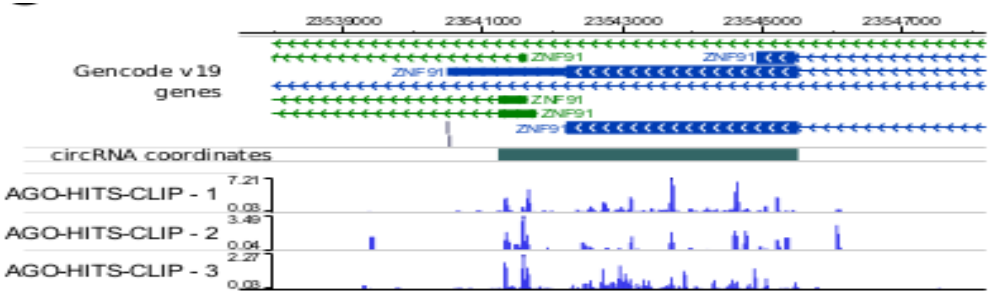








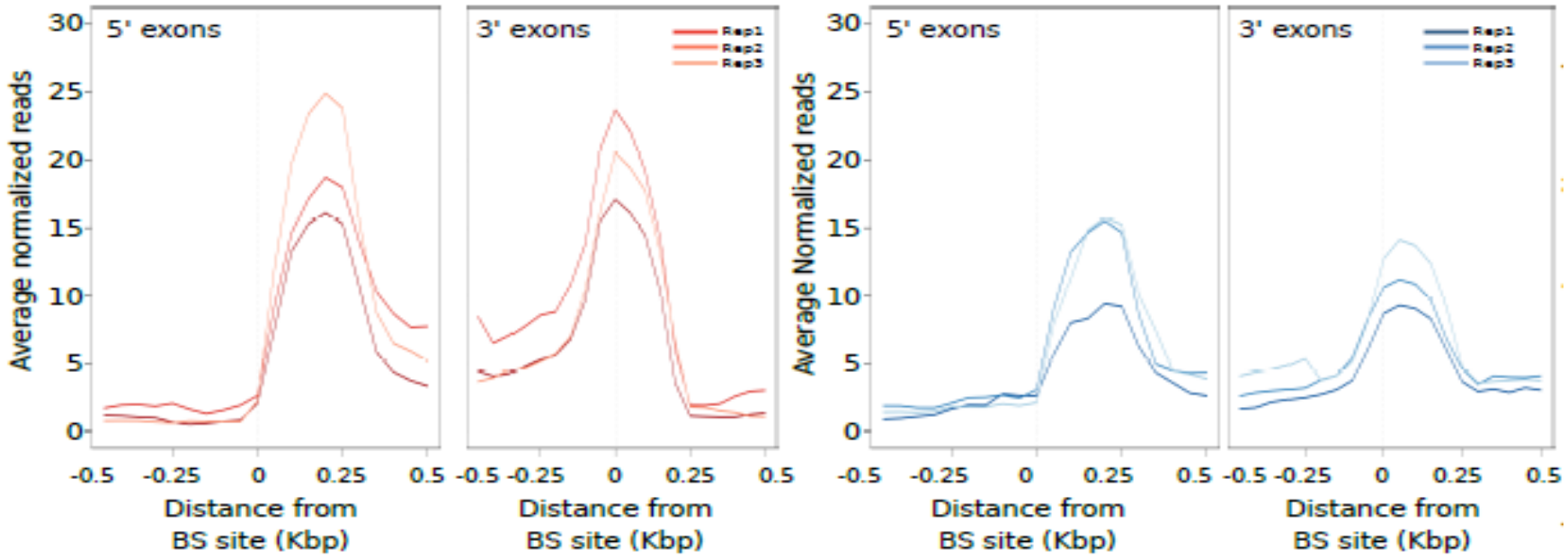
CM7 HOST GENES ARE AGO ENRICHED



AGO Enrichment by HITS-CLIP analysis

Host genes

Control genes



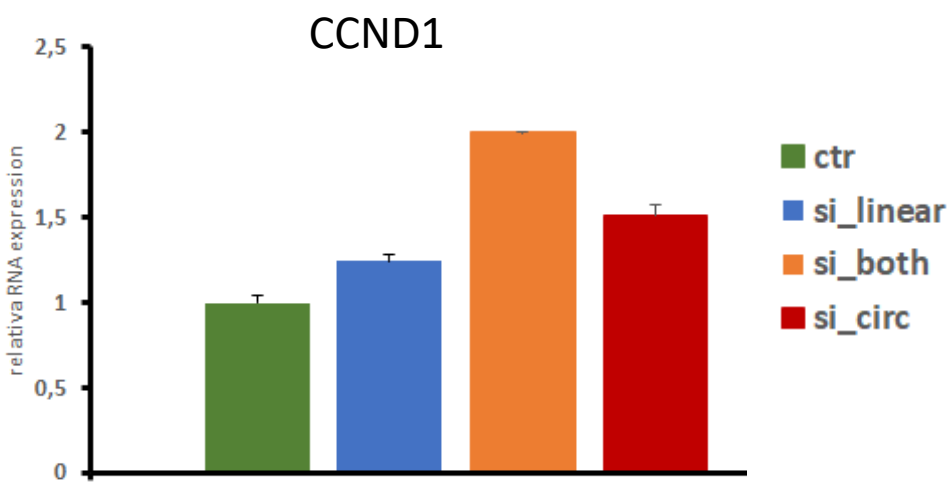
ceRNA mechanism?

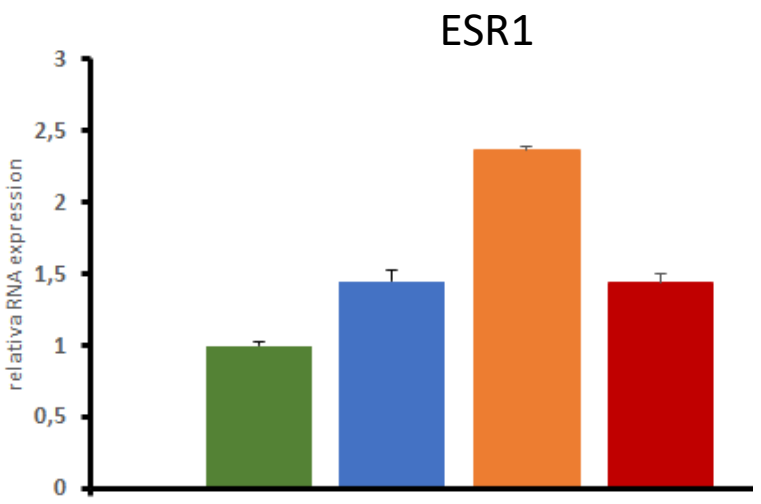
circCDYL miRNA prediction

Circ_CDYL_4	283,64	hsa-miR-185-5p
Circ_CDYL_4	283,64	hsa-miR-3615
Circ_CDYL_4	283,64	hsa-miR-27b-3p
Circ_CDYL_4	283,64	hsa-miR-27a-3p
Circ_CDYL_4	283,64	hsa-miR-193b-3p
Circ_CDYL_4	283,64	hsa-miR-505-3p
Circ_CDYL_4	283,64	hsa-miR-1307-5p
Circ_CDYL_4	283,64	hsa-miR-342-5p
Circ_CDYL_4	283,64	hsa-miR-497-5p
Circ_CDYL_4	283,64	hsa-miR-1247-5p

		Validation methods						
		Strong evidence			Less strong evidence			
miRNA	Target	Reporter assay	Western blot	qPCR	Microarray	NGS	pSILAC	Other
hsa-miR-193b-3p	CCND1	✔	✔	✔	✔	✔		✔
hsa-miR-193b-3p	ESR1	✔			✔			✔
hsa-miR-193b-3p	PLAU	✔	✔	✔	✔	✔		✔
hsa-miR-193b-3p	PRAP1	✔	✔					
hsa-miR-193b-3p	MCL1	✔	✔	✔	✔			✔
hsa-miR-193b-3p	ETS1	✔	✔	✔				✔

miR 193b targets upon CDYL Knockdown





CONCLUSIONS

circRNAS are :

- Non polyadenylated, more stable
- Formed by backsplicing
- Less abundant tan mRNAs
- Predominantly cytoplasmic

BIOGENESIS:

- Transcribed by a faster POLII
- Backsplicing depends on canonical splice sites
- They can arise from exon-skipping
- Regulation by cis elements, Intron Pairing: Alu elements
- Regulation by RBPs: QKI, FUS, hnRNPL, MBL, ADAR1

HOST GENES:

- Are longer
- Have much more number of isoforms
- Have the first intron longer

POSSIBLE FUNCTIONS:

- miRNA sponges?¿
- Translation?¿
- Biomarkers?¿

→ We know nothing!