Examples of lncRNAs

REGULATION OF TRANSLATION BY ANTISENSE
lncRNA Uchl

-- SINEUP--

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]

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Most of the mammalian genome is transcribed[1–3]. This generates a vast repertoire of transcripts that includes protein-coding messenger RNAs, long non-coding RNAs (lncRNAs) and repetitive sequences, such as SINEs (short interspersed nuclear elements). A large percentage of ncRNAs are nuclear-enriched with unknown function[4]. Antisense lncRNAs may form sense–antisense pairs by pairing with a protein-coding gene on the opposite strand to regulate epigenetic silencing, transcription and mRNA stability[5–10]. Here we identify a nuclear-enriched lncRNA antisense to mouse ubiquitin carboxy-terminal hydrolase L1 (*Uchl1*), a gene involved in brain function and neurodegenerative diseases[11]. Antisense *Uchl1* increases UCHL1 protein synthesis at a post-transcriptional level, hereby identifying a new functional class of lncRNAs. Antisense *Uchl1* activity depends on the presence of a 5′ overlapping sequence and an embedded inverted SINEB2 element. These features are shared by other natural antisense transcripts and can confer regulatory activity to an artificial antisense to green fluorescent protein. Antisense *Uchl1* function is under the control of stress signalling pathways, as mTORC1 inhibition by rapamycin causes an increase in UCHL1 protein that is associated to the shuttling of antisense *Uchl1* RNA from the nucleus to the cytoplasm. Antisense *Uchl1* RNA is then required for the association of the overlapping sense protein-coding mRNA to active polysomes for translation. These data reveal another layer of gene expression control at the post-transcriptional level.

HOW IS THE PAPER STRUCTURED….

GENERAL INTRODUCTION

HOW DOES IT WORK??
→FUNCTIONAL MECHANISM

JUST A PICULIAR PHENOMENON OR IMPORTANT?
→SHOW BIOLOGIAL RELEVANCE IN A KNOWN BIOLOGICAL PROCESS

STEP 1: IDENTIFY INTERESTING ANTISENSE TRANSCRIPTS LINKED TO NEURODEGENERATION

HOW CAN WE FIND THOSE ANTI-SENSE RNAs???

1. **Make a list of genes that have an importance for neurodegeneration**

→ Check published literature

→ Use gene expression data and pick genes that are strongly up/downregualted in disease

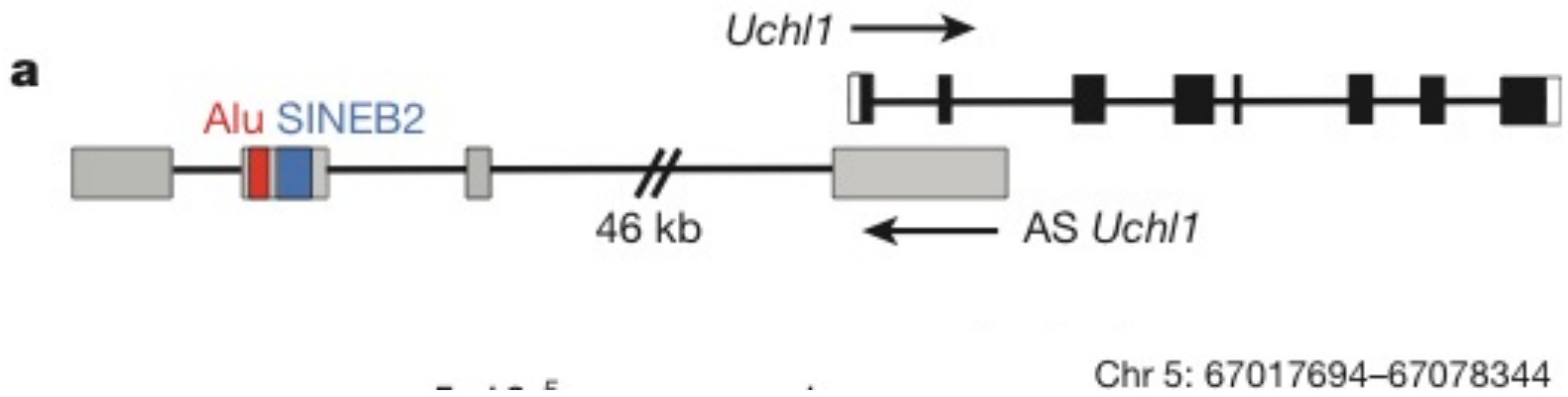
2. Take RNA-seq data from the brain and use bioinformatics to identify transcripts that run in antisense to genes with importance for neurodegenration

Type of strategy:

→candidate approach or

→"educated" guess

STEP 2: PICK BEST CANDIDATE: Uchl1

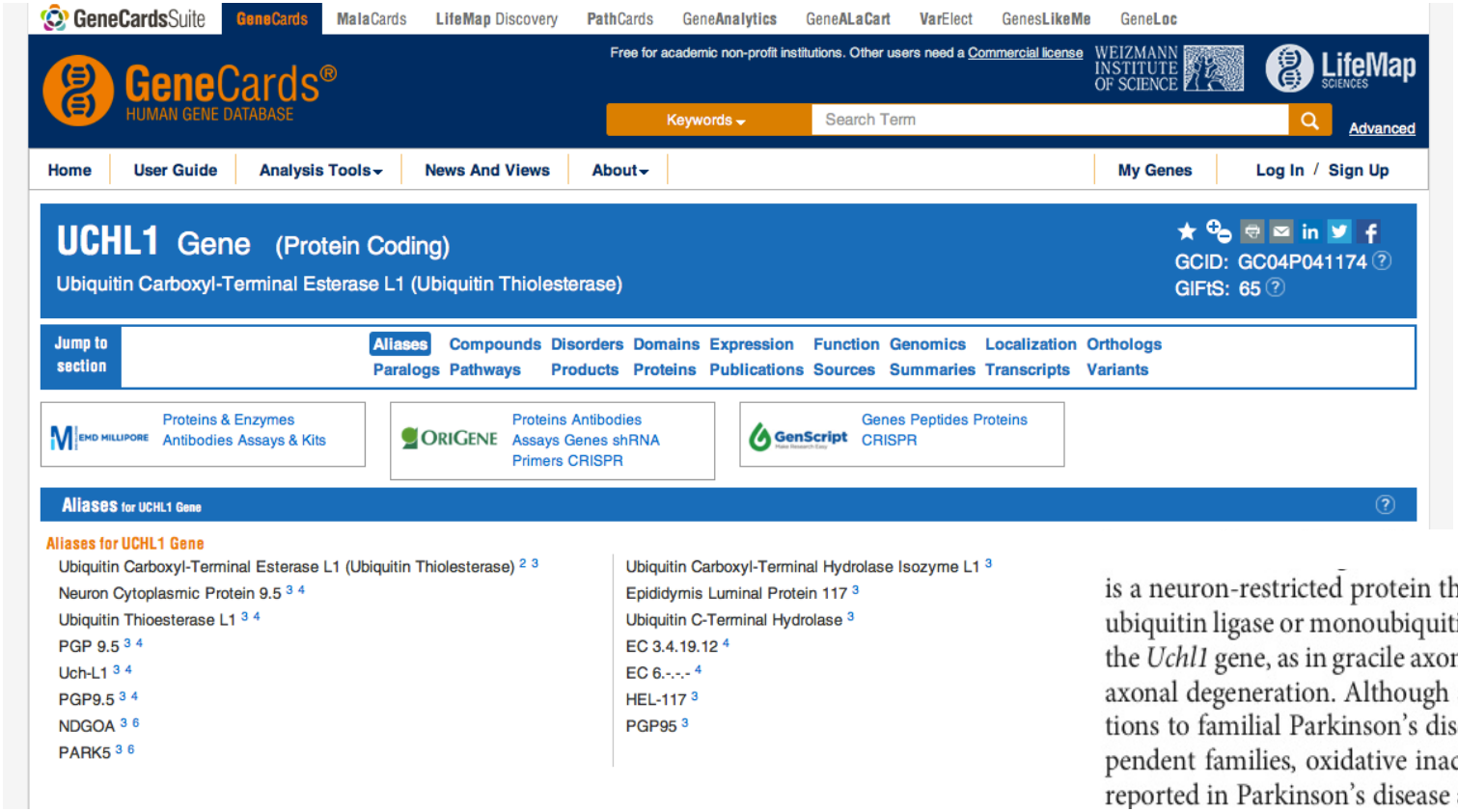


Can we classify the class of lncRNAs AS Uchl1 belongs to??

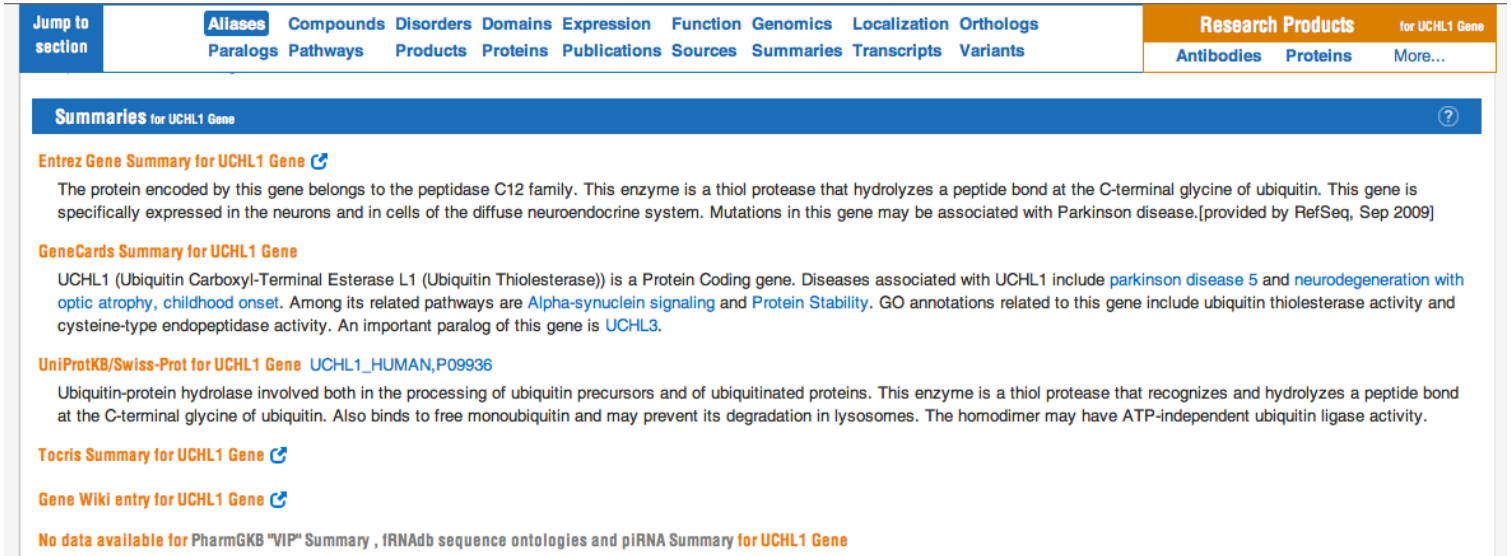
-Antisense lncRNA
-Genic lncRNA
-Convergent transcription with Uchl1
-Spliced
-SPECIAL FEATURE: 2 repetitive seqeuences:
SINEB1 (F1 subclass) = Alu
SINEB2 (B3 subclass)

