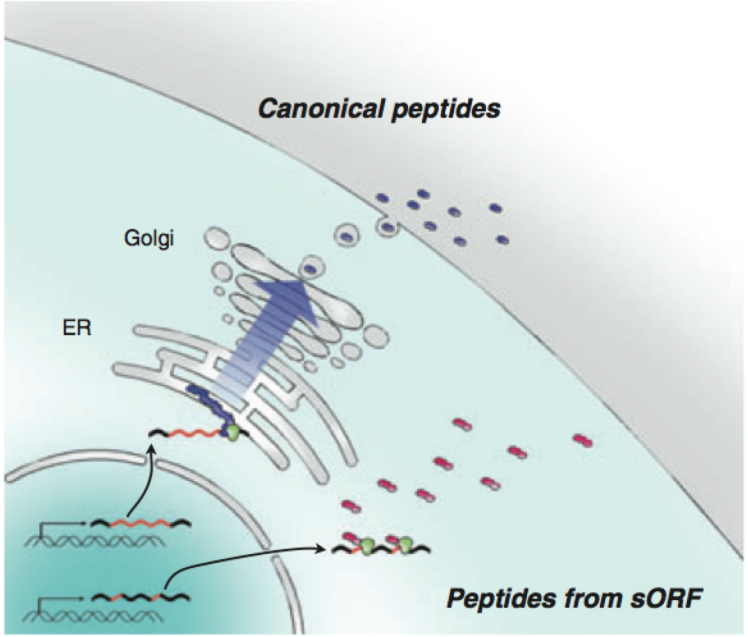
Cytoplasmic lncRNAs

Functions of lncRNAs

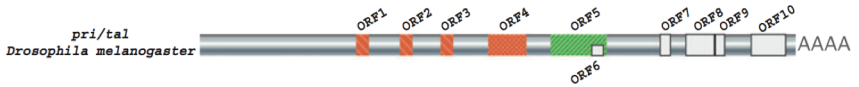
Cytoplasmic lncRNAs

Apparently non-coding RNAs are shown to be translated in functional small peptides.



Canonical peptides are produced by processing a long precursor in the ER and are released into the extracellular space *via* trans-Golgi.

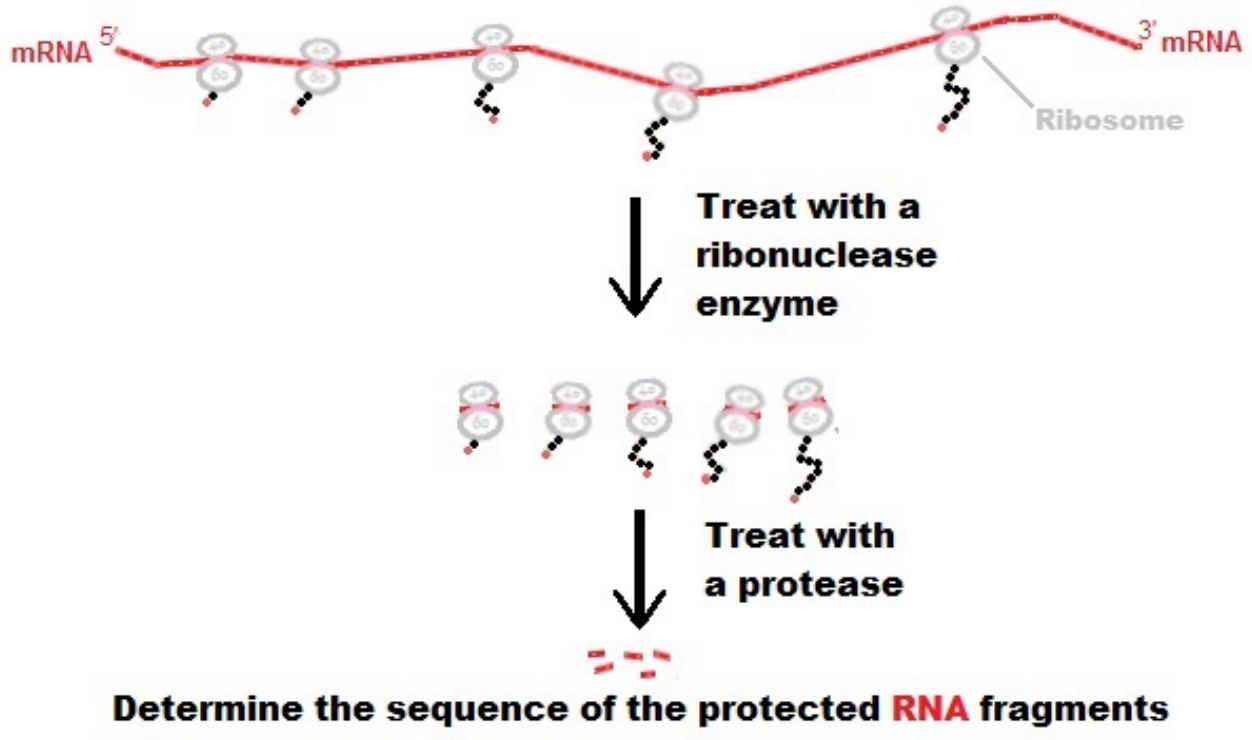
sORF (small open reading frame: 11-100aa) encoded small peptides are direclty produced in the cytoplasm in a non-canonical translation process.

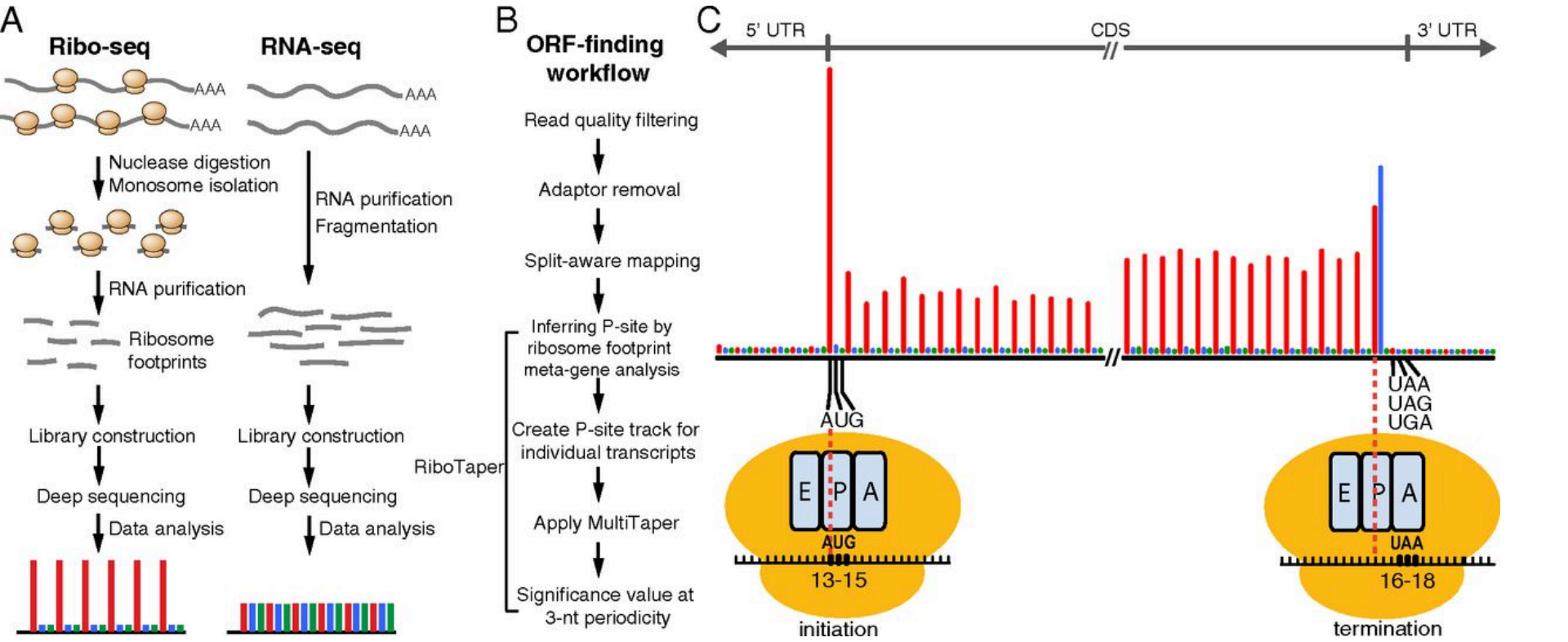


The EMBO Journal Vol 33, 2014

Identification of small ORFs in vertebrates using ribosome footprinting and evolutionary conservation

Ariel A Bazzini,……..& Antonio J Giraldez***




A
Ribo-seq
RNA-seq
AAA
AAA
AAA
AAA
Nuclease digestion
Monosome isolation
RNA purification
Fragmentation
RNA purification
Ribosome
footprints
Library construction
Library construction
Deep sequencing
Deep sequencing
Data analysis
Data analysis
B
ORF-finding
workflow
Read quality filtering
Adaptor removal
Split-aware mapping
Inferring P-site by
ribosome footprint
meta-gene analysis
RiboTaper
Create P-site track for
individual transcripts
Apply MultiTaper
Significance value at
3-nt periodicity
C
5' UTR
CDS
3' UTR
AUG
UAA
UAG
UGA
E
P
A
AUG
13-15
initiation
E
P
A
UAA
16-18
termination

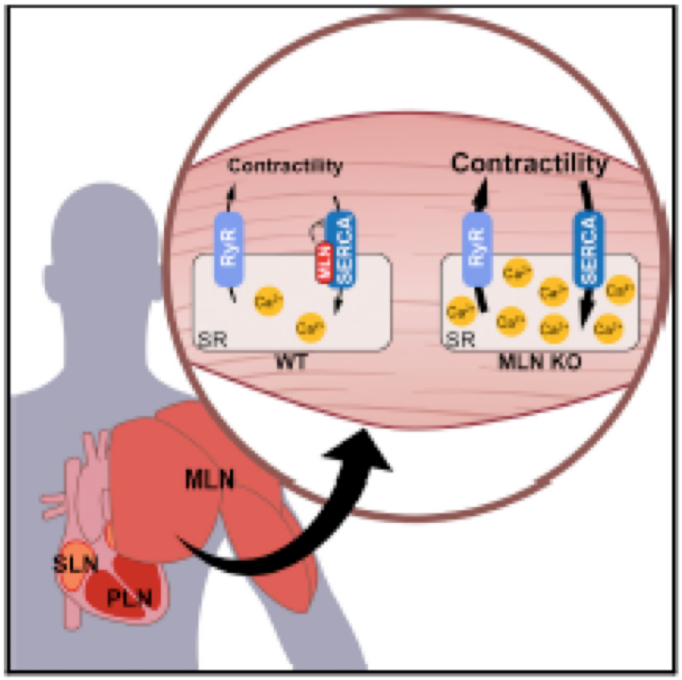
lncRNAs can encode for short peptides

Article

Cell

A Micropeptide Encoded by a Putative Long Noncoding RNA Regulates Muscle Performance

Graphical Abstract



Authors
Douglas M. Anderson,
Kelly M. Anderson, ...,
Rhonda Bassel-Duby, Eric N. Olson

Correspondence
eric.olson@utsouthwestern.edu

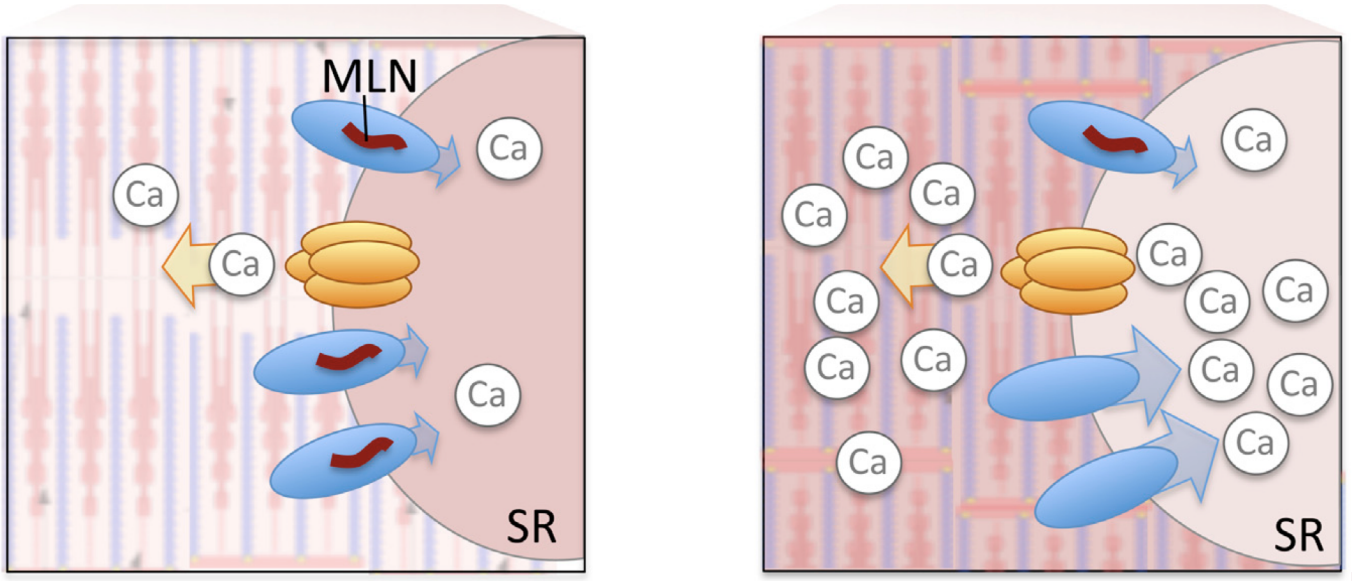
In Brief

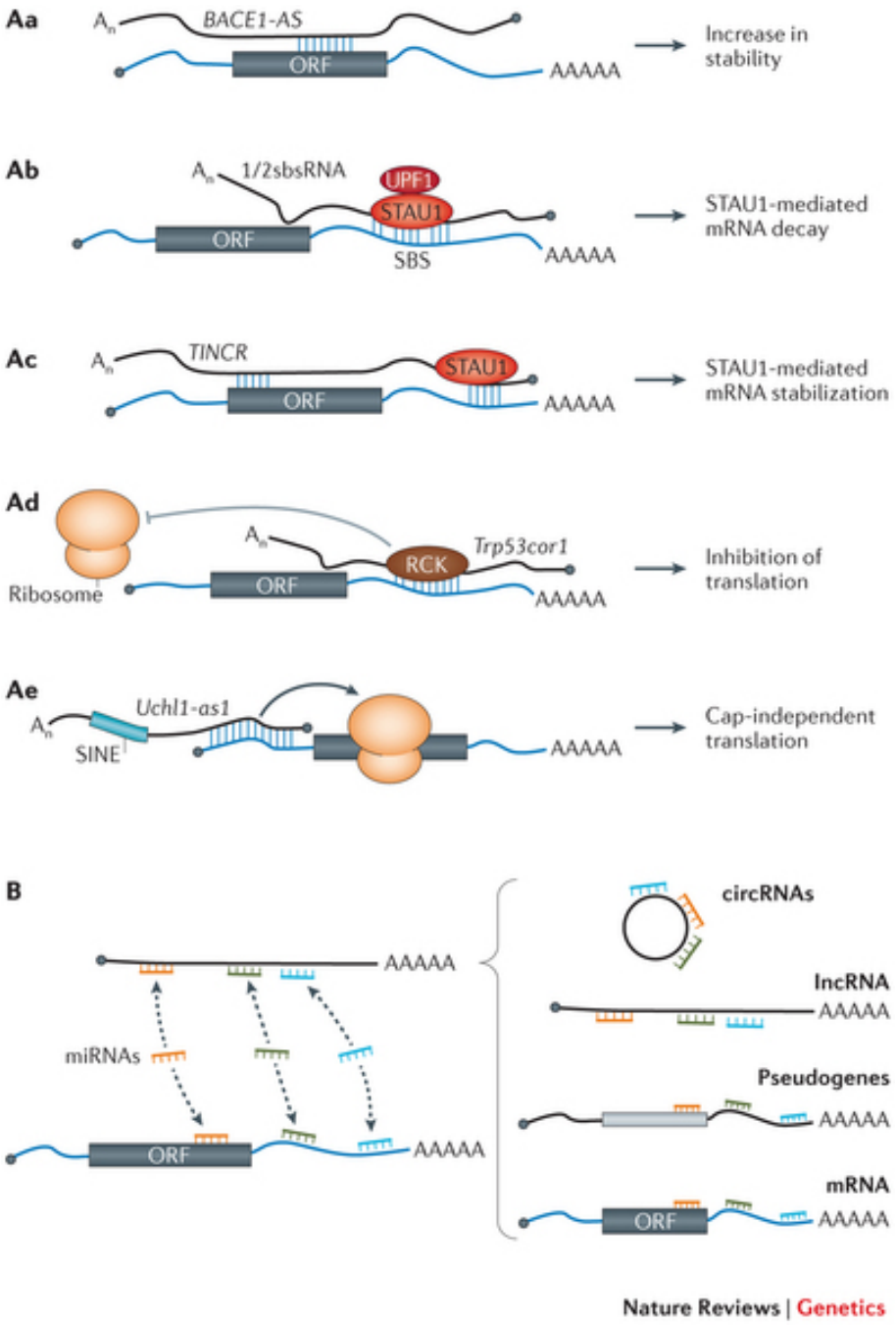
Myoregulin is a skeletal muscle-specific micropeptide that regulates muscle performance by modulating intracellular calcium handling.