SINE ELEMENTS:
(= short interspersed elements)
Retrotransposons that depend on LINE elements for transposition
Alu elements are a subclass of SINE elements
SINEs represent ca.: 13% of the human genome

STEP 3: What is Uchl1?? – Lets get some information: http://www.genecards.org

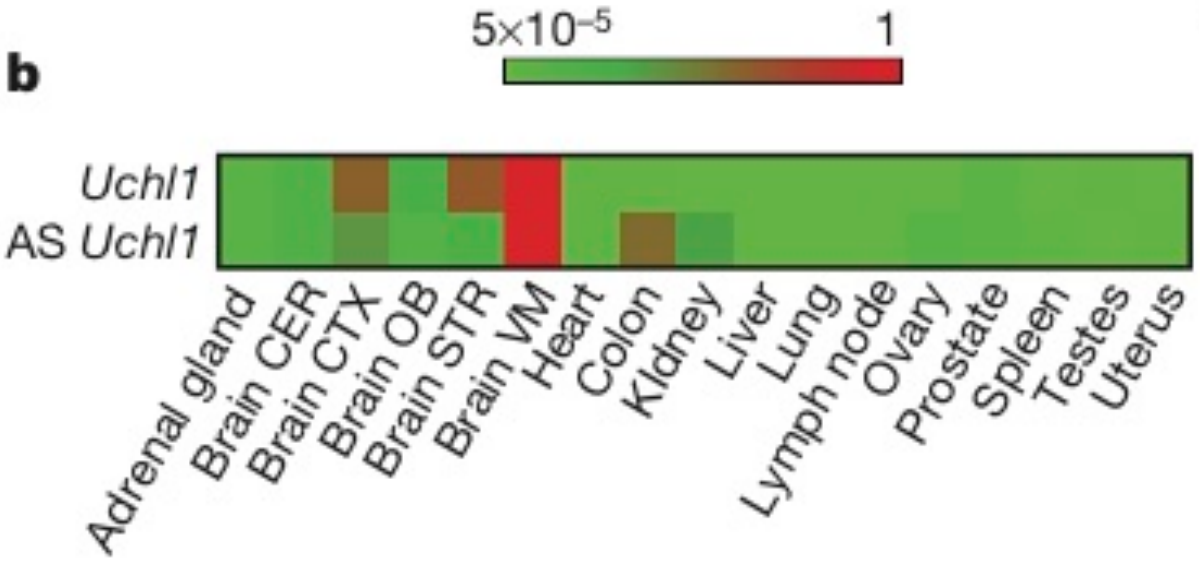


UCHL1 is a neuron-restricted protein that acts as a deubiquitinating enzyme, ubiquitin ligase or monoubiquitin stabilizer[12]. An in-frame deletion in the *Uchl1* gene, as in gracile axonal dystrophy mice, leads to ataxia and axonal degeneration. Although an association of *UCHL1* gene mutations to familial Parkinson's disease has not been confirmed in independent families, oxidative inactivation of UCHL1 protein has been reported in Parkinson's disease and Alzheimer's disease brains[13–15].



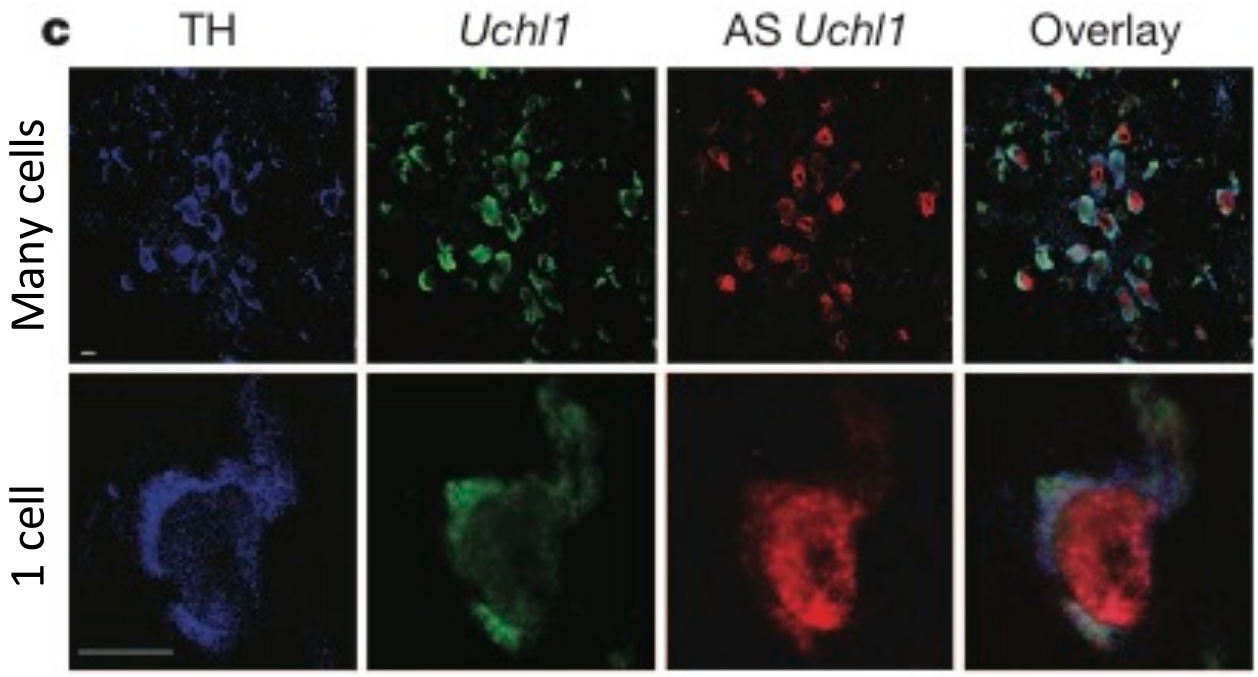
STEP 4: WHERE CAN WE FIND CO-EXPRESSION OF Uchl1 and AS Uchl1:

→ In this compartment we can study the functional interaction of these RNAs



Gene expression array heat map
RED: high expression
GREEN: low expression

Expression of Uchl1; AS Uchl1 restricted to neuronal cells



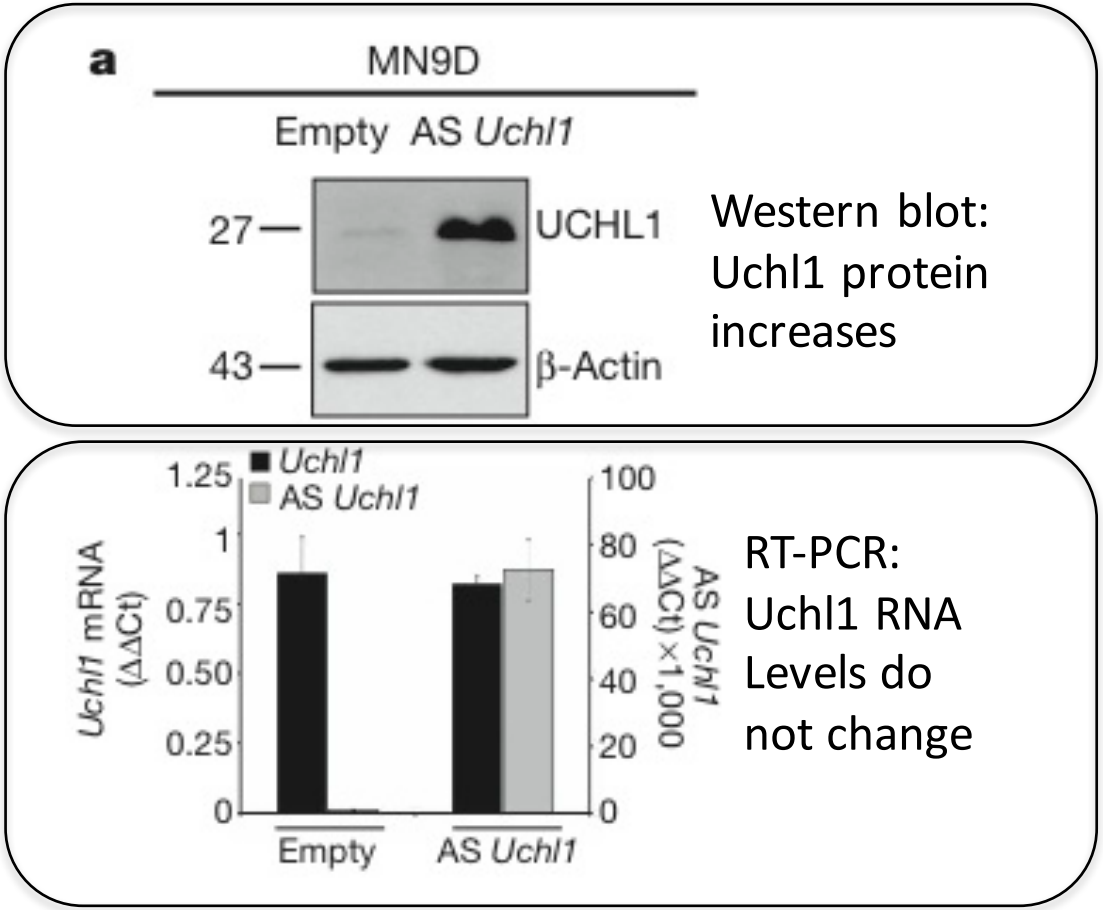
RNA-FISH on dopaminergic neurons in the brain
FISH probe Uchl1: green (cytoplasma)
FISH probe AS Uchl1: red (nucleus)
TH: maker for the identification of dopaminergic neuron

Dopaminergic neurons of the midbrain are the main source of dopamine (DA) in the mammalian central nervous system. Their loss is associated with one of the most prominent human neurological disorders**, Parkinson's disease (PD).** Dopaminergic neurons are found in a 'harsh' region of the brain, the substantia nigra pars compacta, which is DA-rich and contains both redox available neuromelanin and a high iron content. Although their numbers are few, these dopaminergic neurons play an important role in the control of multiple brain functions including voluntary movement and a broad array of behavioral processes such as mood, reward, addiction, and stress.