Functional micropeptides can be concealed within RNAs that appear to be noncoding. We discovered a conserved micropeptide, which we named **myoregulin** (MLN), encoded by a skeletal muscle-specific RNA annotated as a putative long noncoding RNA. MLN shares structural and functional similarity with phospholamban (PLN) and sarcolipin (SLN), which inhibit SERCA, the membrane pump that controls muscle relaxation by regulating Ca2+ uptake into the sarcoplasmic reticulum (SR). **MLN interacts directly with SERCA and impedes Ca2+ uptake into the SR.** In contrast to PLN and SLN, which are expressed in cardiac and slow skeletal muscle in mice, MLN is robustly expressed in all skeletal muscle. Genetic deletion of MLN in mice enhances Ca2+ handling in skeletal muscle and improves exercise performance.
These findings identifyMLNas an important regulator of skeletal muscle physiology and highlight the possibility that additional micropeptides are encoded in the many RNAs currently annotated as noncoding.

lncRNAs can encode for short peptides

This 46-aa micropeptide had been missed for years, hidden in an uncharacterized vertebrate transcript annotated as a long noncoding RNA (lncRNA). The tiny 138-bp open reading frame (ORF) embedded in the third exon of the MLN transcript is heavily conserved between mouse and human and is expressed exclusively in skeletal muscle during embryogenesis and into adulthood





Long non-coding RNAs: new players in cell differentiation and development

Alessandro Fatica & Irene Bozzoni

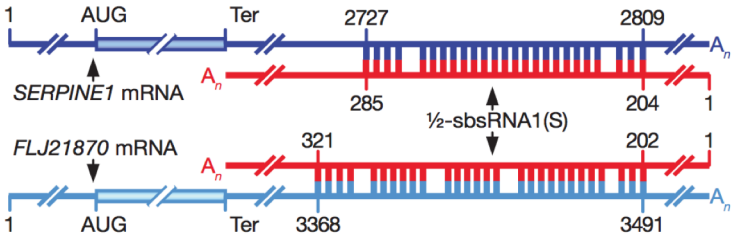
Nature Reviews Genetics

| A common recognition mechanism is through base pairing of complementary regions between the long non-coding RNA (lncRNA) and their target RNA sequence. Aa | Base pairing between specific regions of the human β-site APP-cleaving enzyme 1 (BACE1) mRNA and its antisense transcript BACE1-AS induces stabilization of the target mRNA and increases BACE1 protein expression. Ab | Staufen double-stranded RNA-binding protein 1 (STAU1)-mediated mRNA decay is induced when intermolecular base pairing is formed between an Alu element (or short interspersed element (SINE) in mice) in the 3′ untranslated region of the mRNA and an Alu element within a long half-STAU1-binding site RNA (1/2sbsRNA). This mRNA decay mechanism also involves the RNA helicase up-frameshift 1 (UPF1). Ac | By contrast, STAU1-mediated mRNA stabilization has been described in the case of tissue differentiation-inducing non-protein coding RNA (TINCR), which recognizes its target mRNAs through a 25 nucleotide-long motif. Antisense recognition has been shown to also control translation. Ad | A repressive effect on translation was shown for the targets of tumour protein p53 pathway corepressor 1 (Trp53cor1) lncRNA, which functions with the RNA helicase RCK. Ae | Translation is induced upon stress induction of ubiquitin carboxy-terminal hydrolase L1 antisense RNA 1 (Uchl1-as1). B Base pairing is also the mode of action of competing endogenous RNAs. In this case, however, the complementarity is between microRNAs (miRNAs) and different targets (including circular RNAs (circRNAs, lncRNAs21, pseudogene transcripts and mRNAs). The effect of these interactions is that protein-coding RNAs and non-coding RNAs can crosstalk to each other by competing for miRNA binding through their miRNA recognition motifs. ORF, open reading frame; SBS, STAU1-binding site.

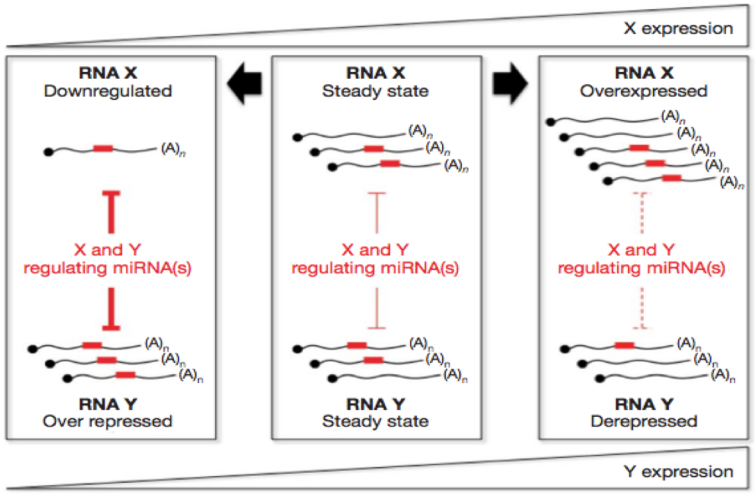
Functions of lncRNAs

Cytoplasmic lncRNAs

lncRNAs can regulate mRNA expression in a sequence-dependent manner



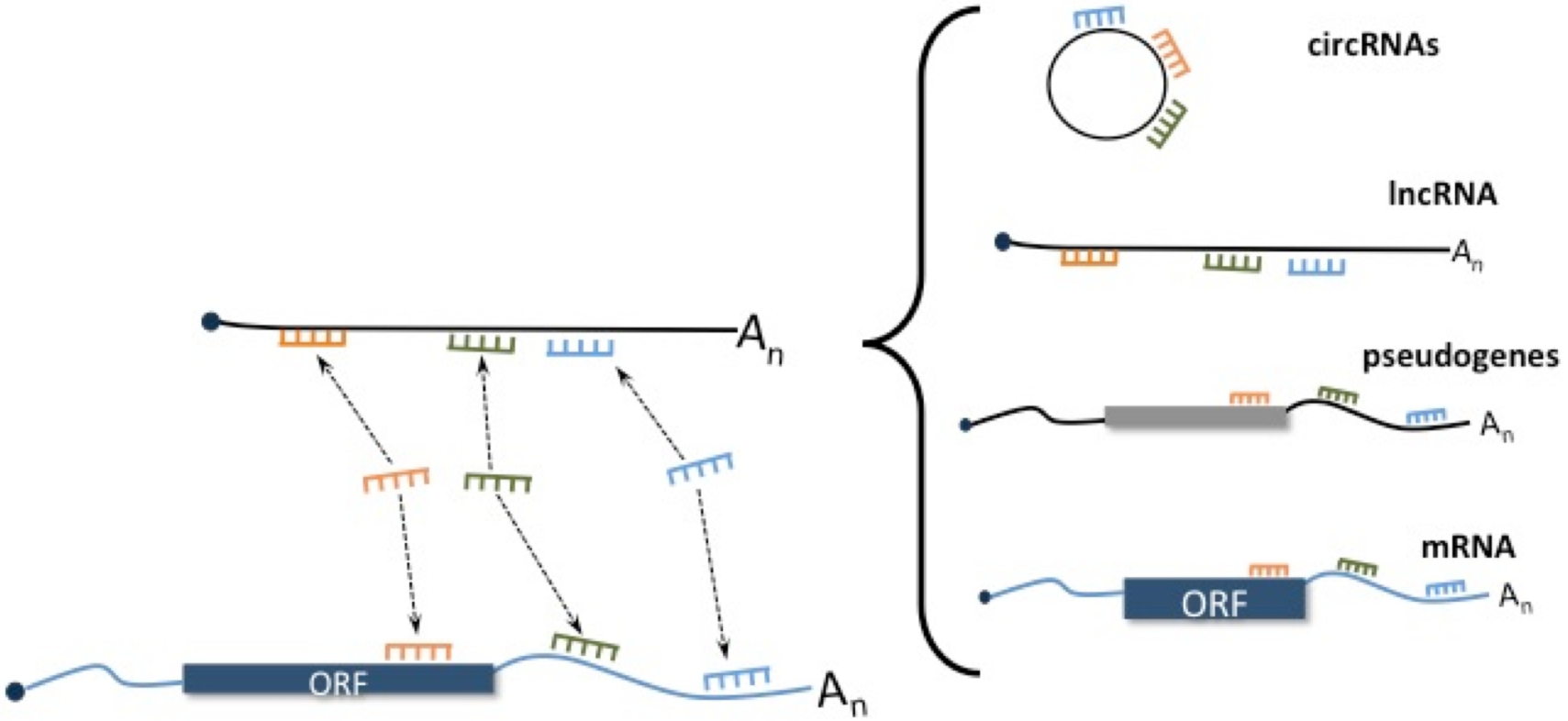
lncRNAs carrying Alu sequences induce mRNA decay by duplexing with their partially complementary 3'UTR and recruiting Staufen-1 on their targets.



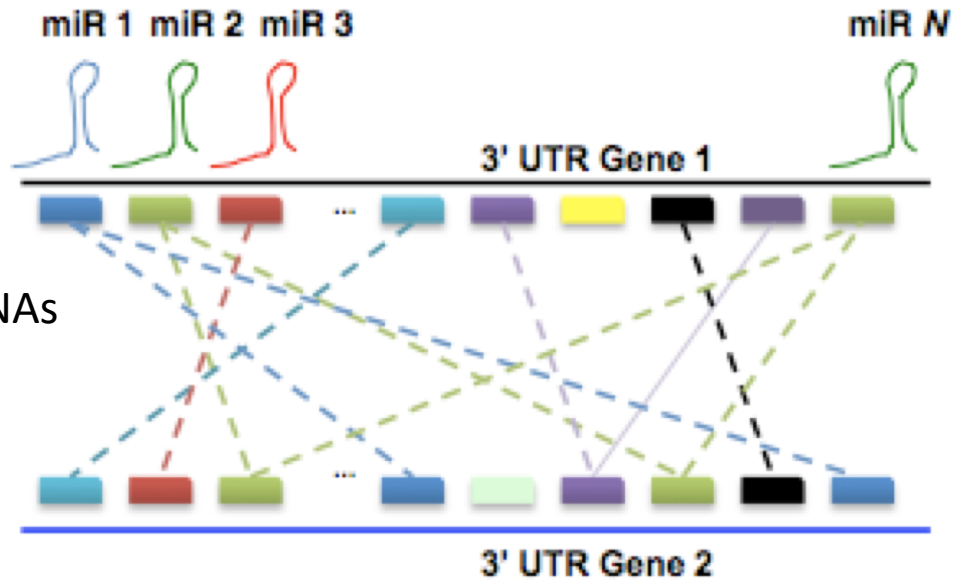
Pseudogene non-coding transcripts can sequester microRNAs that target their coding counterparts.
Also mRNAs can act as "sponges" for miRNA level regulation.

Tumor suppressor PTEN mRNA and its pseudogene-derived transcript PTENP1 are both targeted by miR-19b and miR-20a. PTENP1 is lost in many types of cancer.

Competing endogenous RNAs



Protein coding RNA transcripts can cross talk by competing for common miRNAs



competing endogenous RNAs

ceRNAs

Karreth et al., *Cell* 147, October 14, 2011

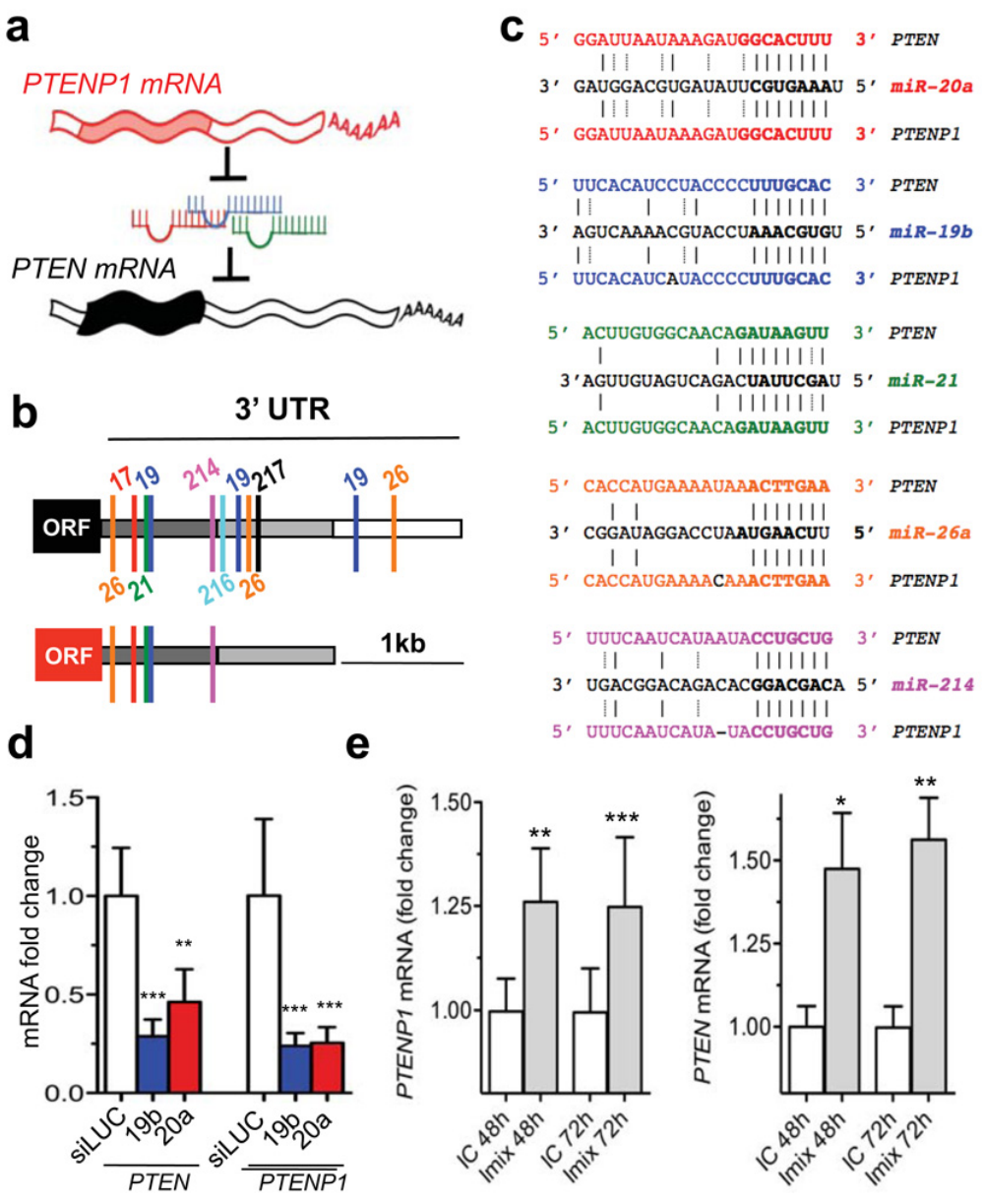
Tay et al., *Cell* 147, October 14, 2011

Sumazin et al., *Cell* 147, October 14, 2011

Nature. 2010 June 24; 465(7301): 1033–1038.

A coding-independent function of gene and pseudogene mRNAs regulates tumour biology

Laura Poliseno,……Pier Paolo Pandolfi1

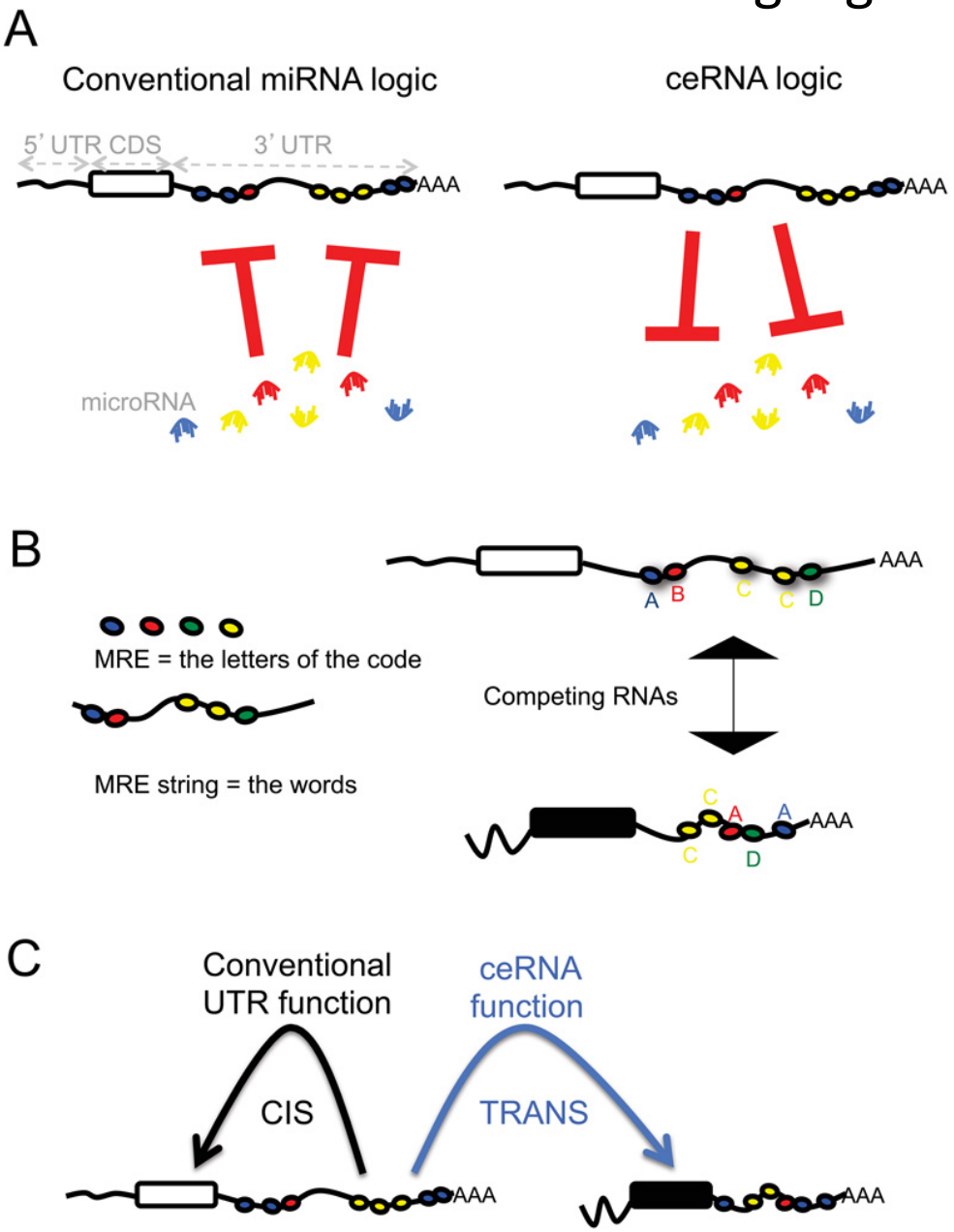


PTENP1 is targeted by PTEN-targeting microRNAs a. Working hypothesis: PTEN is protected from microRNA binding by PTENP1. microRNAs: colored squiggles; 5'and 3'UTRs: open rectangles; open reading frames: filled rectangles. b. PTEN (upper) and PTENP1 (lower) 3'UTRs contain a highly conserved (dark grey) followed by a poorly conserved (light grey) domain. PTEN-targeting microRNA seed matches within in the high homology region are conserved between PTEN and PTENP1. c. Binding of PTEN-targeting microRNAs to PTENP1. Seeds and seed matches: bold; canonical pairings: solid lines; non-canonical pairings (G:U): dotted lines. d. PTEN-targeting miR-19b and miR20a decrease PTEN and PTENP1 mRNA abundance. e. miR-17 and miR-19 family inhibitors derepress PTENP1 abundance (left). PTEN is used as positive control (right)

Cell. 2011 Aug 5;146(3):353-8.