STEP 5: WHAT FUNCTION DOES Uchl1 HAVE IN NEURONAL CELLS

LETS INCREASE AS Uchl1 EXPRESSION IN A DOPAMERGIC NEURONAL CELL LINE

MN9D cells transiently transfected with a plasmid encoding AS Uchl1 → superhigh expression of AS Uchl1 lncRNA

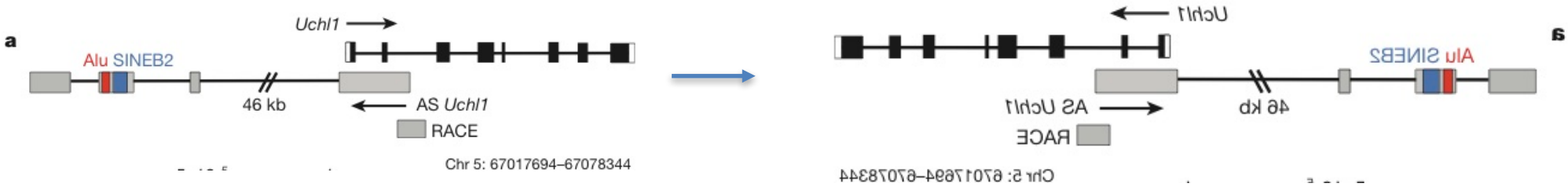


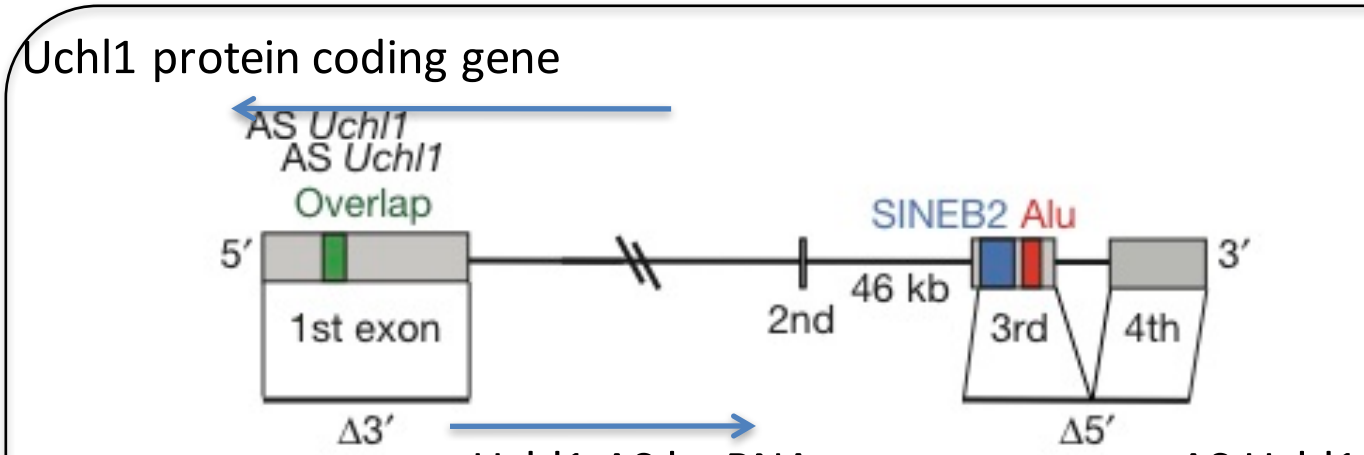
Western blot: Uchl1 protein increases

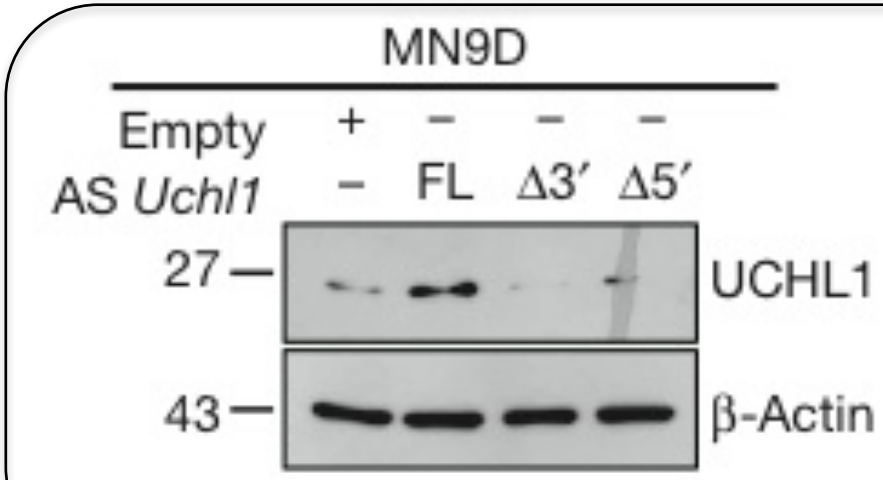
RT-PCR: Uchl1 RNA Levels do not change

Conclusion: AS Uchl1 regulates Uchl1 on the protein level

STEP 5: WHICH PARTS OF AS Uchl1 ARE IMPORTANT TO CONTROL Uchl1 PROTEIN LEVELS



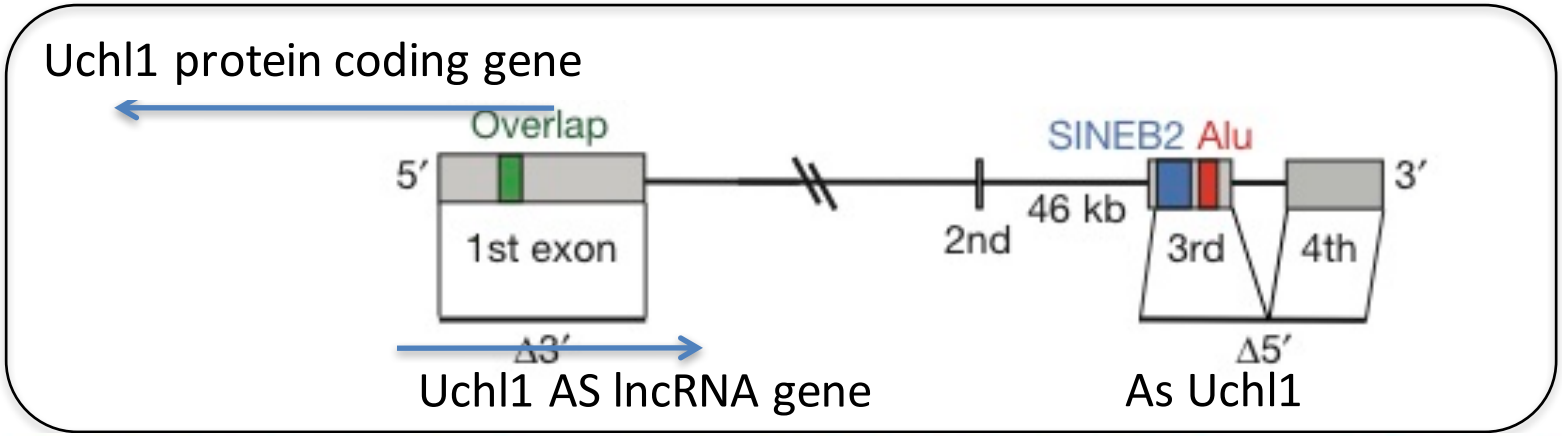


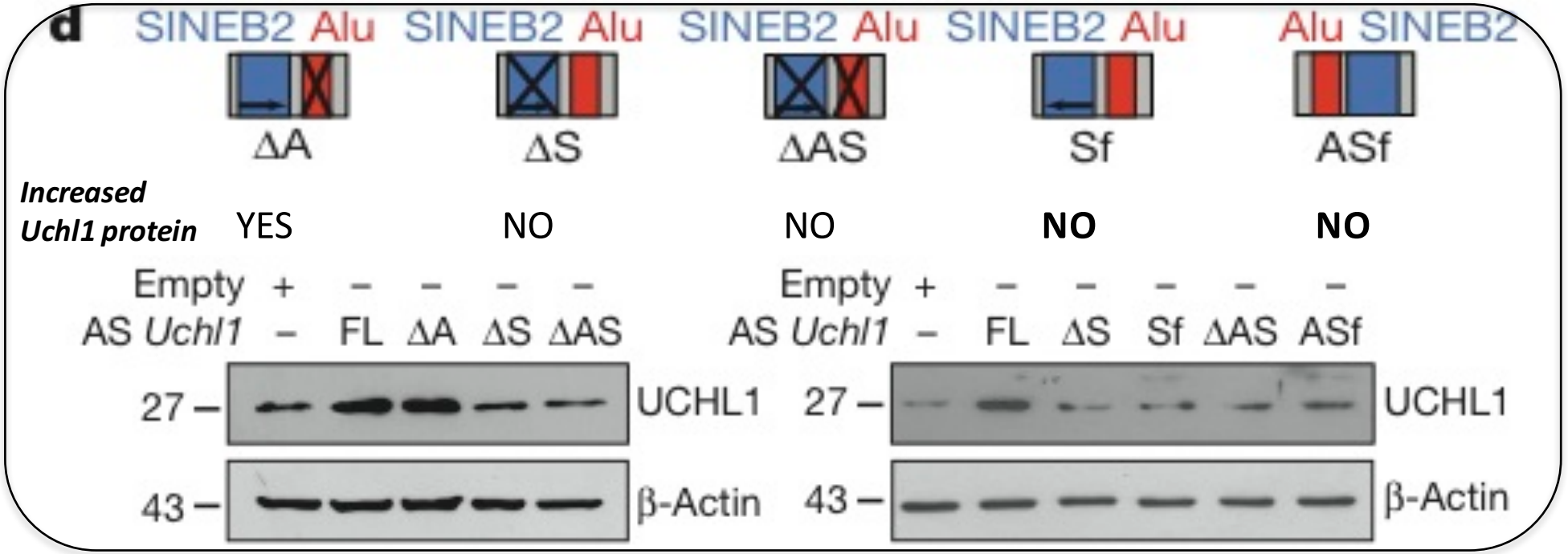


Uchl1 3’ and 5’ region are important for the regulation of Uchl1