A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language?

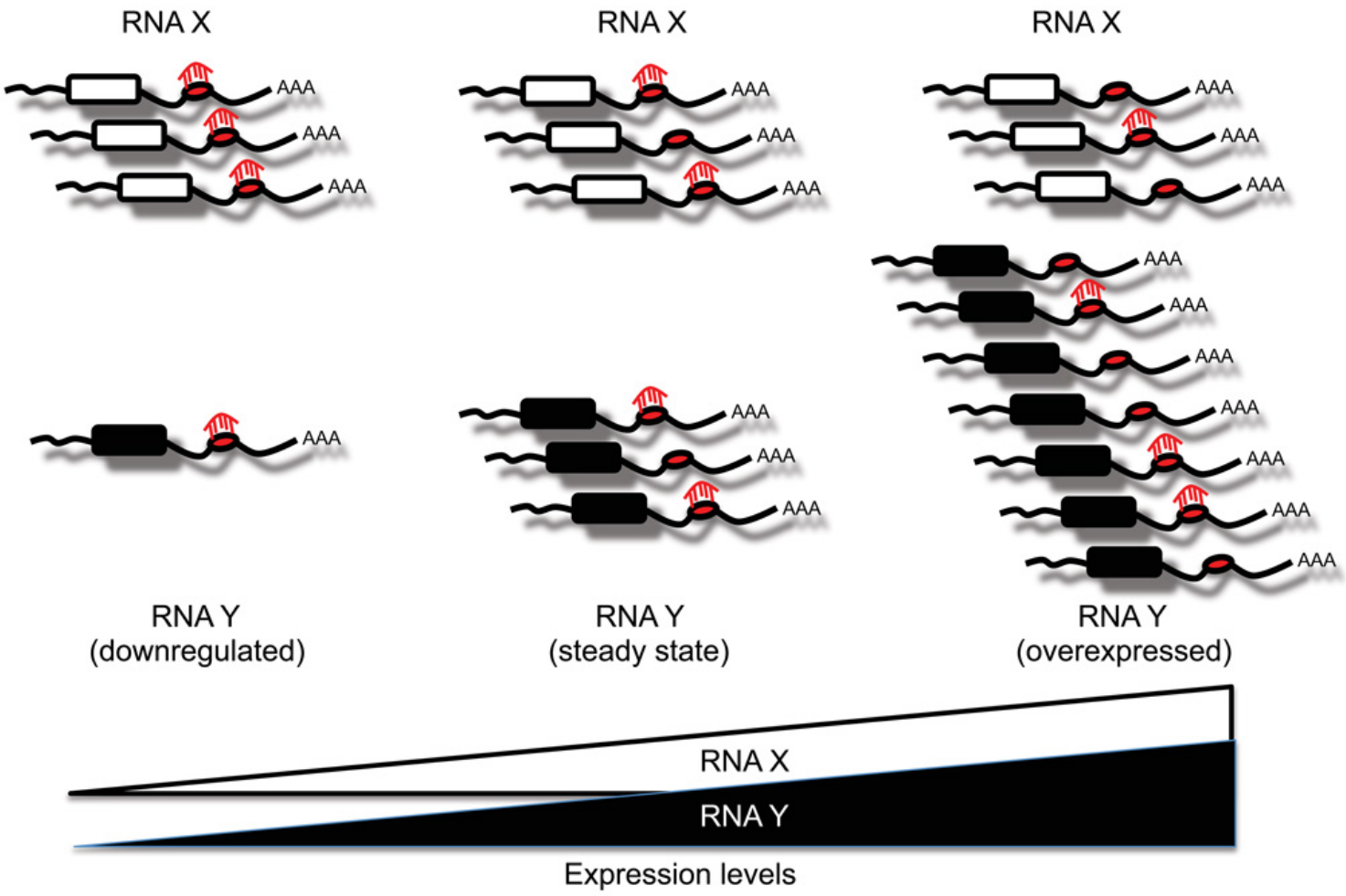
Salmena L, …….Pandolfi PP.

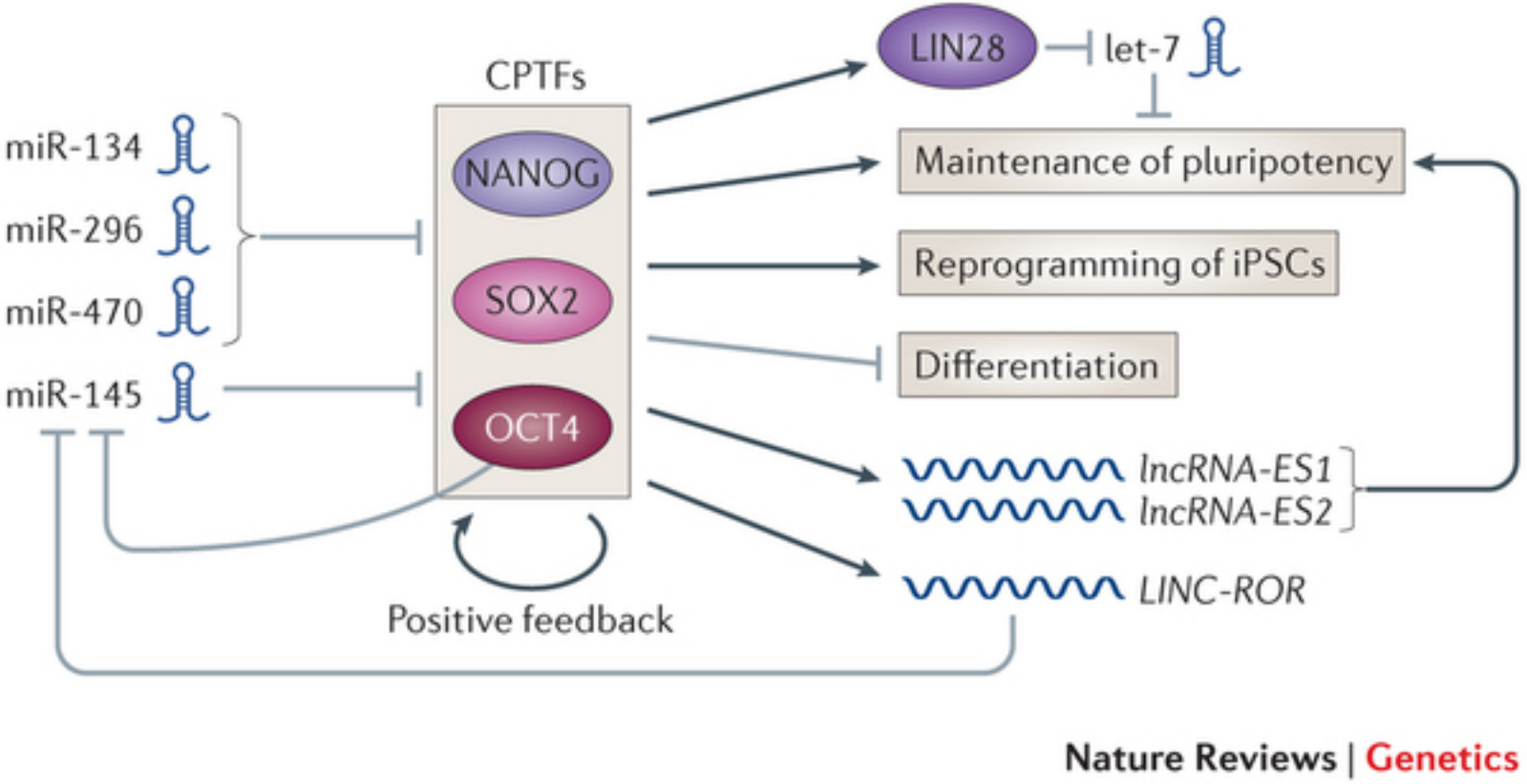


Cell. 2011 Aug 5;146(3):353-8.

A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language?

Salmena L, …….Pandolfi PP.



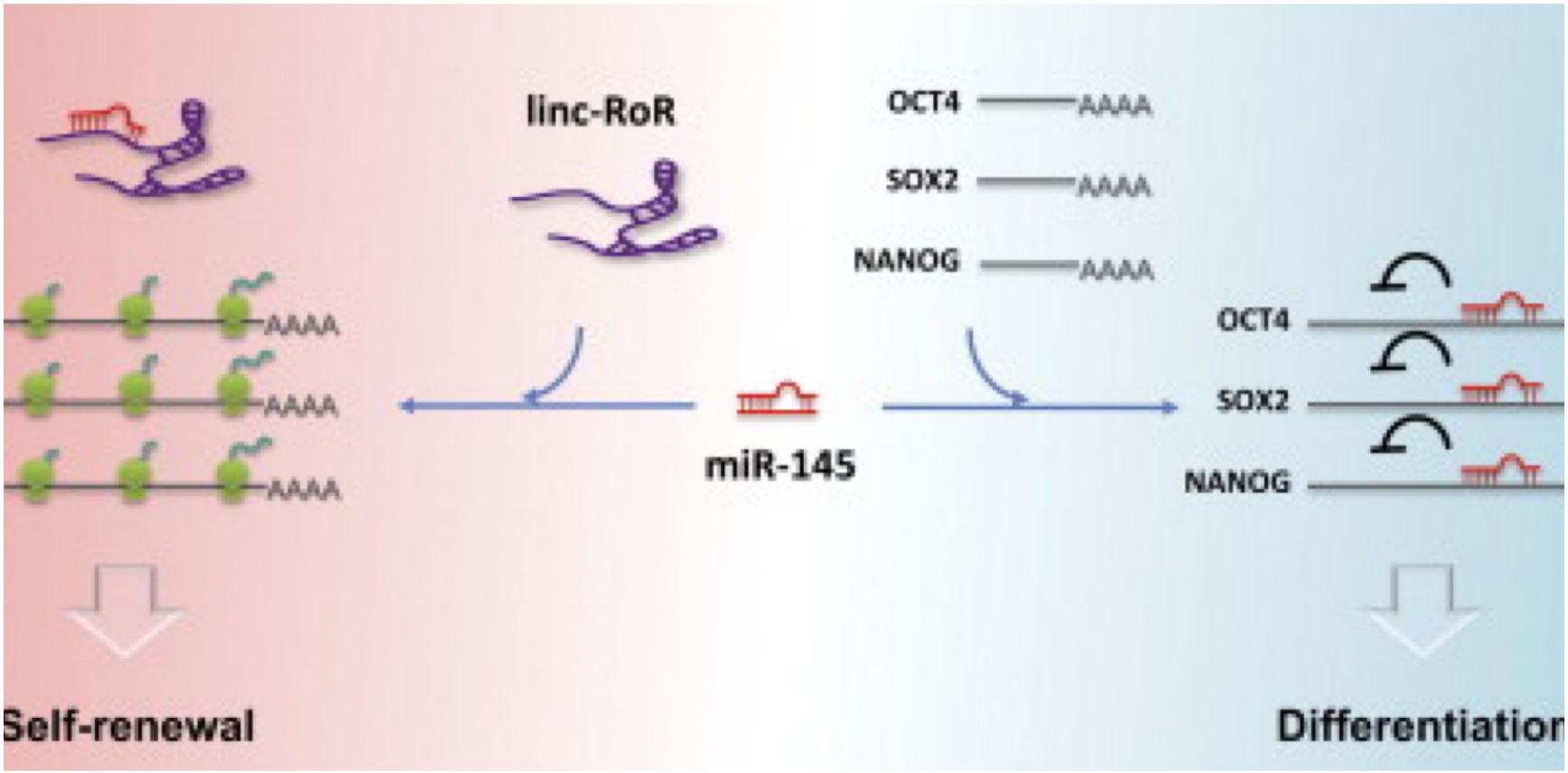


A schematic representation of the regulatory circuitries that involve the homeobox protein NANOG, sex-determining region Y-box 2 (SOX2) and OCT4 core pluripotency transcription factors (CPTFs), microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) in pluripotency control is shown. Several miRNAs were described as being necessary and sufficient to control self-renewal and pluripotency in human embryonic stem cells or to trigger differentiation by a direct link with CPTFs. LINC-ROR (long intergenic non-protein coding RNA, regulator of reprogramming) contributes to this circuitry by maintaining high levels of CPTFs — it competes for miR-145 binding through its miR-145-recognition motif. When present, LINC-ROR prevents miR-145 from repressing the translation of CPTFs and therefore ensures the stem cell fate; when LINC-ROR is downregulated, the synthesis of CPTFs is repressed. Notably, within this circuitry, CPTFs activate their own synthesis through a positive feedback loop, thus reinforcing the regulatory circuit. Additionally, lncRNA-ES1 and lncRNA-ES2 contribute to maintaining pluripotency of embryonic stem cells by repressing SOX2 neural targets. iPSCs, induced pluripotent stem cells.

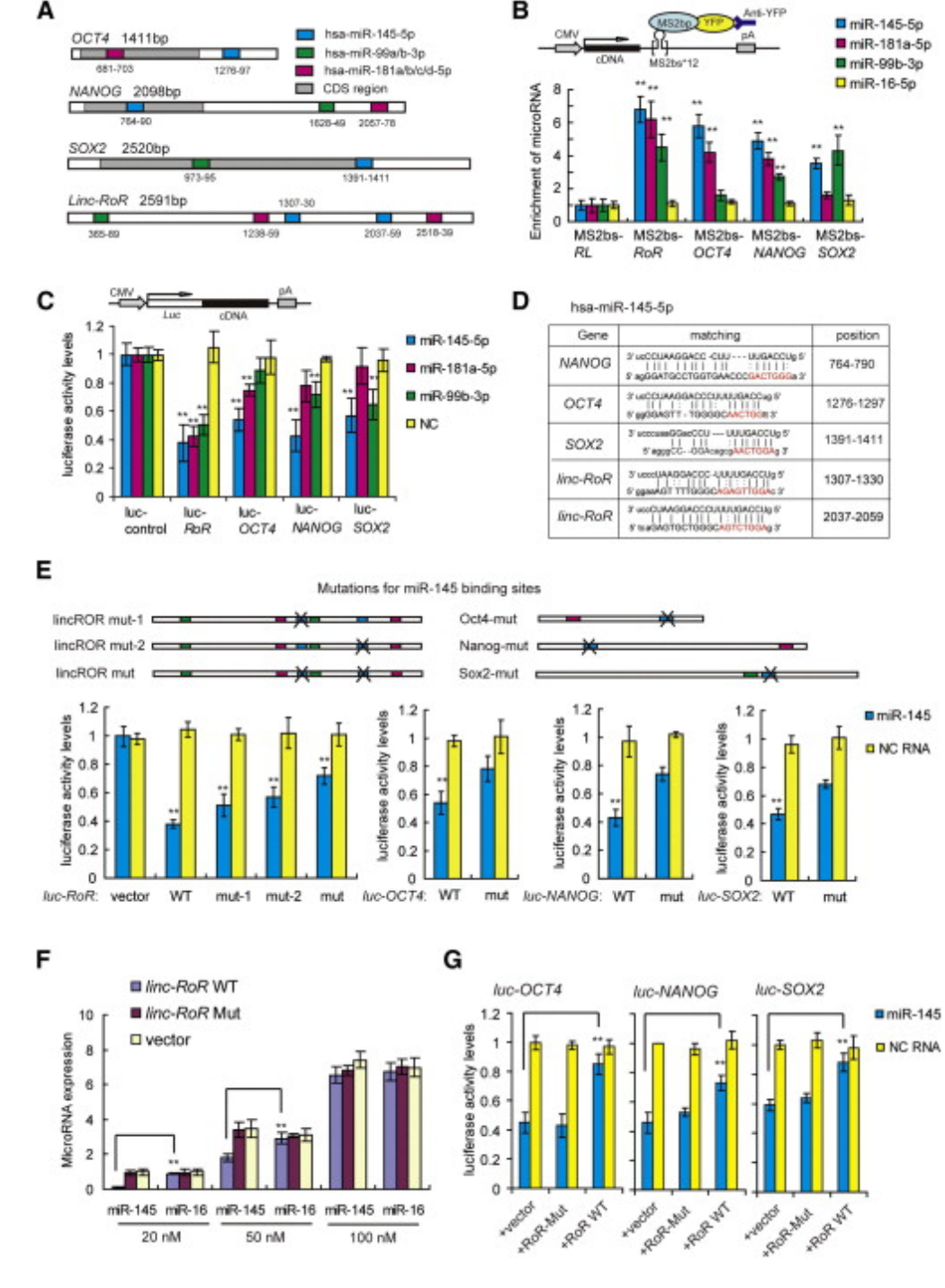
Endogenous miRNA Sponge lincRNA-RoR Regulates Oct4, Nanog, and Sox2 in Human Embryonic Stem Cell Self-Renewal.