STEP 5: WHICH PARTS OF AS Uchl1 ARE IMPORTANT TO CONTROL Uchl1 PROTEIN LEVELS

More deletion constructs in overexpression experiments



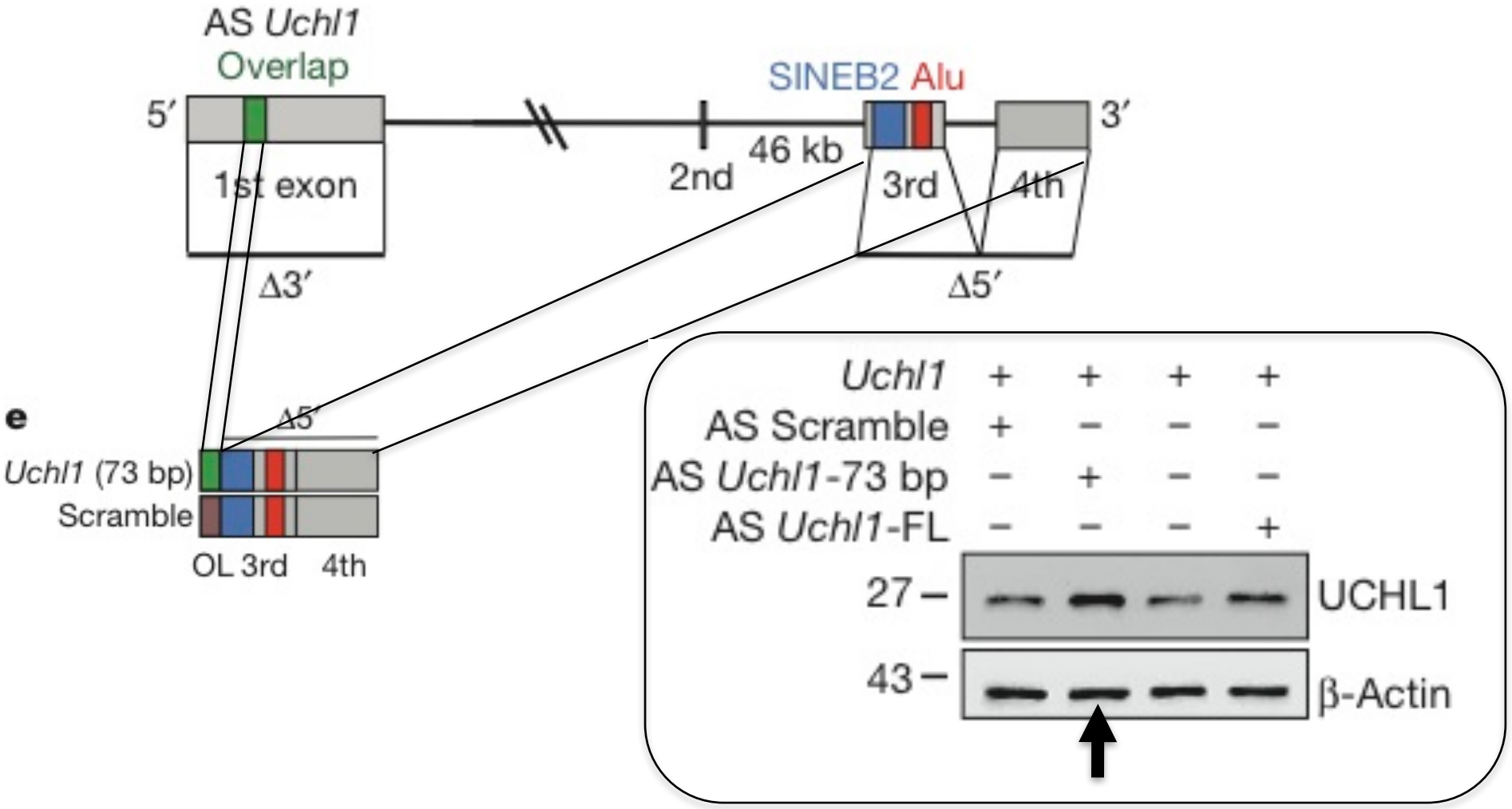


AS Uchl1 mutants have a DOMINANT NEGATIVE effect

CONLCUSION: SINEB2 element in anti-sense orientation is important for Uchl1 regulation

STEP 5: WHICH PARTS OF AS Uchl1 ARE IMPORTANT TO CONTROL Uchl1 PROTEIN LEVELS

Lets make a construct that contains only essential pieces of AS Uchl1



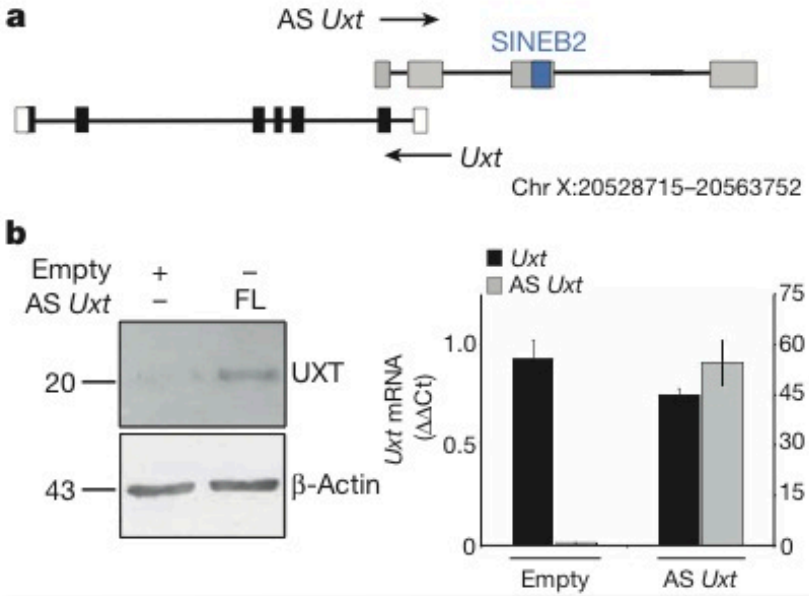
ESSENTIAL PIECES: 1. 73nt AS Uchl1 RNA that overlaps with Uchl1 protein coding gene
2. SINEB2 (+ Alu element)

1+2 ARE SUFFICIENT TO INCREASE Uchl1 PROTEIN EXPRESSION

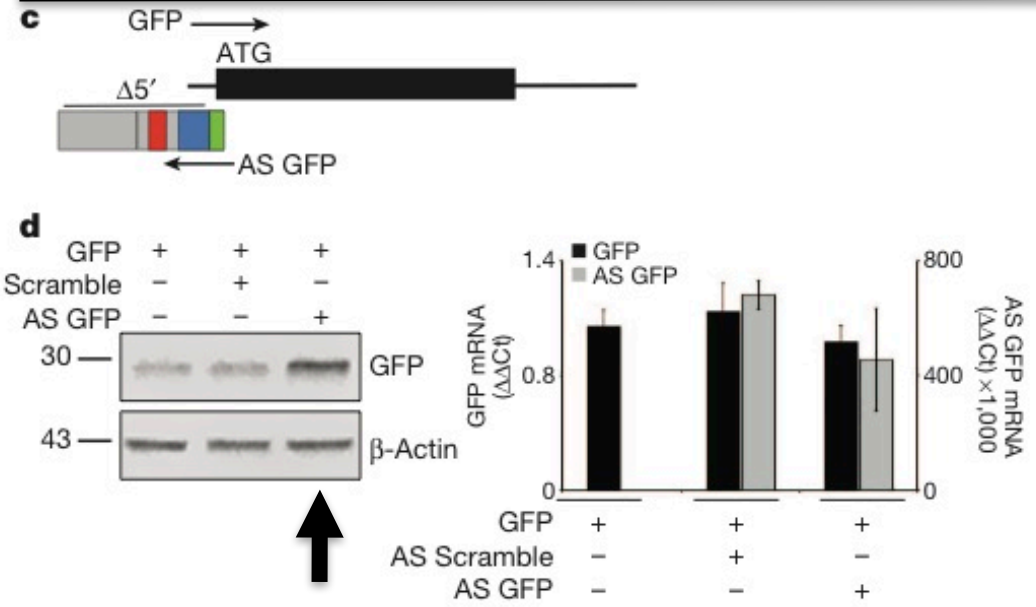
STEP6: LOOKS NICE, BUT DOES IT ALSO WORK FOR OTHER GENES???????

Lets check another example: Utx and AS Utx represent an identical scenario

1. Sense – antisense transcription
2. SINEB2 element and small overlap of Utx ORF with AS Utx



Overexpression of AS UXT1 increases UXT protein expression but not UXT RNA expression



Make construct that contains the The AS Uchl1 SINEB2 and Alu element and a 70 nt seqeunce that complements with a short piece of GFP

Overexpression of AS Ucl1 – AAS GFP construct increases GFP protein expression but not GFP RNA expression

LETTER

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

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GENERAL INTRODUCTION

HOW DOES IT WORK??
→FUNCTIONAL MECHANISM

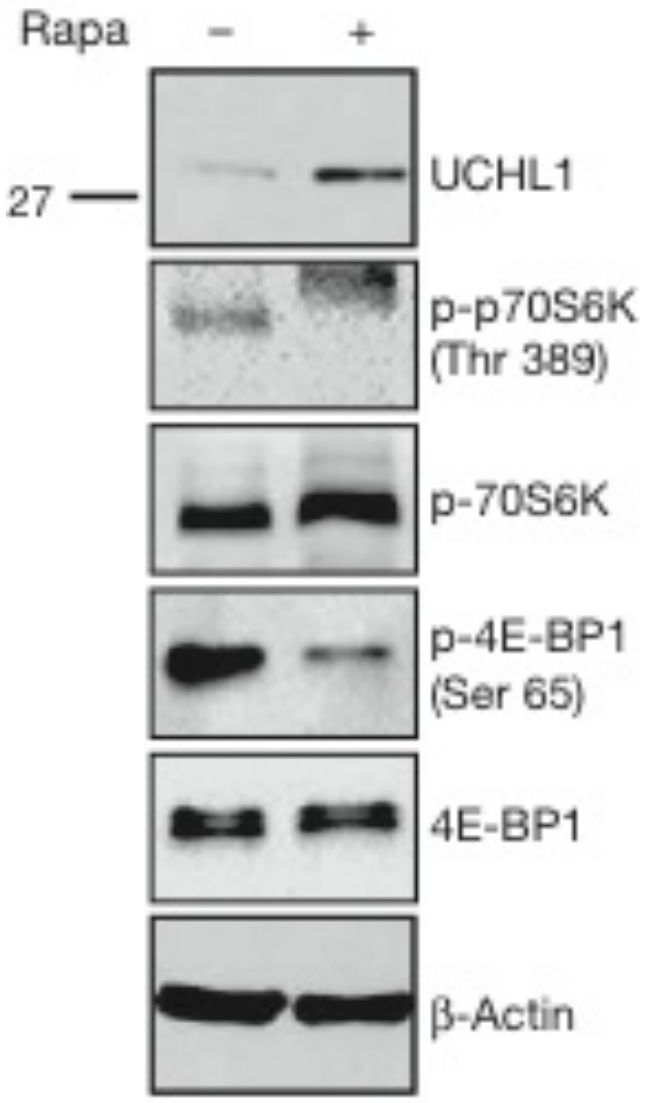
EXPRESSION OF SINEB1/ALU FUSED WITH A SHORT ANTISENSE SEQEUNCE OF A TARGET GENE OF CHOICE INCREASES TARGET PROTEIN EXPRESSION (not RNA)
→→A completely new mechanism of gene regulation

JUST A PICULIAR PHENOMENON OR IMPORTANT?
→SHOW BIOLOGIAL RELEVANCE IN A KNOWN BIOLOGICAL PROCESS

STEP7: LINKING Uchl1 REGUALTION TO PHYSIOLOGICAL PROCESS IN NEURONS

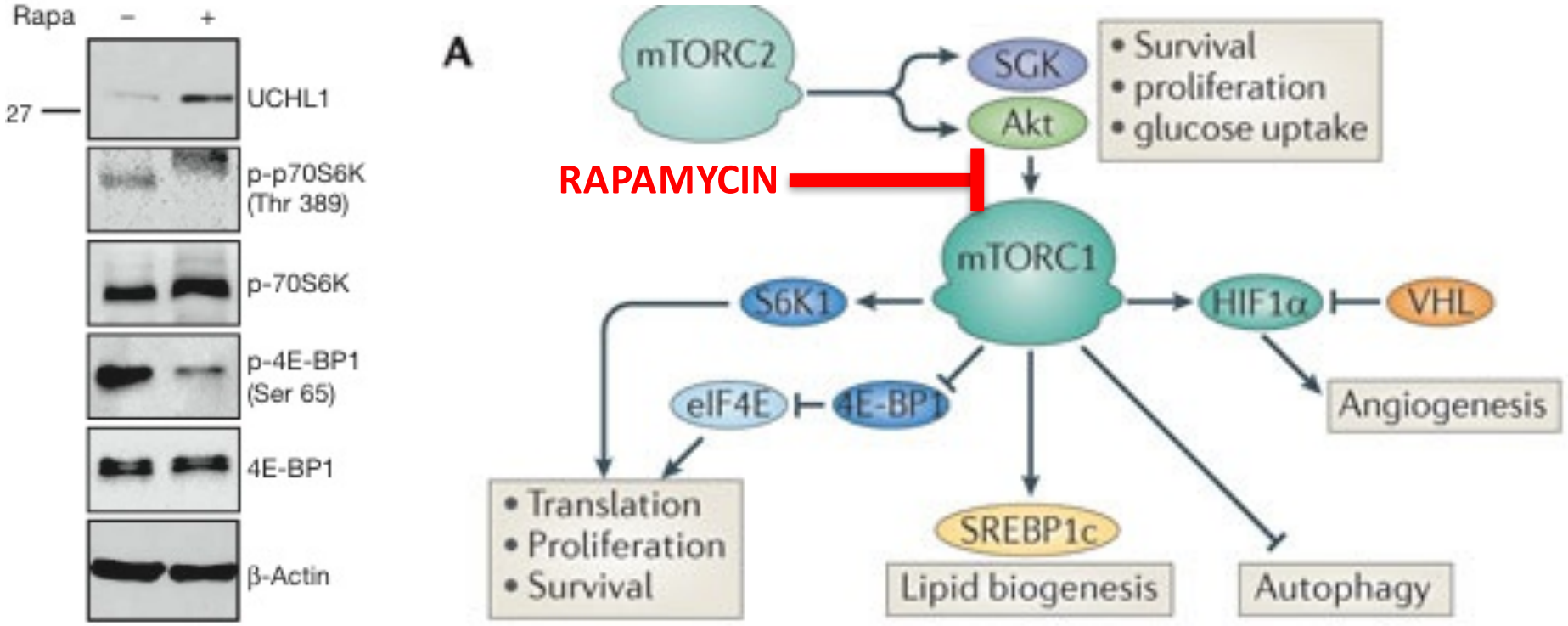
Biological context: treat cells with a series of compounds or stimuli to find condition that increases Uchl1 protein expression → Inhibition of mTOR pathway

To understand how the antisense *Uchl1* transcript operates and the physiological conditions in which it might act, we assayed several stimuli and/or drugs for their ability to modulate UCHL1 protein expression. Inhibition of mTORC1 signalling favoured an increase in UCHL1 levels in a range from 1.5- to 2.5-fold (Fig. 4a). This effect was



STEP7: LINKING Uchl1 REGUALTION TO PHYSIOLOGICAL PROCESS IN NEURONS

Biological context: treat cells with a series of compounds or stimuli to find condition that increases Uchl1 protein expression → Inhibition of mTOR pathway (mTORC1 –> kinase)

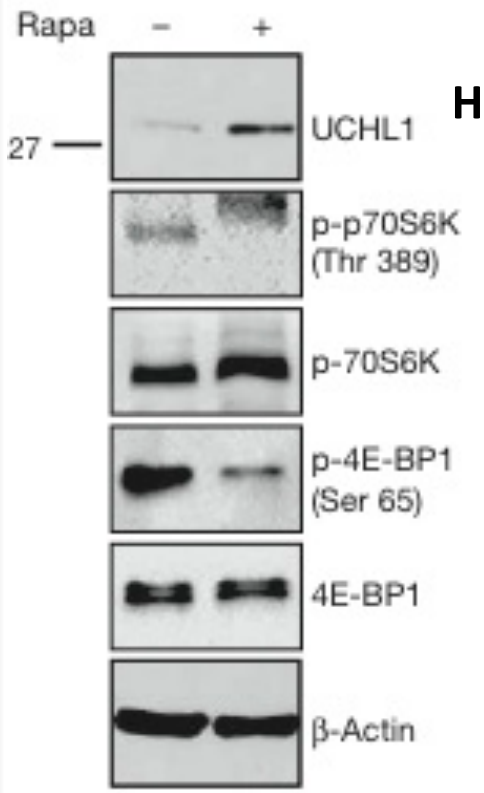


p70S6K: Ribosomal protein S6 kinase

p-4E-BP1: Eukaryotic translation initiation factor 4E-binding protein 1

mTORC1, also known as mammalian target of rapamycin complex 1 or mechanistic target of rapamycin complex 1, is a protein complex that functions as a nutrient/energy/redox sensor and controls protein synthesis.mTOR Complex 1 (mTORC1) is composed of mTOR itself, regulatory-associated protein of mTOR (Raptor), mammalian lethal with SEC13 protein 8 (MLST8) and the recently identified PRAS40 and DEPTOR. This complex embodies the classic functions of mTOR, namely as a nutrient/energy/redox sensor and controller of protein synthesis. The activity of this complex is regulated by rapamycin, insulin, growth factors, phosphatidic acid, certain amino acids and their derivatives (e.g., l-leucine and β-hydroxy β-methylbutyric acid), mechanical stimuli, and oxidative stress. The role of mTORC1 is to activate translation of proteins. In order for cells to grow and proliferate by manufacturing more proteins, the cells must ensure that they have the resources available for protein production. Thus, for protein production, and therefore mTORC1 activation, cells must have adequate energy resources, nutrient availability, oxygen abundance, and proper growth factors in order for mRNA translation to begin.