Wang Y, Xu Z, Jiang J, Xu C, Kang J, Xiao L, Wu M, Xiong J, Guo X, Liu H.

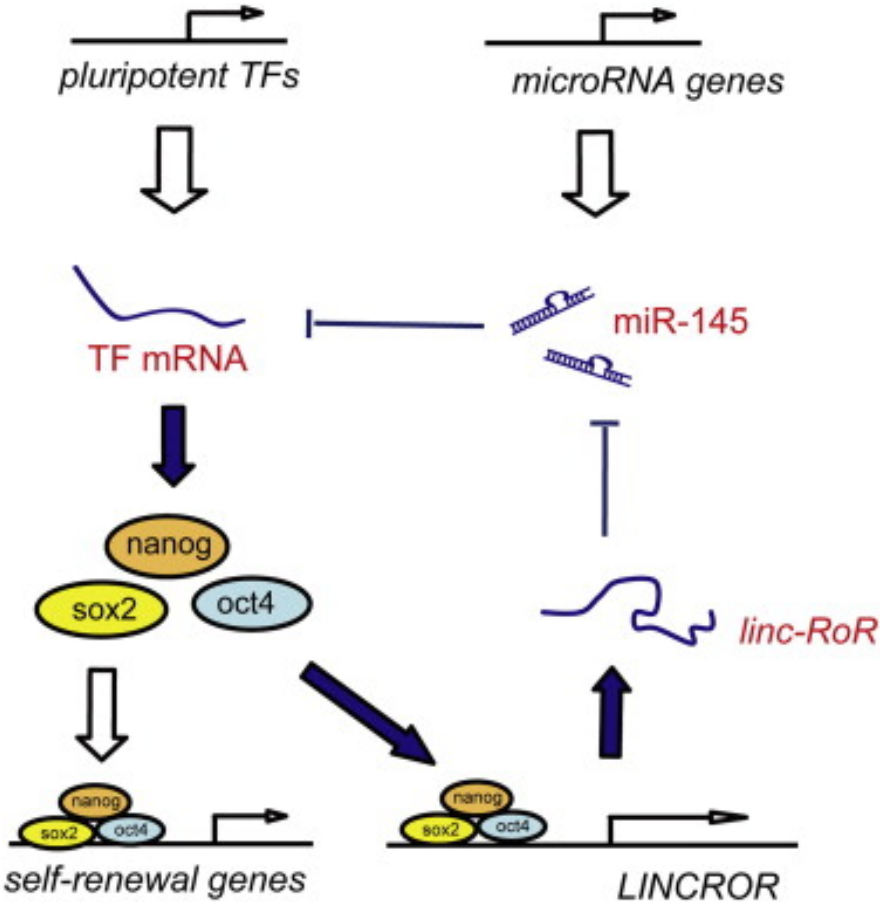
Dev Cell. 2013 25:69-80.

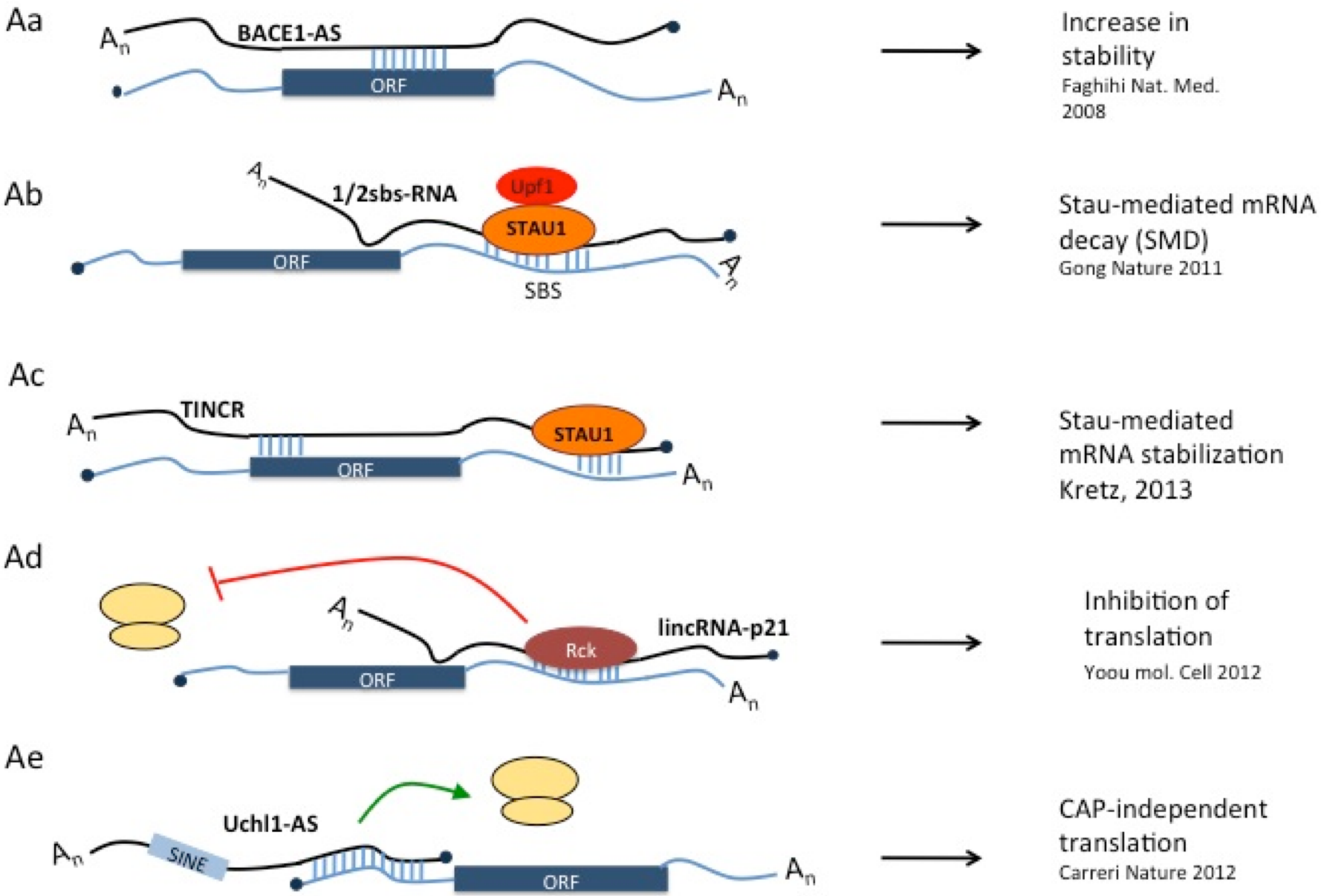


A Competition for miR-145 between linc-RoR and mRNAs Encoding the Core TFs. The presence of linc-RoR in hESCs traps miR-145, preventing it from repressing the translation of the core pluripotency factors and ensuring the stem cell fate. The disappearance of linc-RoR in differentiating hESCs releases miR-145, allowing it to repress the translation of core pluripotency factors.

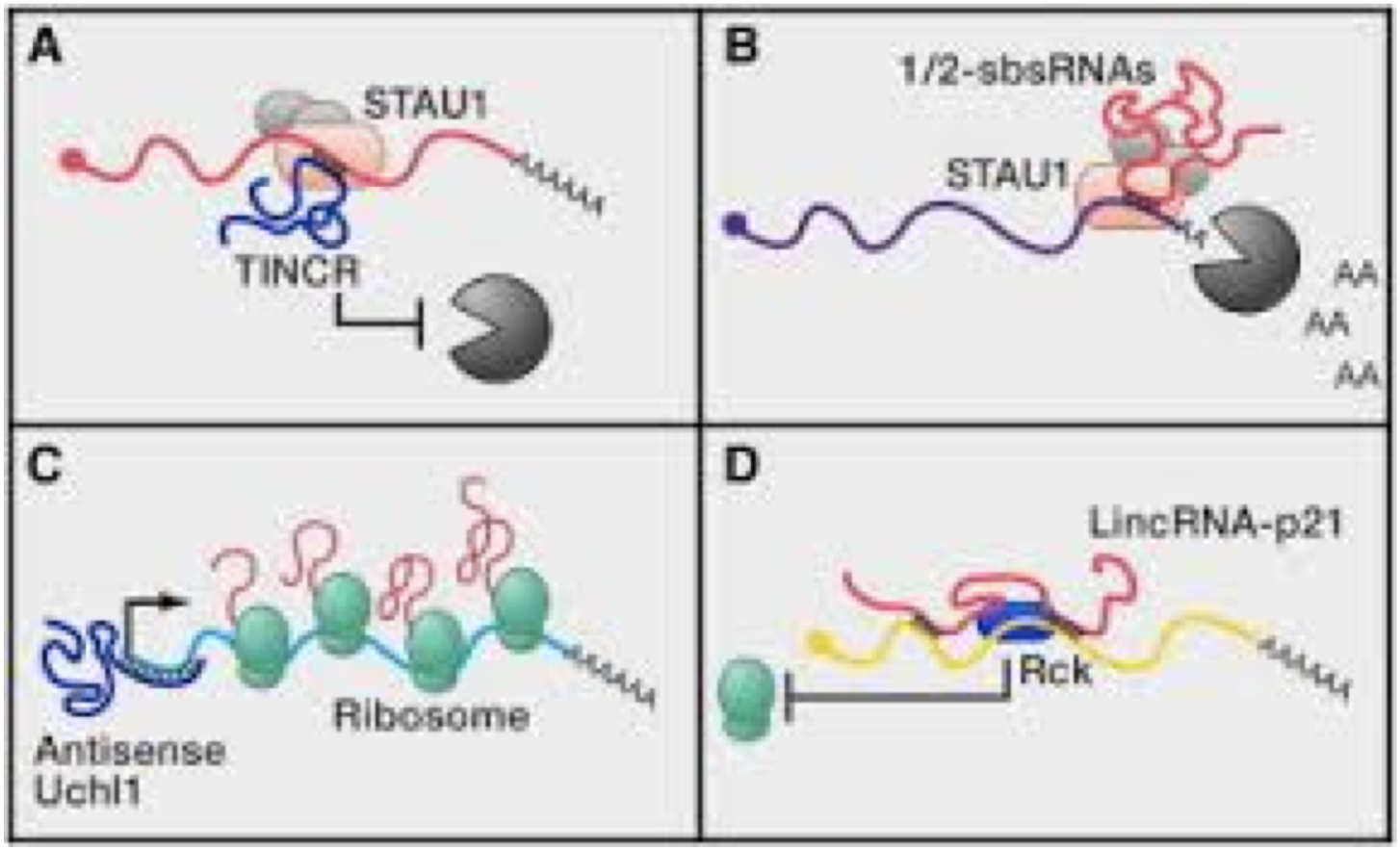

A
OCT4 1411bp
681-703
1278-97
NANOG 2098bp
764-90
1628-49
2057-78
SOX2 2520bp
973-95
1391-1411
Linc-RoR 2591bp
1307-30
365-89
1238-59
2037-59
2518-39
hsa-miR-145-5p
hsa-miR-99a/b-3p
hsa-miR-181a/b/c/d-5p
CDS region
B
CMV
cDNA
MS2bs*12
MS2bp
YFP
Anti-YFP
pA
miR-145-5p
miR-181a-5p
miR-99b-3p
miR-16-5p
Enrichment of microRNA
MS2bs-RL
MS2bs-RoR
MS2bs-OCT4
MS2bs-NANOG
MS2bs-SOX2
C
CMV
Luc
cDNA
pA
miR-145-5p
miR-181a-5p
miR-99b-3p
NC
luciferase activity levels
luc-control
luc-RoR
luc-OCT4
luc-NANOG
luc-SOX2
D
hsa-miR-145-5p
Gene
matching
position
NANOG
764-790
OCT4
1276-1297
SOX2
1391-1411
linc-RoR
1307-1330
linc-RoR
2037-2059
E
Mutations for miR-145 binding sites
lincROR mut-1
lincROR mut-2
lincROR mut
Oct4-mut
Nanog-mut
Sox2-mut
miR-145
NC RNA
luciferase activity levels
luc-RoR: vector WT mut-1 mut-2 mut
luc-OCT4: WT mut
luc-NANOG: WT mut
luc-SOX2: WT mut
F
linc-RoR WT
linc-RoR Mut
vector
MicroRNA expression
miR-145 miR-16 miR-145 miR-16 miR-145 miR-16
20 nM
50 nM
100 nM
G
luc-OCT4
luc-NANOG
luc-SOX2
miR-145
NC RNA
luciferase activity levels
+vector
+RoR-Mut
+RoR WT

(A) The prediction for miRNA-binding elements on linc-RoR, OCT4, NANOG, and SOX2 transcripts by Miranda.
(B) The binding ability of linc-RoR, OCT4, NANOG, and SOX2 full-length transcripts to miRNAs, which were precipitated by cDNA combined with MS2-binding sequences (MS2bs) and its binding protein MS2BP-YFP. The immunoprecipitated miRNAs were assayed by quantitative real-time PCR and normalized to U6; MS2bs-RL and miR-16 were used as negative controls. RL, Renilla luciferase.
(C–E) The target validation using luciferase reporters in HEK293 cells. The relative luciferase activities of luciferase reporters containing wild-type (WT) or mutant (Mut) transcripts were assayed 48 hr after cotransfection with the indicated microRNAs or scramble negative control RNA (NC). Luc, firefly luciferase; pA, polyadenylation signal; Control, the basal luciferase reporter without inserts. (D) Comparison summary of miR-145 target sites in the mRNA of linc-RoR, OCT4, NANOG, and SOX2. The red nucleotides (target sites) were deleted in the mutant constructs.
(F) Linc-RoR facilitated miR-145 degradation. MiRNA levels were assayed by quantitative real-time PCR in HEK293 cells cotransfected with different concentrations of miR-145 mimics and WT or mutant linc-RoR. MiR-16 was used as a negative control.
(G) Coexpression of wild-type linc-RoR rescued the relative luciferase activities of luciferase reporters containing OCT4, NANOG, and SOX2 when cotransfected with miR-145. Blank vector (vector) and mutant linc-RoR were used as controls.
Data are represented as mean ± SEM. $**p < 0.01$, n = 3. See also Figure S3 and Table S1.


pluripotent TFs
microRNA genes
TF mRNA
miR-145
nanog
sox2
oct4
linc-RoR
nanog
sox2
oct4
nanog
sox2
oct4
self-renewal genes
LINCROR



Fatica & Bozzoni, Figure 2A

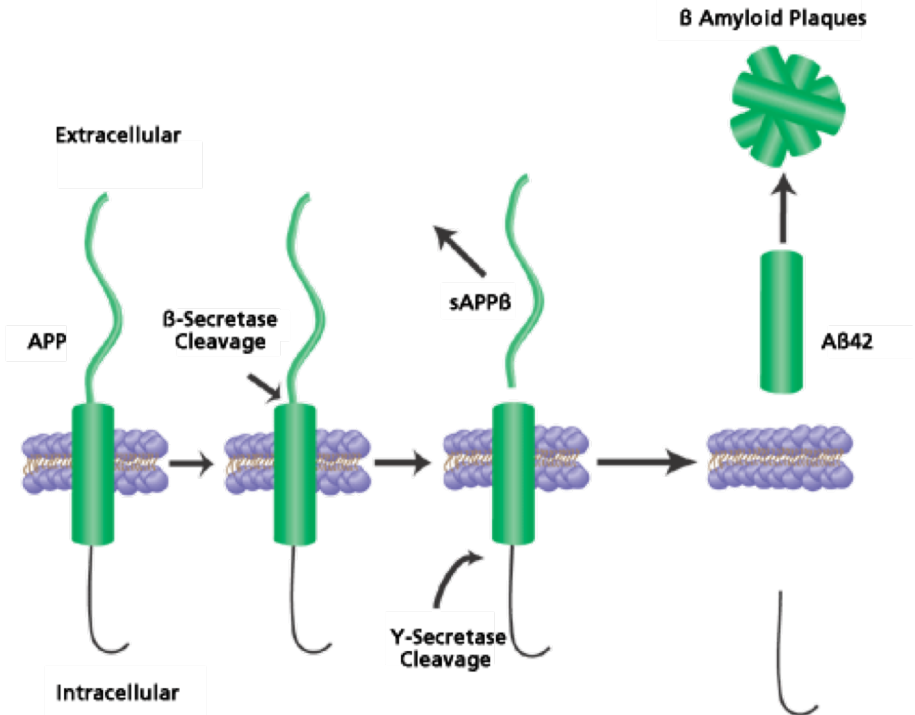

A
STAU1
TINCR
B
1/2-sbsRNAs
STAU1
AA
AA
AA
C
Ribosome
Antisense
Uchl1
D
LincRNA-p21
Rck

Nat Med. 2008 Jul;14(7):723-30. doi: 10.1038/nm1784. Epub 2008 Jun 29.

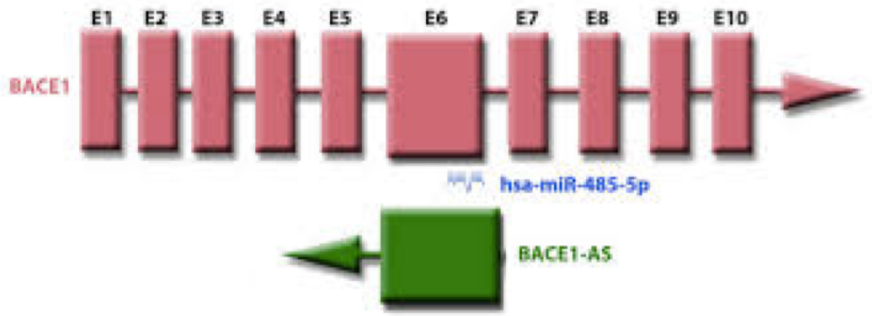
Expression of a **noncoding RNA is elevated in Alzheimer's disease** and drives rapid feed-forward regulation of beta-secretase.

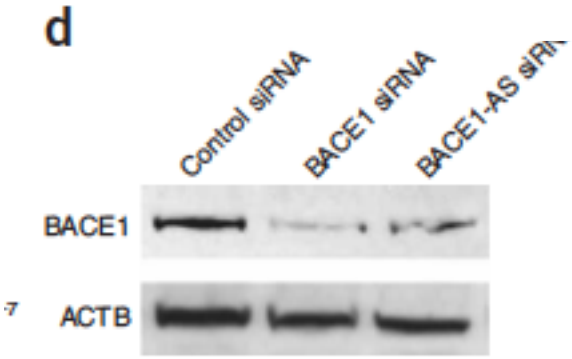
Faghihi et al.

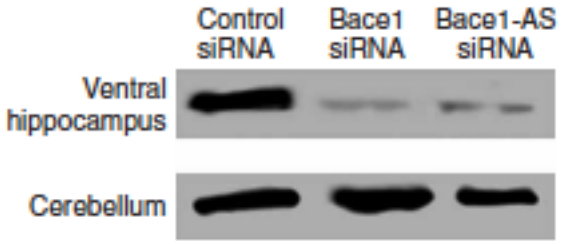
Recent efforts have revealed that numerous protein-coding messenger RNAs have natural antisense transcript partners, most of which seem to be noncoding RNAs. Here we identify a conserved noncoding antisense transcript for beta-secretase-1 (BACE1), a crucial enzyme in Alzheimer's disease pathophysiology. The BACE1-antisense transcript (BACE1-AS) regulates BACE1 mRNA and subsequently BACE1 protein expression in vitro and in vivo**. Upon exposure to various cell stressors including amyloid-beta 1-42 (Abeta 1-42), expression of BACE1-AS becomes elevated, increasing BACE1 mRNA stability and generating additional Abeta** 1-42 through a post-transcriptional feed-forward mechanism. BACE1-AS concentrations were elevated in subjects with Alzheimer's disease and in amyloid precursor protein transgenic mice. These data show that BACE1 mRNA expression is under the control of a regulatory noncoding RNA that may drive Alzheimer's disease-associated pathophysiology. In summary, we report that a long noncoding RNA is directly implicated in the increased abundance of Abeta 1-42 in Alzheimer's disease.



BACE1-AS regulates BACE1 RNA and protein in vitro e in vivo







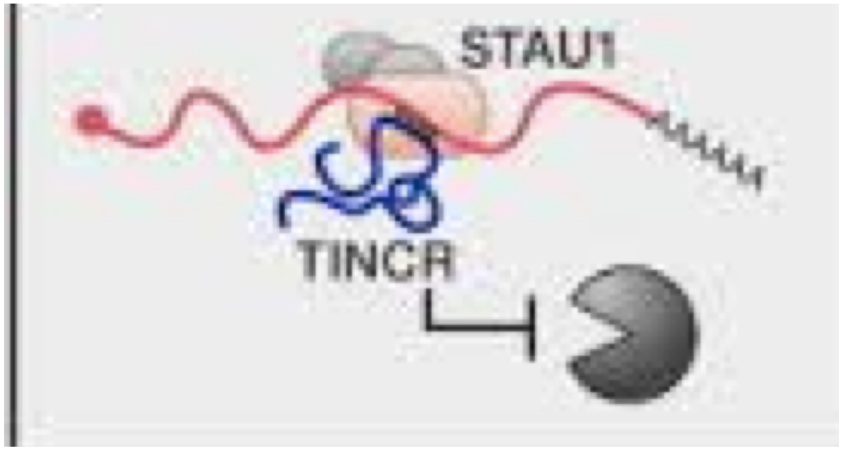
Nature. 2013 Jan 10;493(7431):231-5.

Control of somatic tissue differentiation by the long non-coding RNA TINCR.

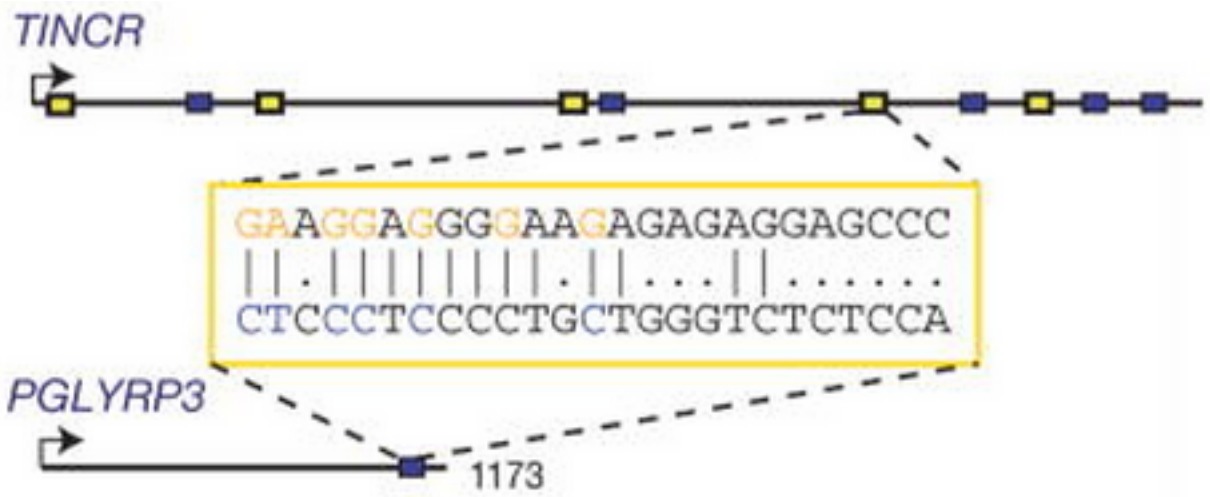
Kretz et al.

Abstract

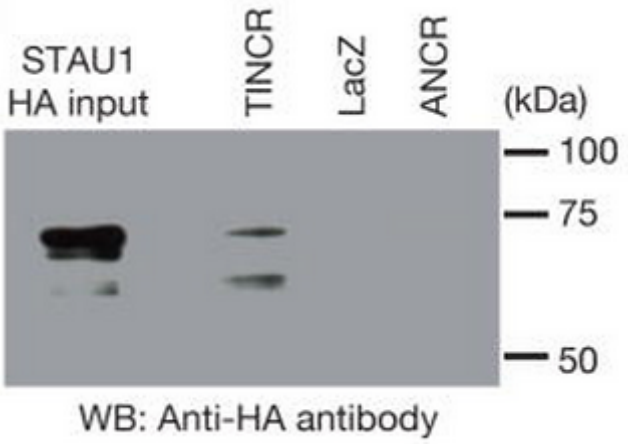
Several of the thousands of human long non-coding RNAs (lncRNAs) have been functionally characterized; however, potential roles for lncRNAs in somatic tissue differentiation remain poorly understood. Here we show that a **3.7-kilobase lncRNA**, terminal differentiation-induced ncRNA (TINCR), controls human **epidermal differentiation** by a post-transcriptional mechanism. TINCR is required for high messenger RNA abundance of key differentiation genes, many of which are mutated in human skin diseases, including FLG, LOR, ALOXE3, ALOX12B, ABCA12, CASP14 and ELOVL3. TINCR-deficient epidermis lacked terminal differentiation ultrastructure, including keratohyalin granules and intact lamellar bodies. Genome-scale RNA interactome analysis revealed that **TINCR interacts with a range of differentiation mRNAs**. TINCR-mRNA interaction occurs through a **25-nucleotide 'TINCR box**' motif that is strongly enriched in interacting mRNAs and required for TINCR binding. A high-throughput screen to analyse TINCR binding capacity to approximately 9,400 human recombinant proteins revealed direct binding of **TINCR RNA to the staufen1 (STAU1)** protein. STAU1-deficient tissue recapitulated the impaired differentiation seen with TINCR depletion. Loss of UPF1 and UPF2, both of which are required for STAU1-mediated RNA decay, however, did not have differentiation effects. Instead, the TINCR-STAU1 complex seems to mediate stabilization of differentiation mRNAs, such as KRT80. These data identify TINCR as a key lncRNA required for somatic tissue differentiation, which occurs through lncRNA binding to differentiation mRNAs to ensure their expression.



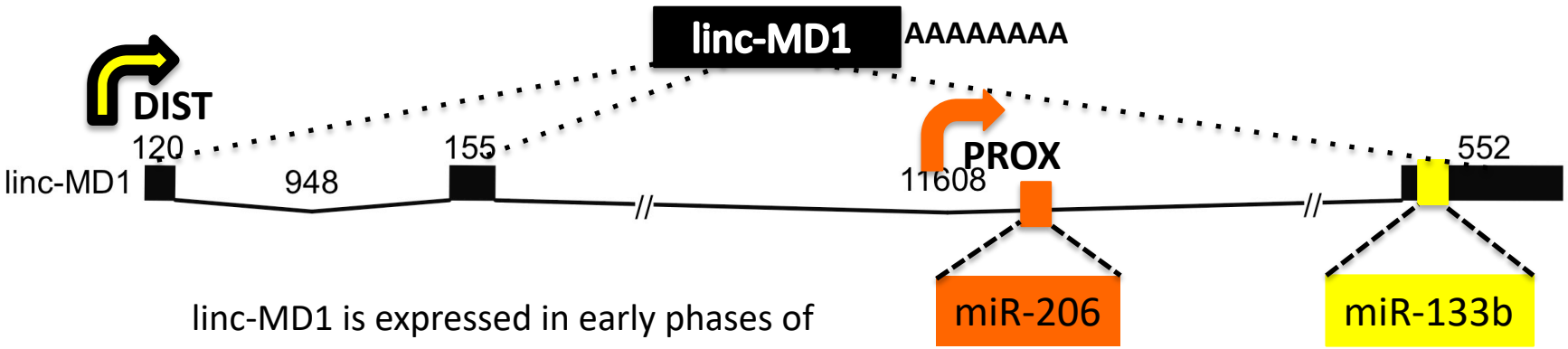
Given its cytoplasmic localization, TINCR control of epidermal barrier genes may occur at the post-transcriptional level through direct association with target mRNAs. To test this, we developed RNA interactome analysis, followed by deep sequencing (RIA-Seq)**. Thirty-eight biotinylated DNA probes were designed in even- and odd-numbered pools. These two pools were used separately in a multiplex fashion for pull-down of endogenous TINCR and associated RNAs** in differentiated keratinocytes



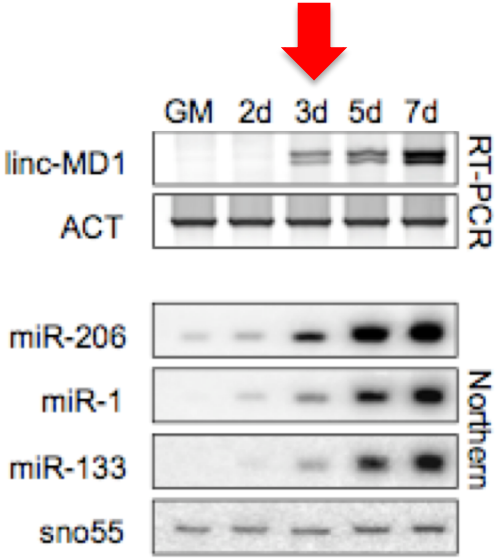
25-nucleotide motif that was strongly enriched in TINCR-interacting mRNAs



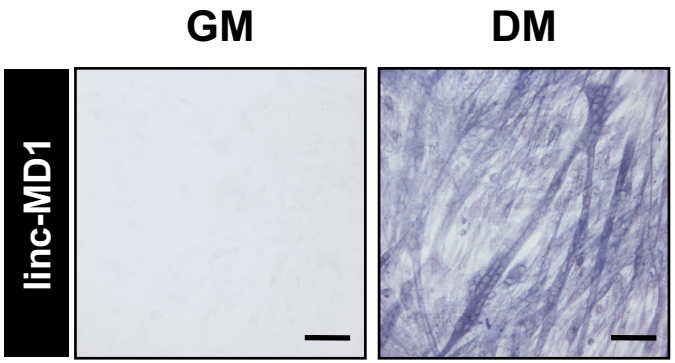
linc-MD1 is a cytoplasmic polyA+ long non-coding RNA



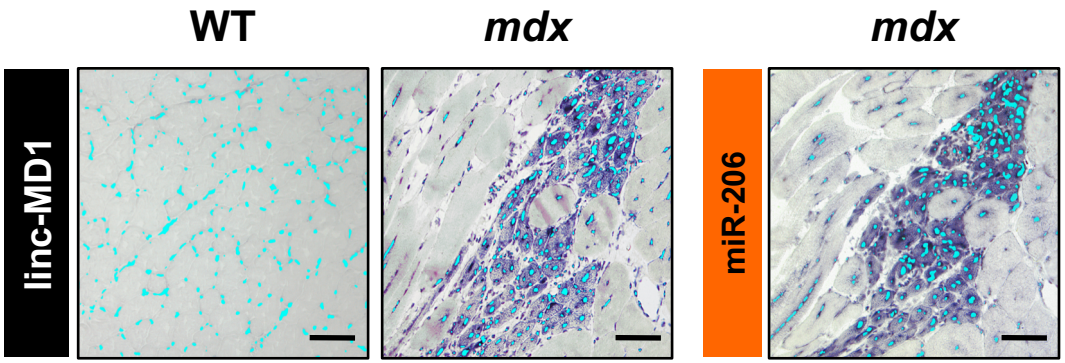
linc-MD1 is expressed in early phases of differentiation from the DIST promoter