STEP7: LINKING Uchl1 REGUALTION TO PHYSIOLOGICAL PROCESS IN NEURONS



Hypothesis:

Rapamycin

↓

AS Uchl1

↓

Uchl1 increase

Rapamycin

↓

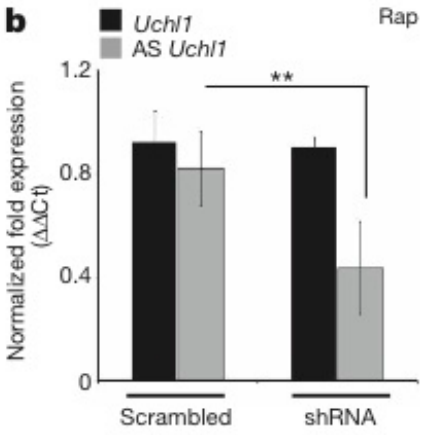
~~AS Uchl1~~

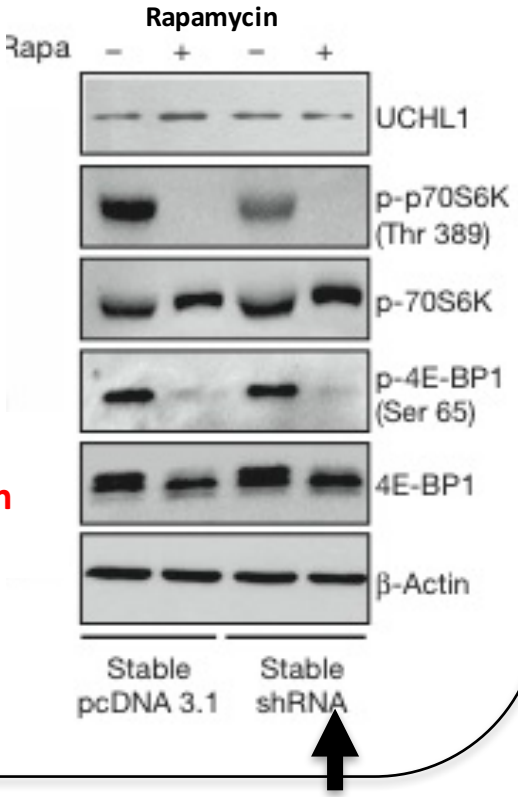
AS Uchl1 Knock-down (siRNA)

↓

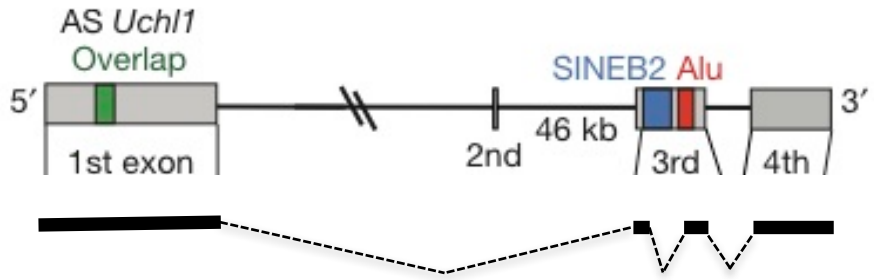
NO Uchl1 increase

Up-regulation of Uchl1 by Rapamycin depends on AS Uchl1





STEP7: LINKING Uchl1 REGUALTION TO PHYSIOLOGICAL MECHANISM IN NEURONS



AS Uchl1 deltaSINEB2
(SINEB2 is essential for Uchl1 AS function)

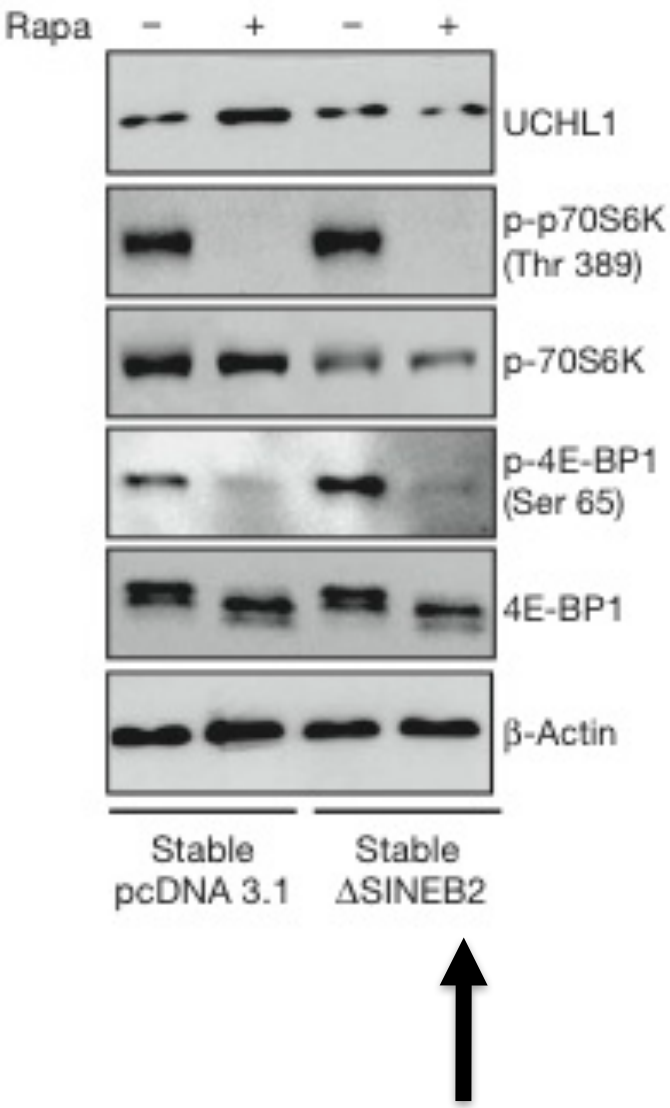
Rapamycin

↓

AS Uchl1 ⊣ Overexpression of delta SINEB2 Uchl1 Has a dominant negative effect on endogenous AS Uchl1 In rapamycin treated cells (blocking the function of endogenous AS Uchl1)

⇣

NO Uchl1 increase



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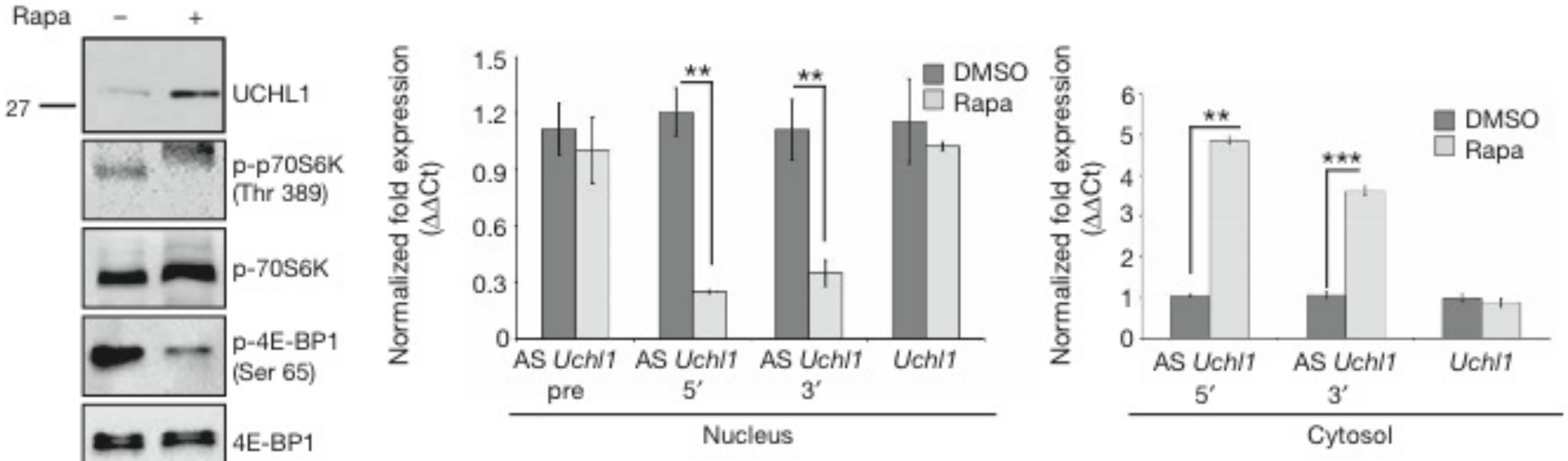


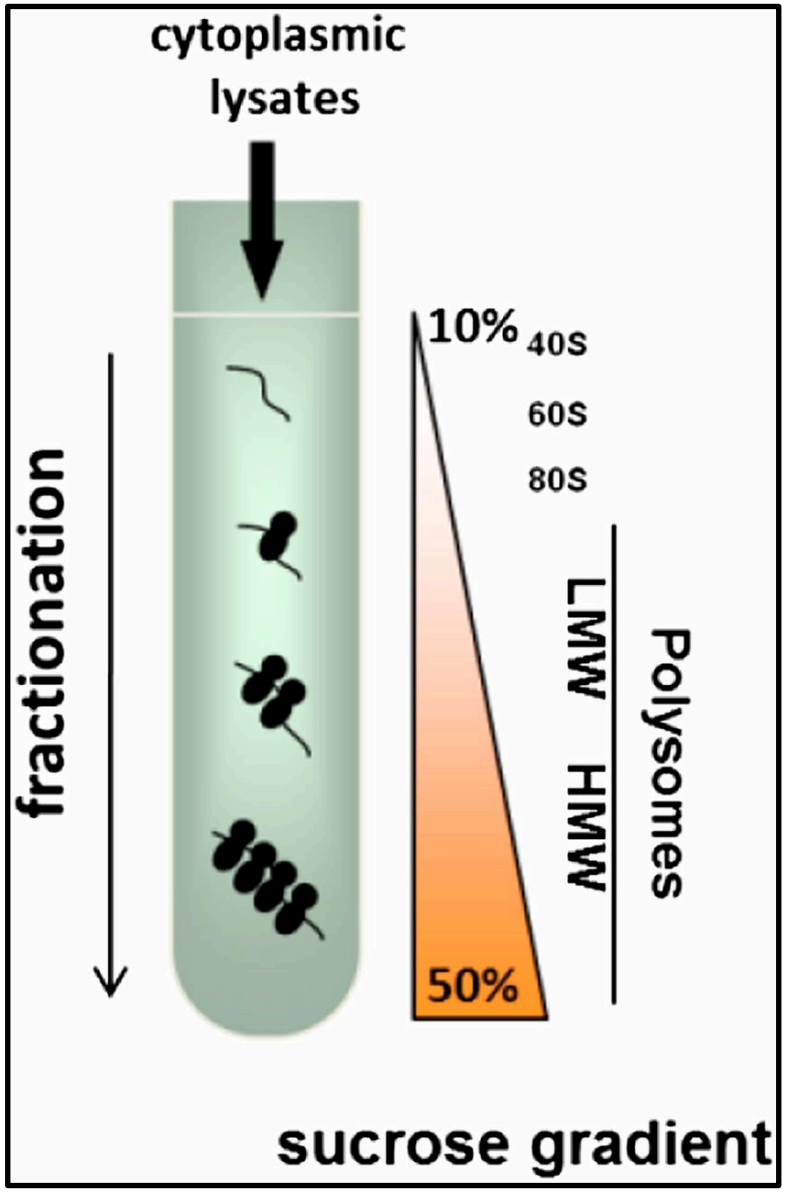
RAPAMYCIN TREATMENT CAUSES ACCUMULATION OF AL Uchl1 IN THE CYTOPLASMA

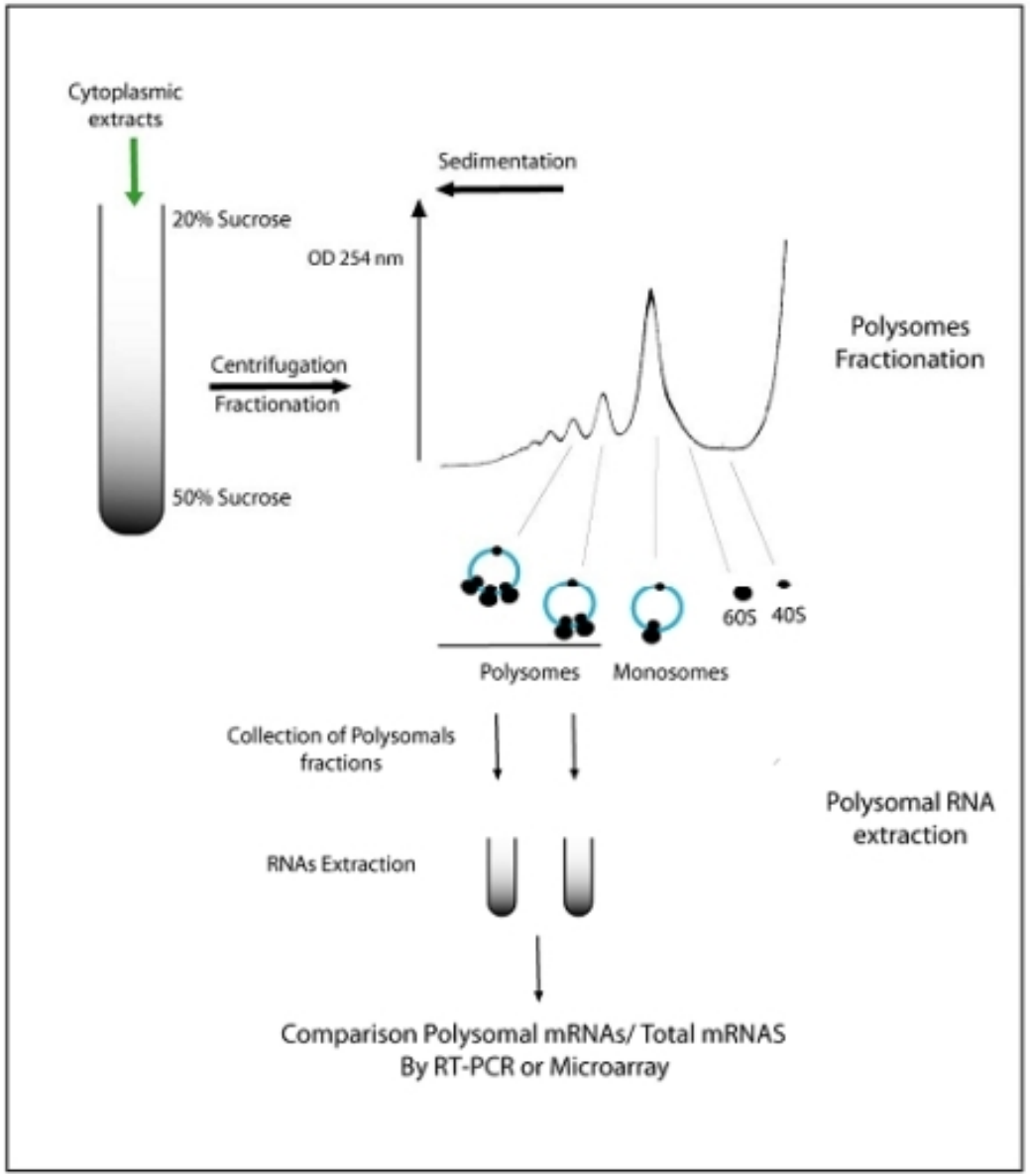
→ ALTERATION IN PROTEIN TRANSLATION

HOW IS UCHL1 PROTEIN TRANLATION AFFECTED???

STEP7: LINKING Uchl1 REGUALTION TO PHYSIOLOGICAL MECHANISMS IN NEURONS - TRANSLATION

PURIFICATION OF RIBOSOMES ON RNA - POLYSOMES





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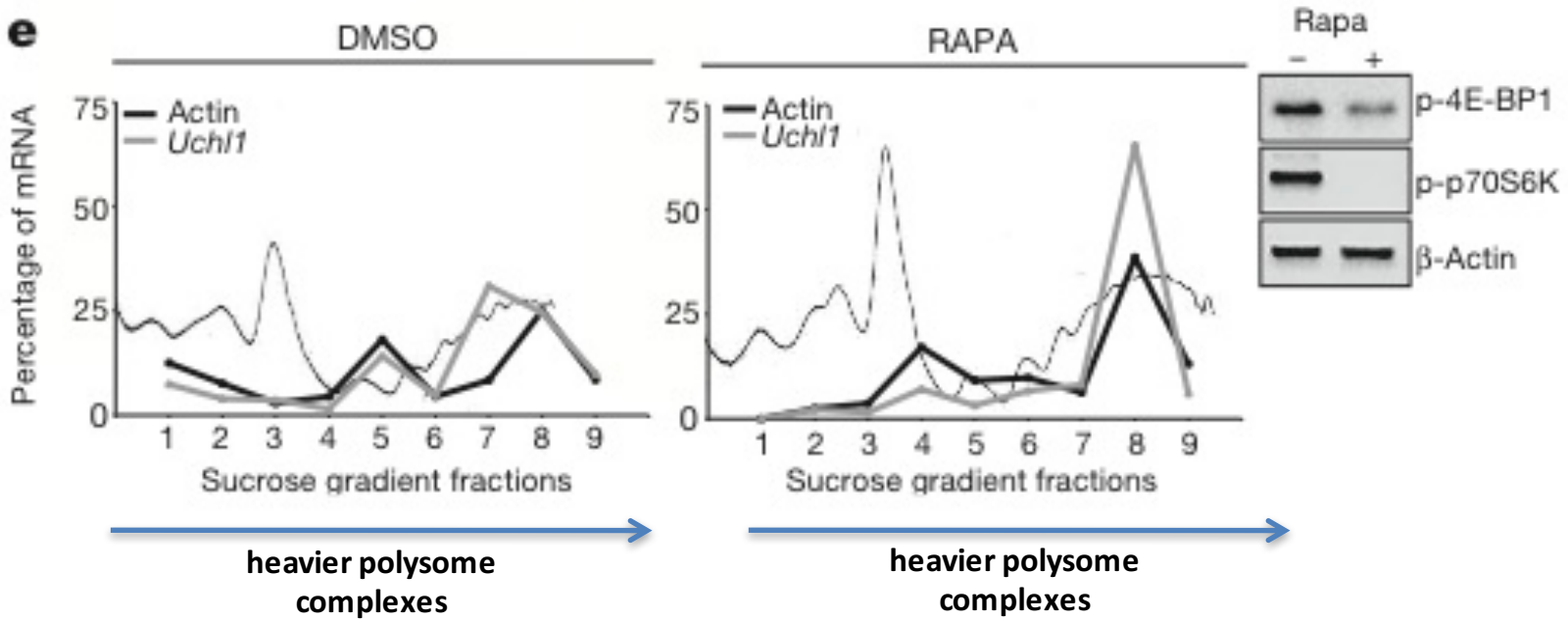
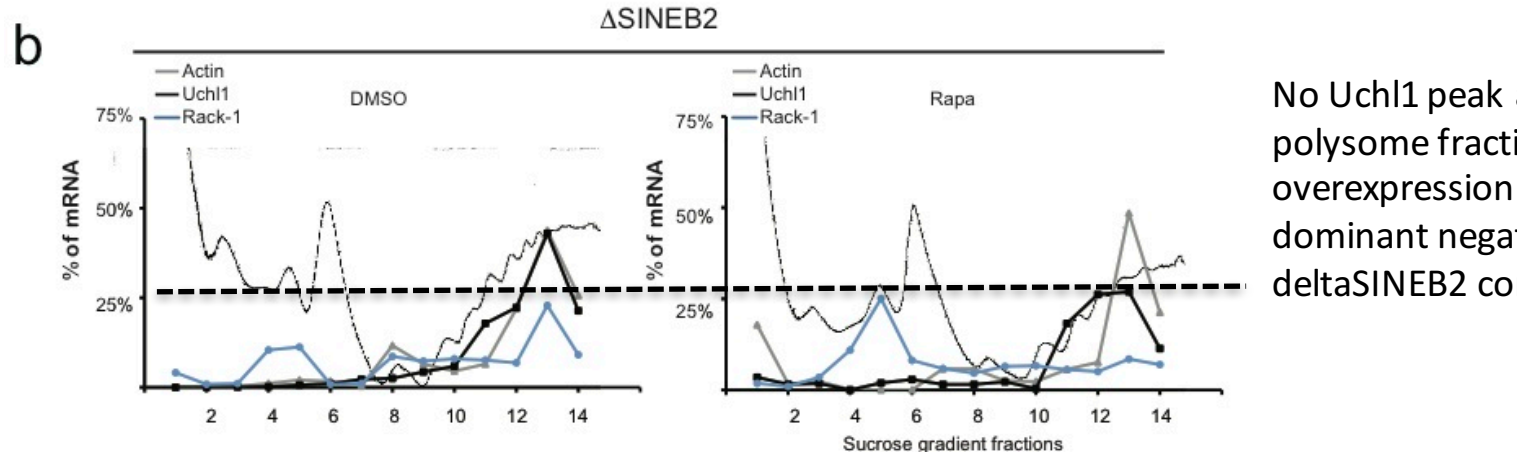