linc-MD1 is expressed in differentiating myoblasts and not in mature fibers

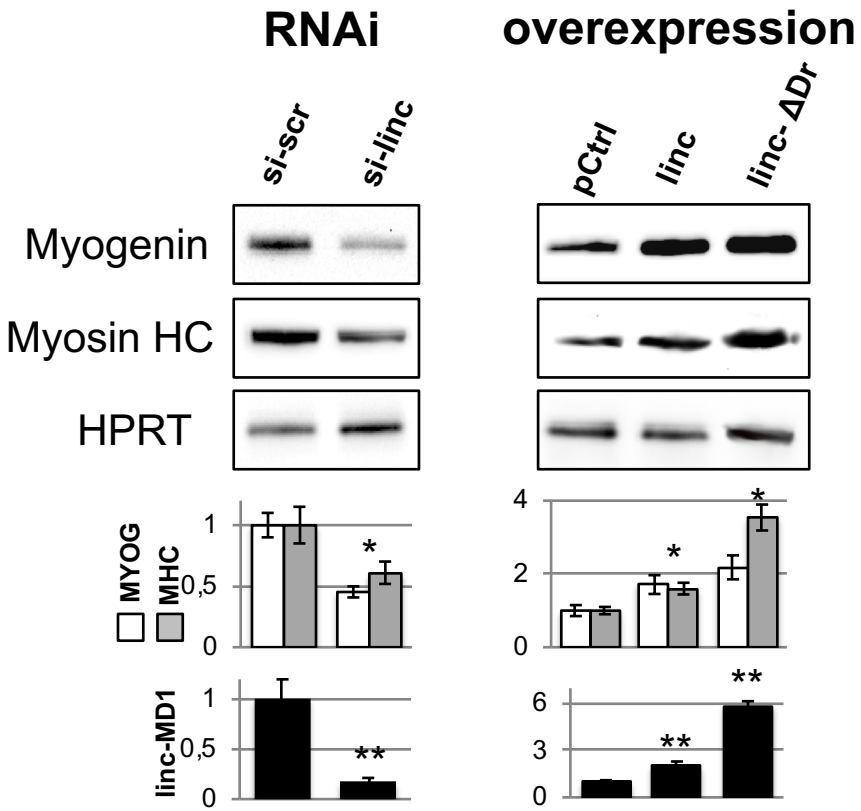


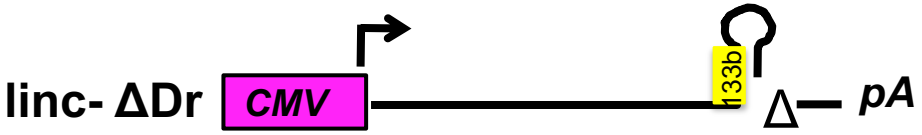
C2 myoblasts



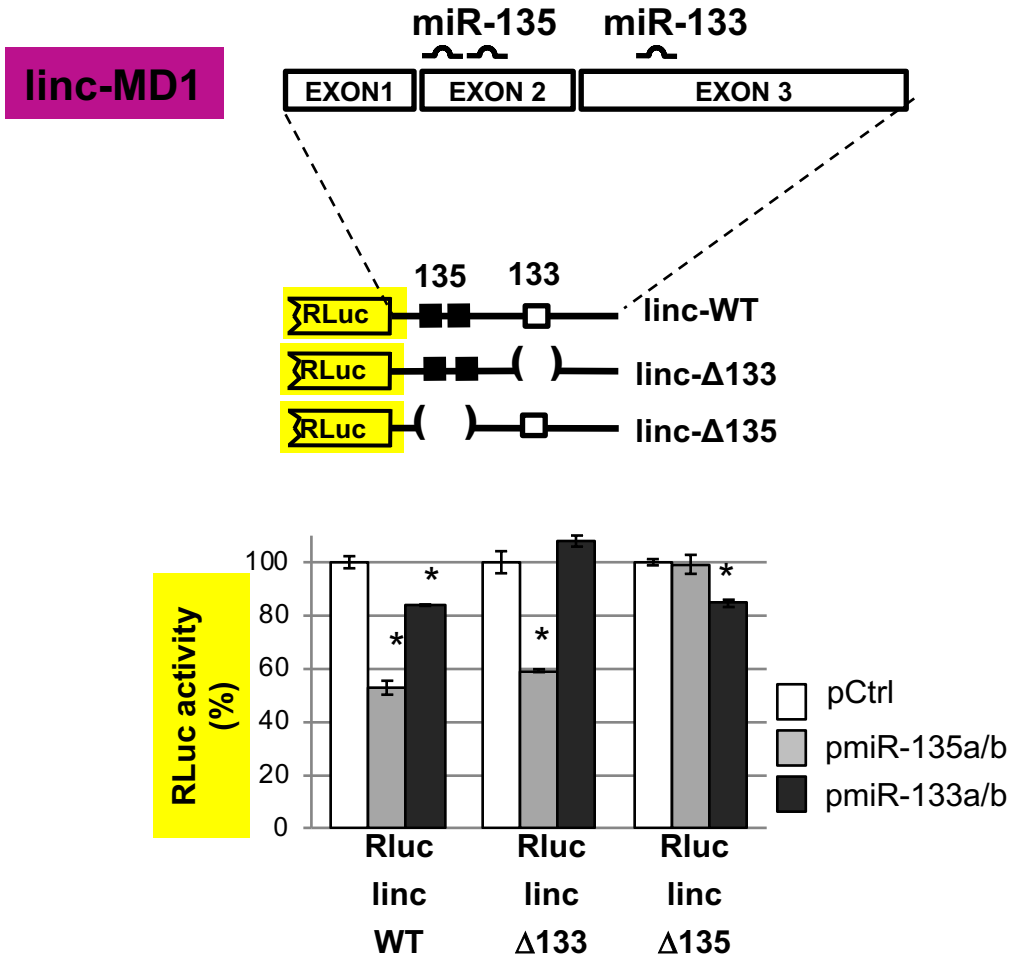
gastrocnemius

linc-MD1 levels affect expression of early myogenic markers

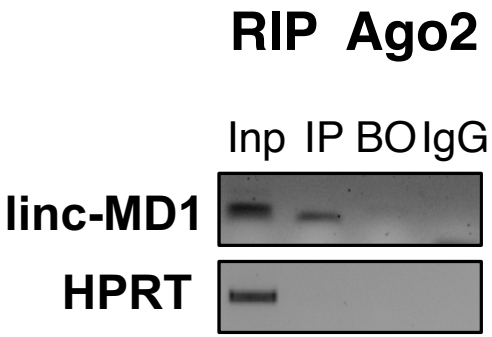


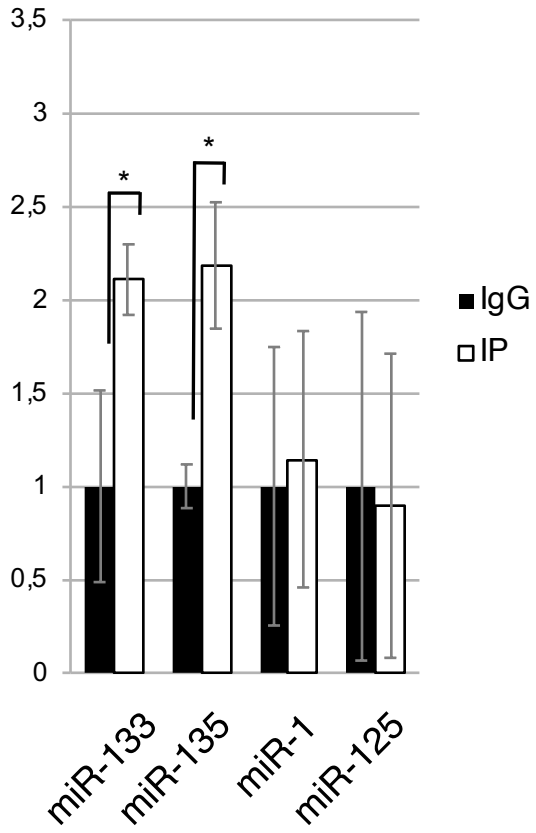


linc-MD1 is target of miR-135 and miR-133

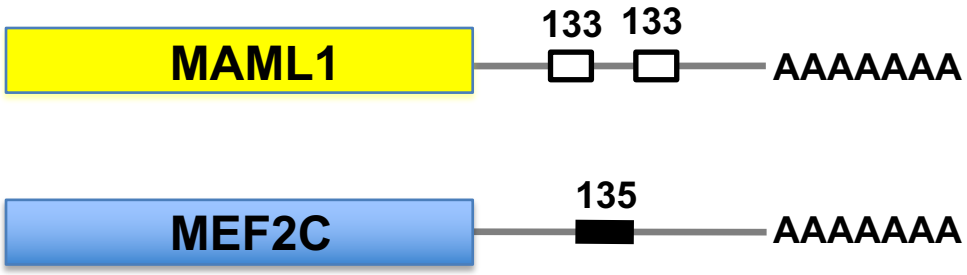


linc-MD1 binds Ago2 and miR-135 and miR-133





the 3’ UTRs of the myogenic factors MAML1 and MEF2C contain miR-135 and miR-133 binding sites



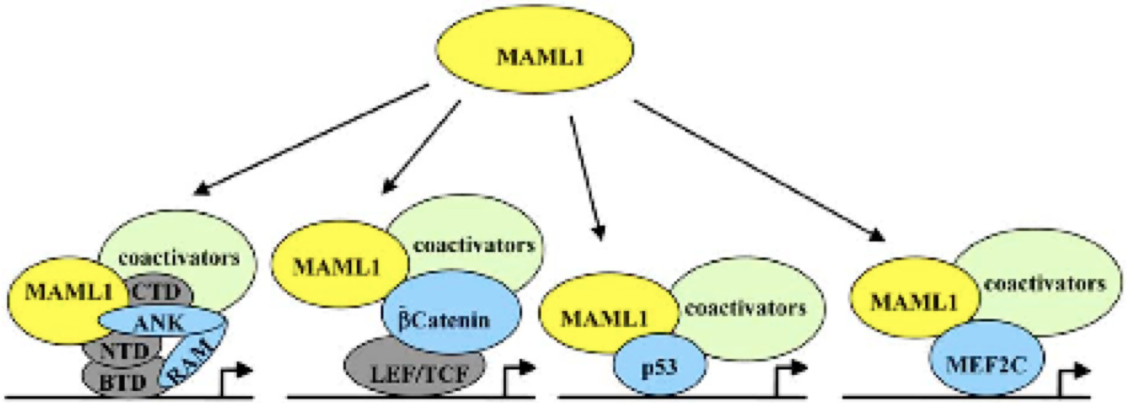
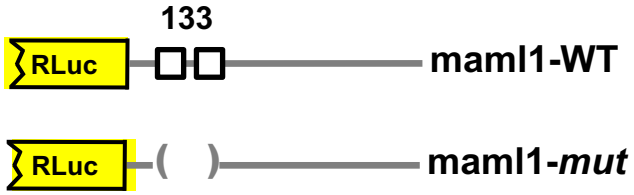
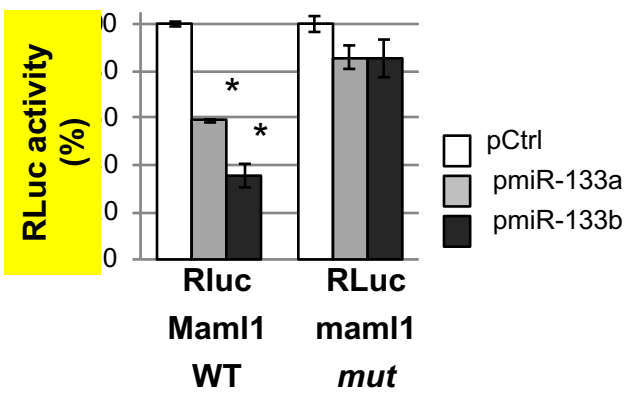
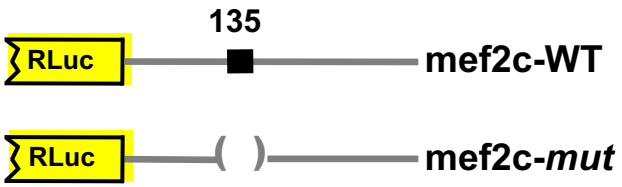


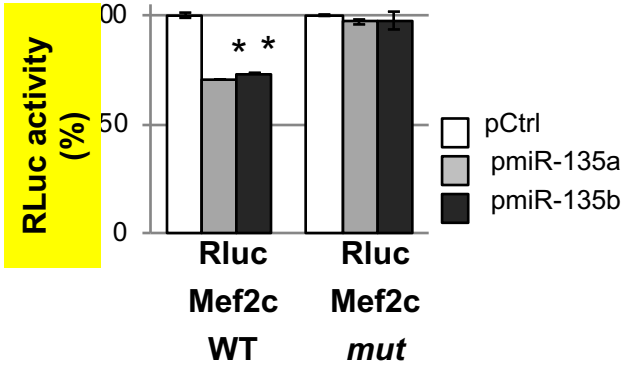
Fig. (1). The MAML1 protein acts as a coactivator for diverse transcription factors. MAML1 is recruited by the ANK (ankyrin repeats) domain of Notch and forms a complex with the DNA-binding protein CSL (composed of NTD (N-terminal domain), CTD (C-terminal domain), and BTD (β-trefoil domain)). The RAM (RBP-Jk associated molecule) domain of Notch interacts with the BTD of CSL. In addition, MAML1 is recruited by β-catenin, p53 and MEF2C to regulate various signalling pathways. Most likely, additional coactivators are working cooperatively with MAML1 in gene regulation.

the myogenic factors MAML1 and MEF2C are target of miR-133 and miR-135

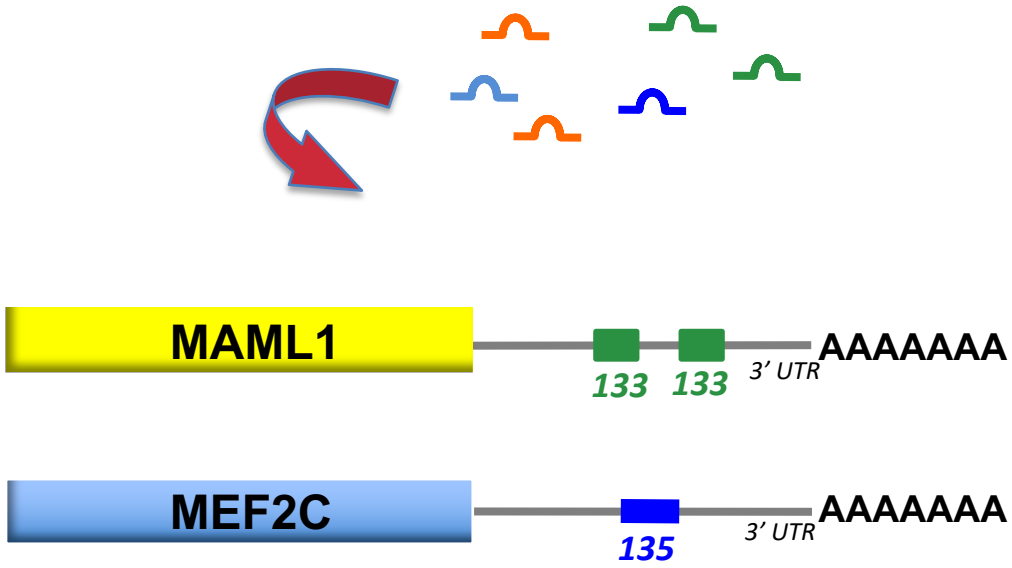






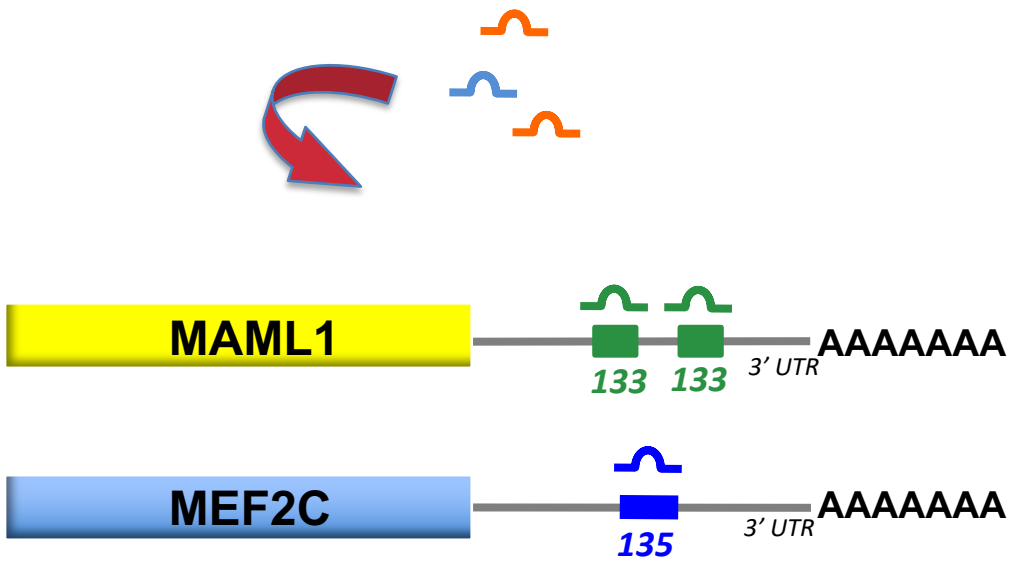


Competition for miRNA binding



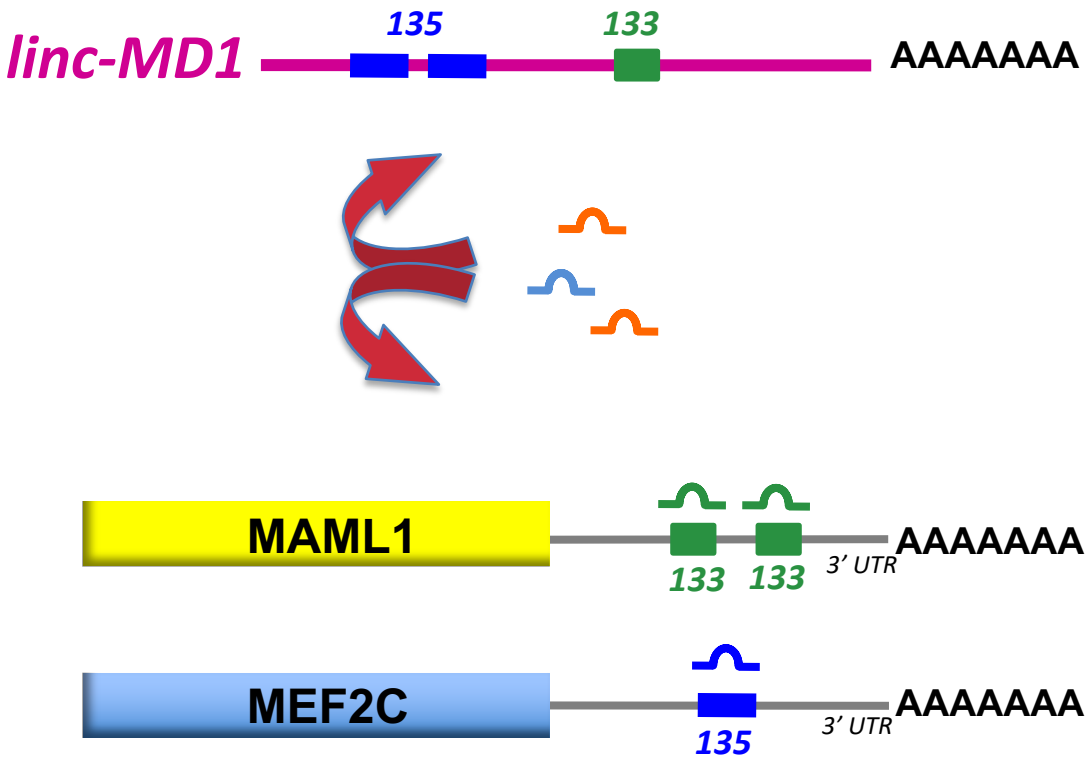
MAML1 and MEF2C are myogenic factors controlling muscle differentiation