Under Rapamycin conditions Uchl1 mRNA shifts to heavy ribosome fraction
→ More Ribosomes per Uchl1 mRNA = more translation



No Uchl1 peak at heavy polysome fraction upon overexpression of dominant negative deltaSINEB2 construct

CONCLUSION

RAPAMYCIN

↓

Translocation of AS Uchl1
to cytoplasma

↓

Interaction with Uchl1
Dependent on sense-antisense orientation
of Uchl1 and AS-Uchl1 (72nt at 5' end of AS Uchl1)
and SINEB2 element in AS Uchl1

⇣

MECHANISM
NOT KNOWN

⇣

Increased association
of Uchl1 to polysomes

Antisense *Uchl1* is the representative member of a new functional class of lncRNAs that are part of S–AS pairs in the mammalian genome that require overlap at the 5′ end and the action of a SINEB2 repeat. This new function for SINEB2 sequences in the cytoplasm adds to their well-established role in the nucleus as inhibitors of RNA polymerase II[16]. Stress-dependent nucleocytoplasmic shuttling of lncRNAs may be a common strategy to regulate translation, as CTN-RNA, another nuclear-retained lncRNA, was found to have a cryptic protein-coding sequence at its 3′ end when in the cytoplasm[21].
tumorigenesis[22,24]. In genetic and neurochemical models of Parkinson's disease, mTORC1 inhibition protects dopaminergic neurons from apoptosis[25,26].

Antisense lncRNA-mediated translation may be another mechanism to maintain synthesis of pro-survival proteins, such as UCHL1, that are involved in rapamycin neuroprotective function and more generally in cellular response to stress. This mechanism may represent the outcome of an evolutionary pressure on the genomic organization of anti-stress elements to favour gene-specific regulation of translation when CAP-dependent initiation is reduced. Finally, natural and synthetic antisense transcripts with embedded repetitive elements may represent molecular tools to increase translation of selected mRNAs, defining a potential new class of RNA therapeutics.

SINE-UP may allow to escape from Rapamycin induced block of translation

An application for the AS-Uchl1 mechanism for therapeutic applications

“SINEUP”: upregulation of protein expression by using SINEB2 elements

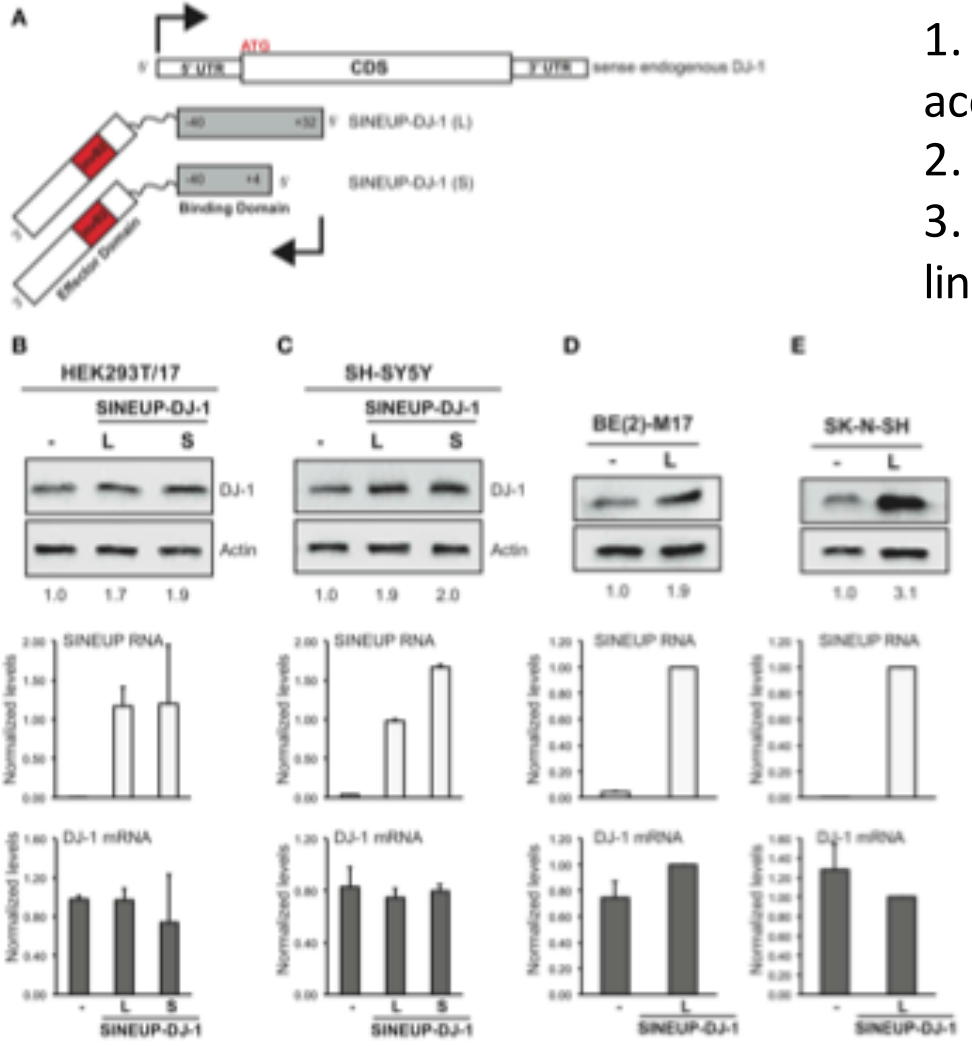
frontiers in Cellular Neuroscience

ORIGINAL RESEARCH
published: 13 May 2015
doi: 10.3389/fncel.2015.00174

SINEUPs are modular antisense long non-coding RNAs that increase synthesis of target proteins in cells

Silvia Zucchelli[1,2†], Francesca Fasolo[1†], Roberta Russo[1], Laura Cimatti[1], Laura Patrucco[2], Hazuki Takahashi[3], Michael H. Jones[4], Claudio Santoro[2], Daniele Sblattero[2], Diego Cotella[2], Francesca Persichetti[2], Piero Carninci[3] and Stefano Gustincich[1]*

Engineered SINEUPs to upregulate gene expression of genes of interest



1. Pairing region designed according to target gene
2. SINEB2 element fused
3. Overexpression in cell lines

CELL guidance systems

ENABLING TECHNOLOGIES
FOR STEM CELL SCIENCE

UP

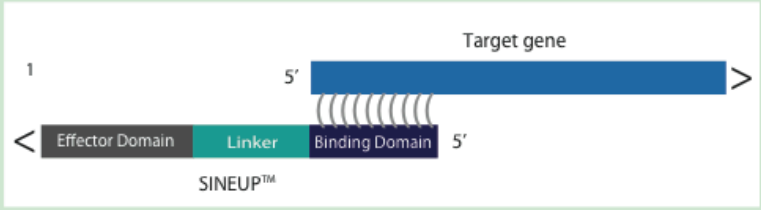
IN

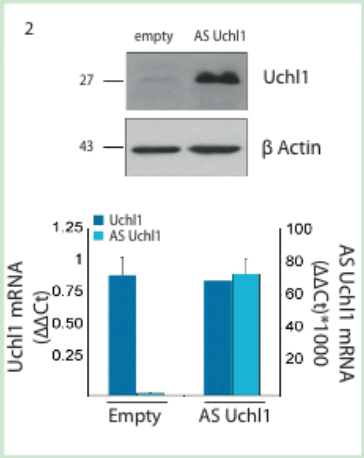
OUT

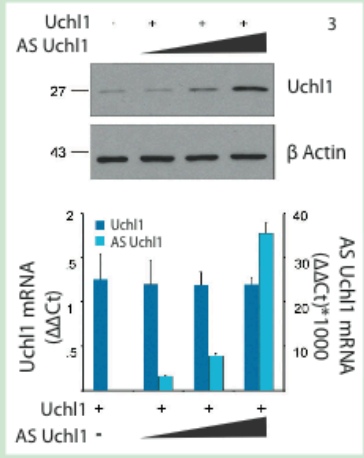
DOWN

Take your research in a new direction

SINEUP™ Technology







(1) SINEUP™ constructs express non-coding RNAs containing a short target gene specific binding domain linked to a common 1105 nt effector domain incorporating a SINEB2 element. The technology was developed following identification and characterization of a long non-coding RNA transcript which contains an antisense sequence to the Uchl1 gene linked to a SINEB2 repeat.
(2) Increased expression of the AS Uchl1 SINEUP™ from a transfected construct results in increased expression of Uchl1 protein without any increase in Uchl1 mRNA levels.
(3) Expression levels of Uchl1 protein can be titrated by modulating expression of an AS Uchl1 SINEUP™ construct.