Competition for miRNA binding



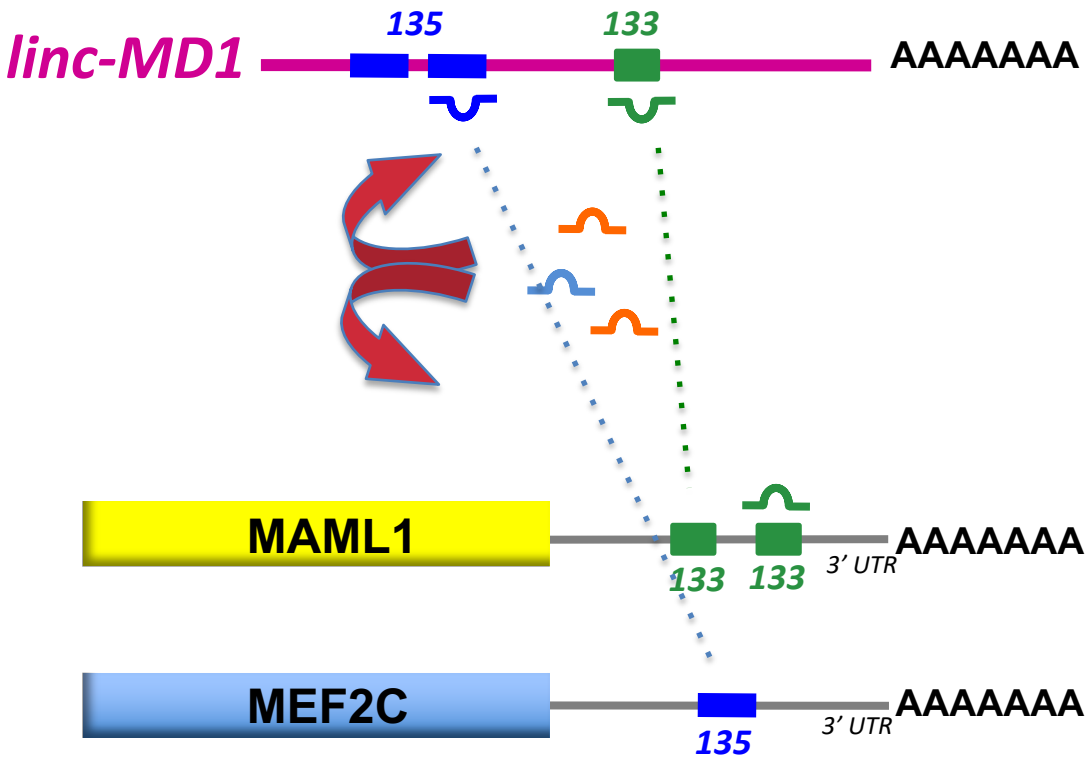
MAML1 and MEF2C are myogenic factors controlling muscle differentiation

Competition for miRNA binding



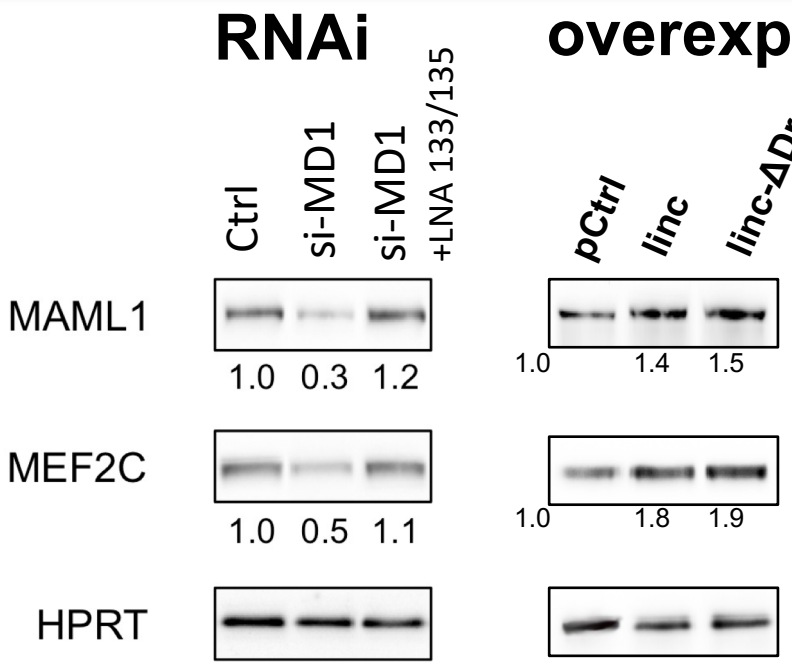
MAML1 and MEF2C are myogenic factors controlling muscle differentiation

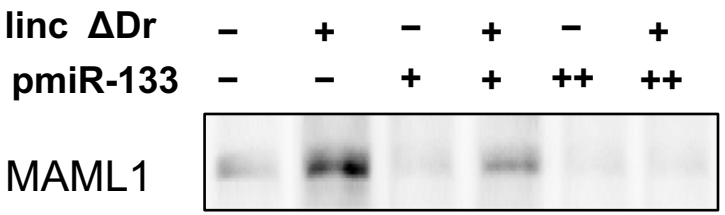
Competition for miRNA binding

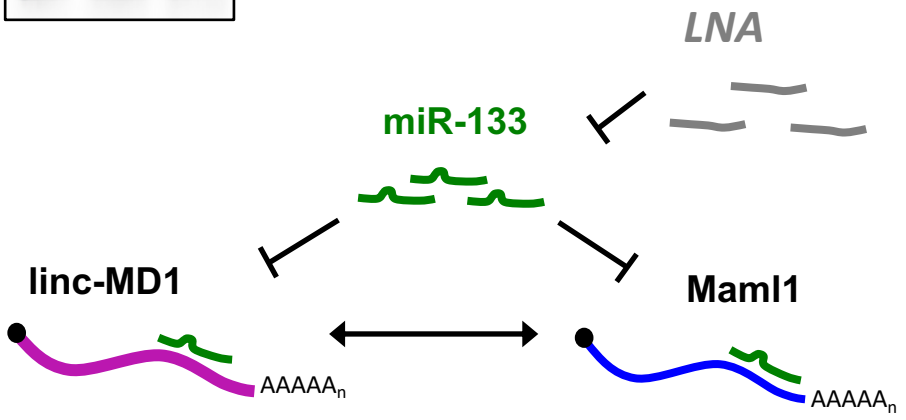


MAML1 and MEF2C are myogenic factors controlling muscle differentiation

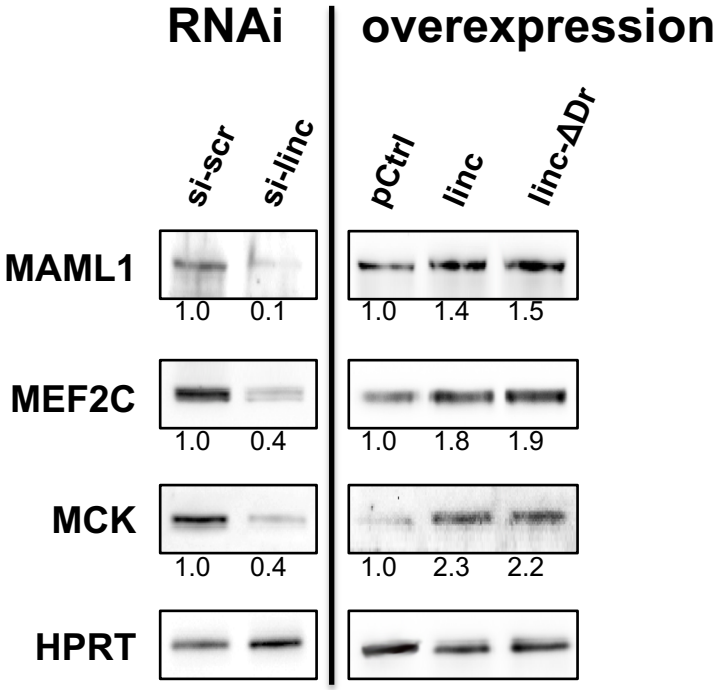
linc-MD1 controls the levels of MAML1 and MEF2C

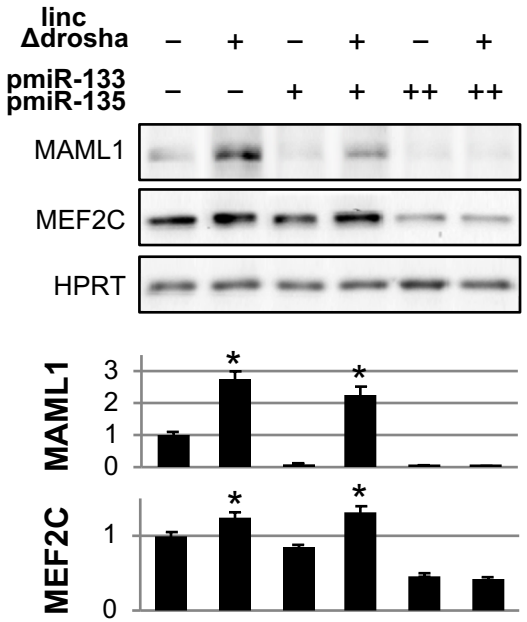




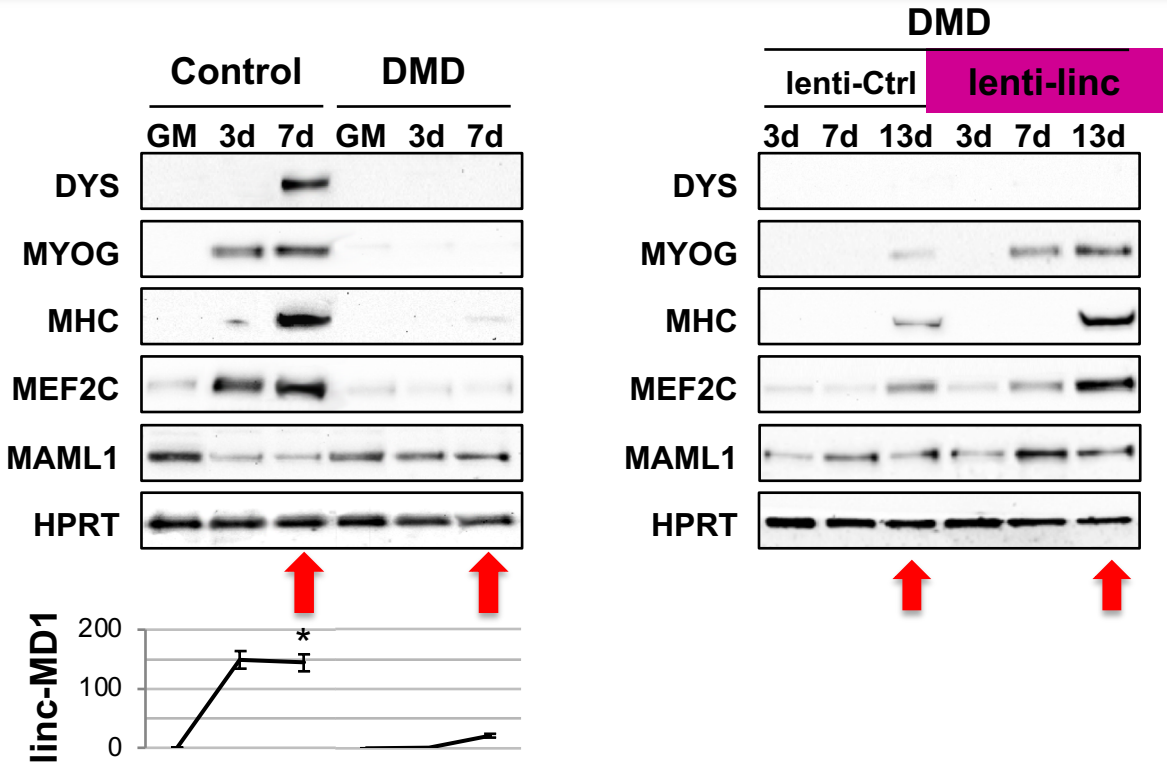


linc-MD1 controls the levels of MAML1 and MEF2C



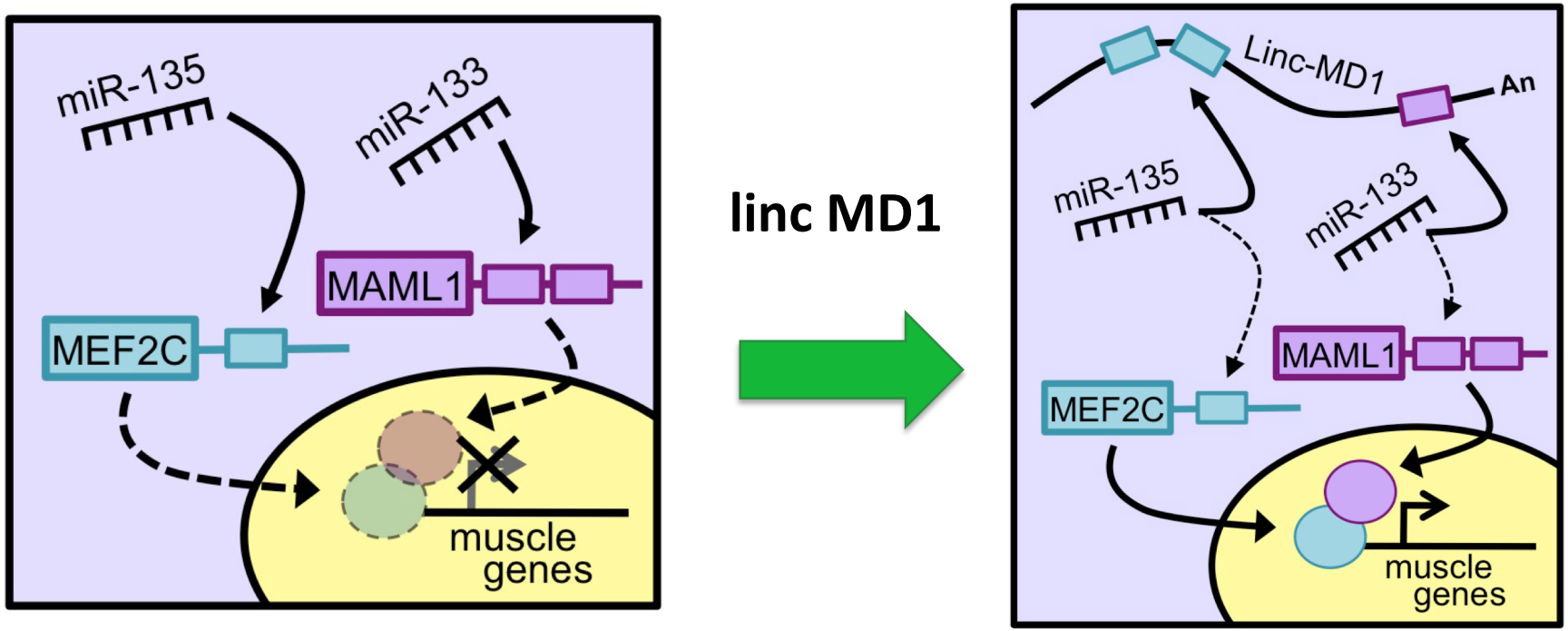


- linc-MD1 controls differentiation in human myoblasts
- it is down-regulated in Duchenne myoblasts



- low levels of linc-MD1 account for the delay in differentiation of DMD myoblasts
- rescue of linc-MD1 restores an almost wt differentiation timing

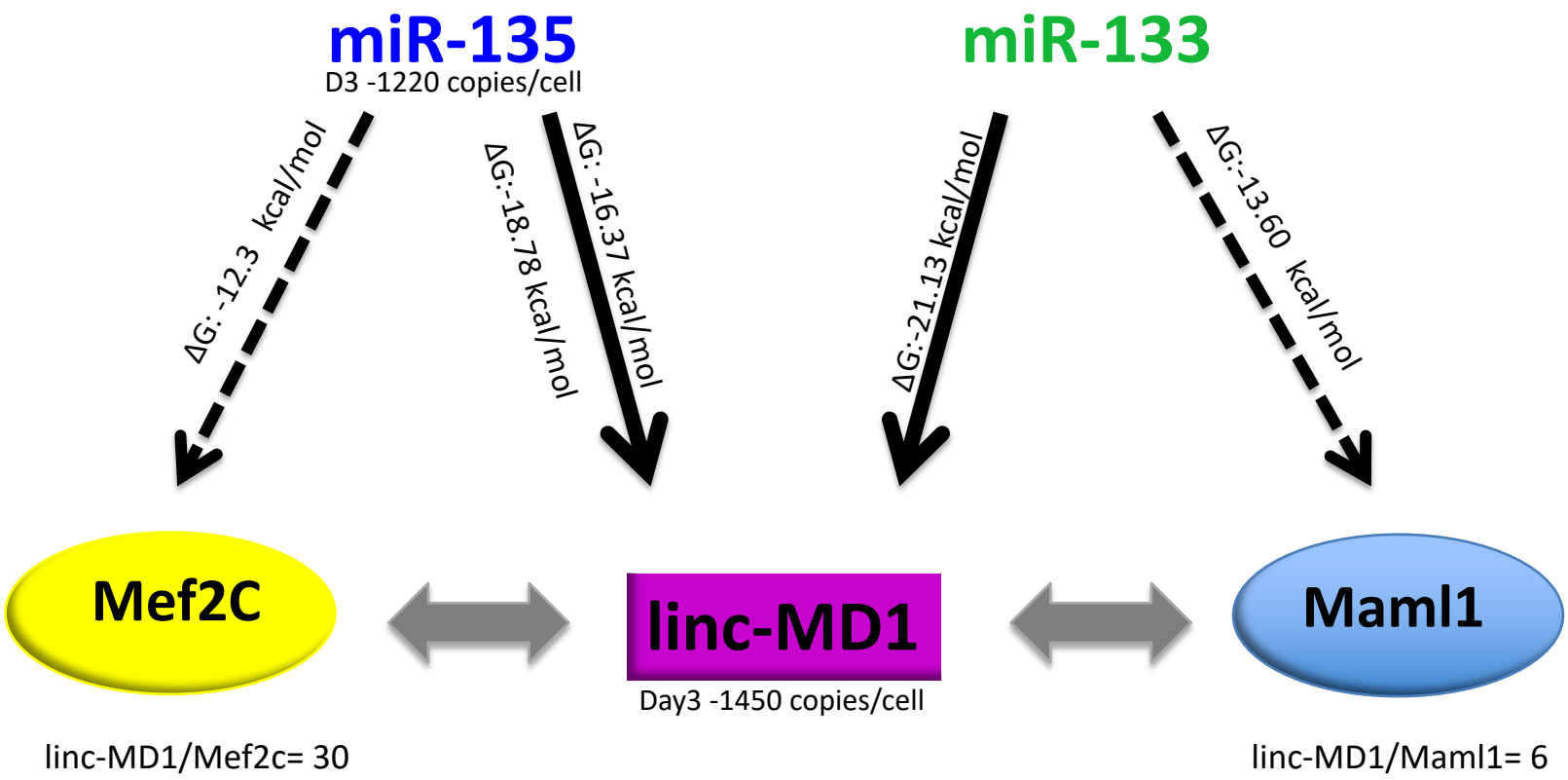
linc-MD1 acts as a sponge for specific miRNAs



differentiation

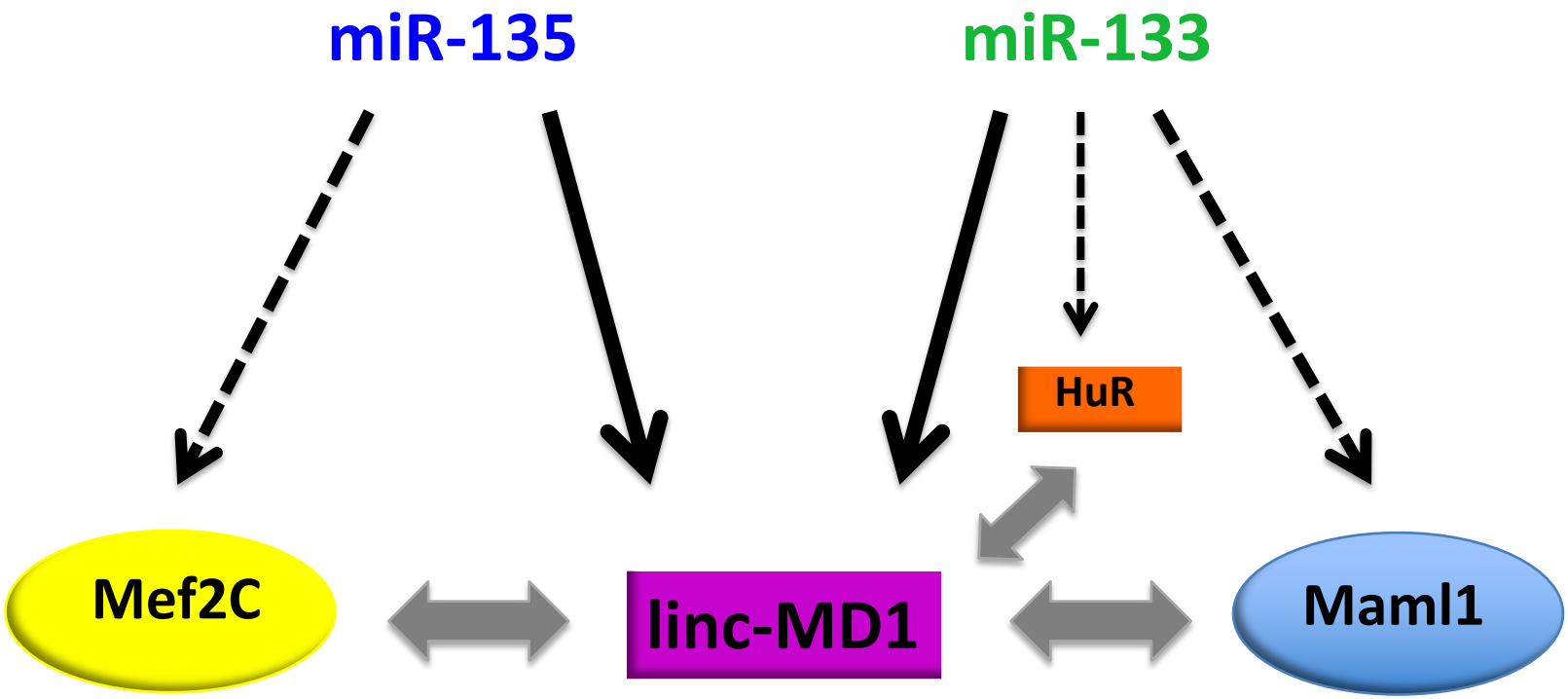
Cesana et al., *Cell* 147, *358-369*, 2011

Crosstalk between coding and non coding RNAs

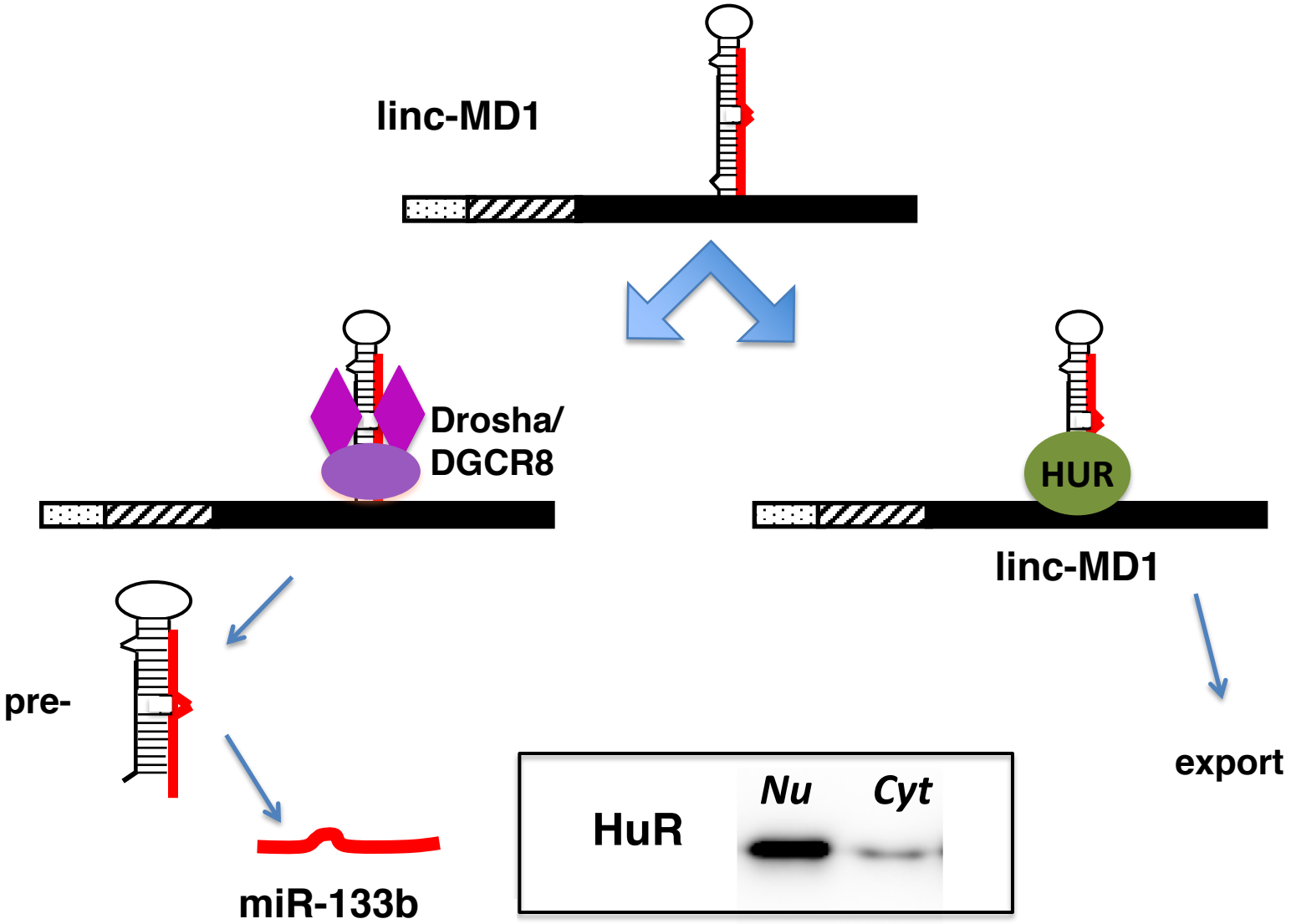


ΔG values were obtained from miRanda (Enright et al., 2003)

Expanding the circuitry……

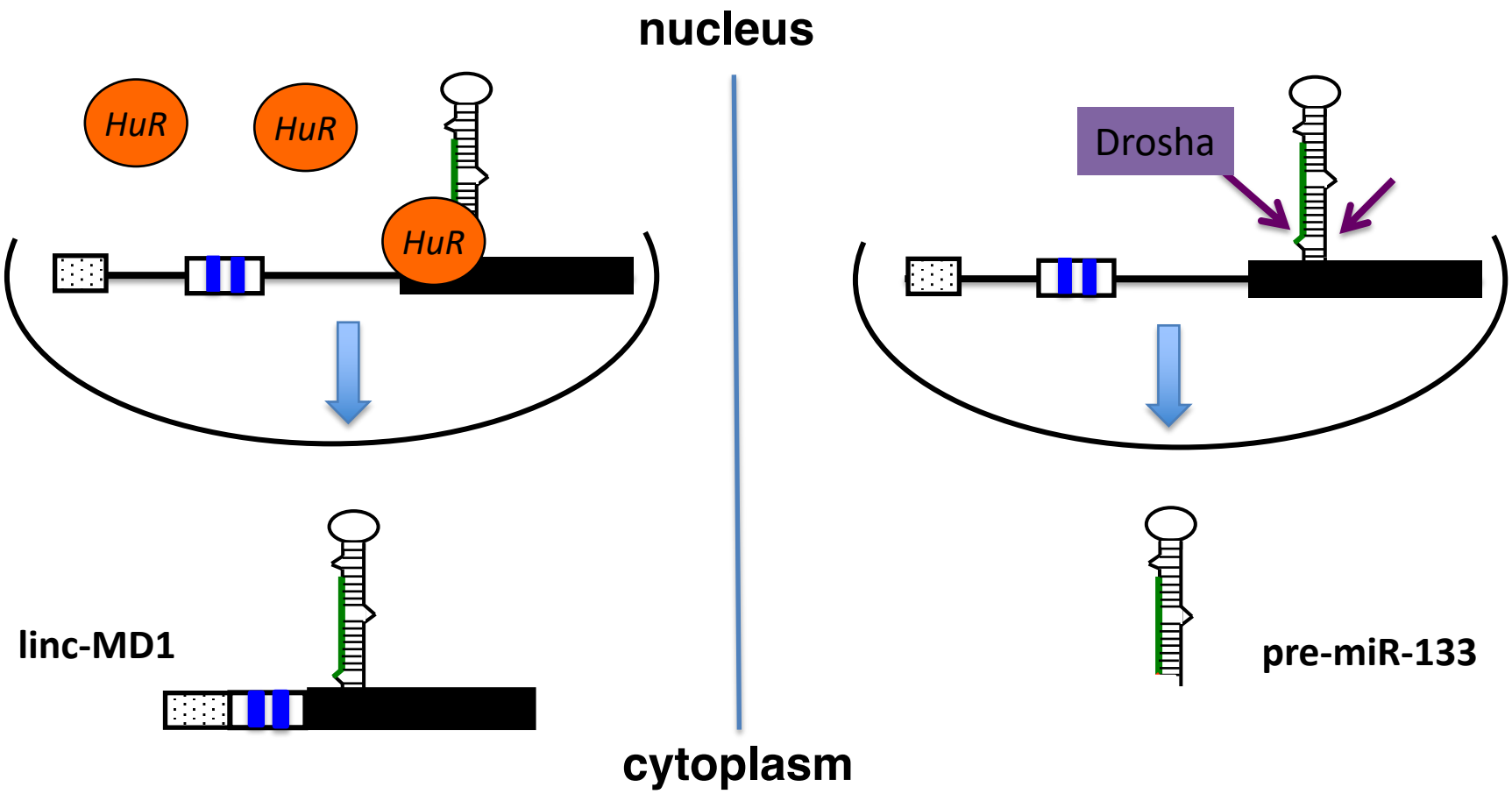


HuR affects the alternative fate of linc-MD1

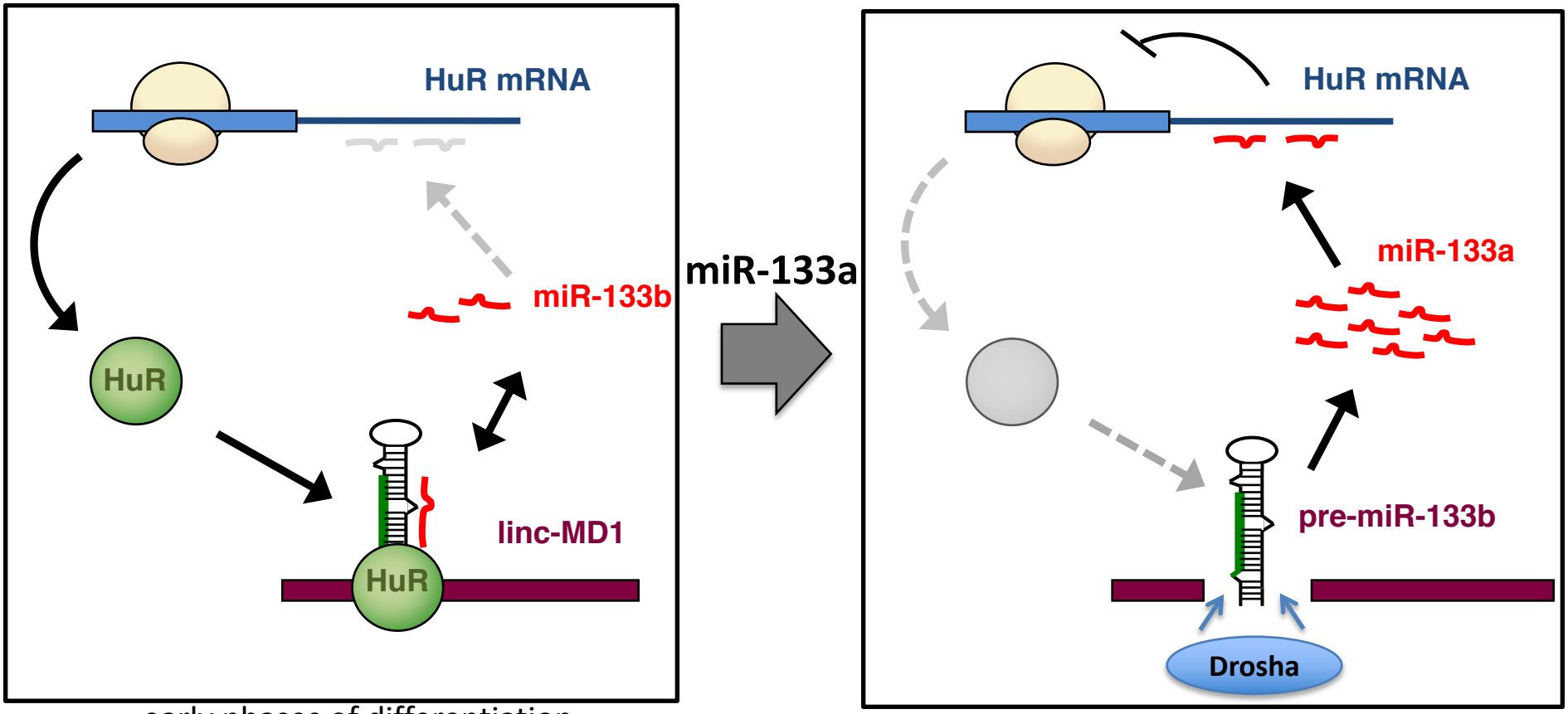


Legnini et al., Mol *Cell* - 2014

Nuclear HuR controls the relative ratio of linc-MD1 versus miR-133b



A feed forward positive loop between HuR and linc-MD1



early phases of differentiation

differentiation

Legnini et al., Mol *Cell* - 2014