III Jornadas de genómica clínica
FCEN-UBA
25 de octubre de 2024

Terapia combinada de una enfermedad hereditaria sobre la base de modulación del splicing alternativo y del estado de la cromatina

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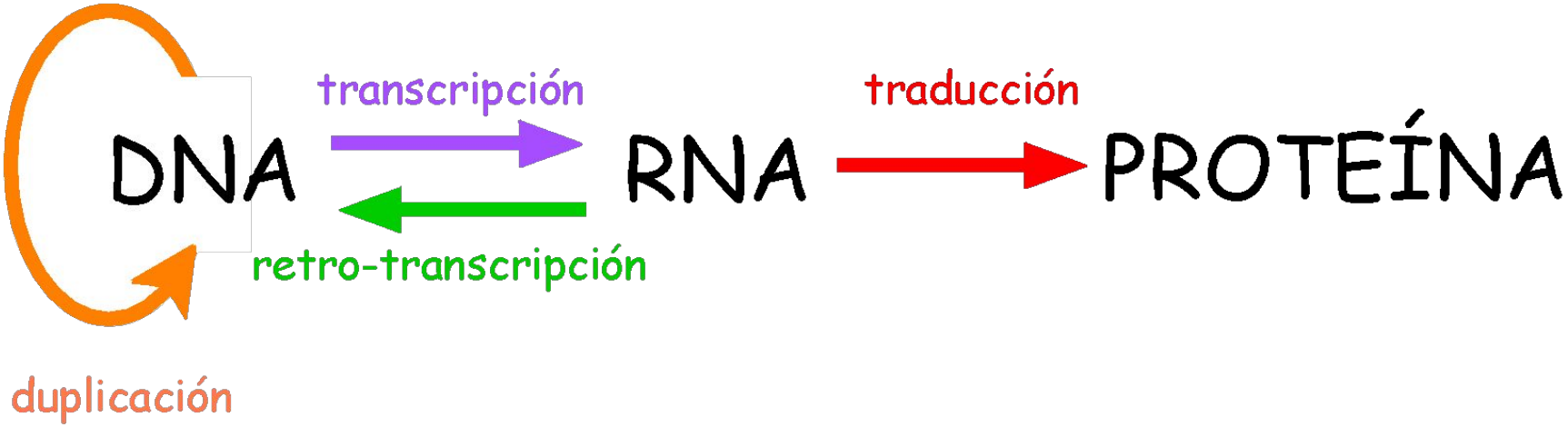
Facultad de Ciencias Exactas y Naturales
Universidad de Buenos Aires - Argentina

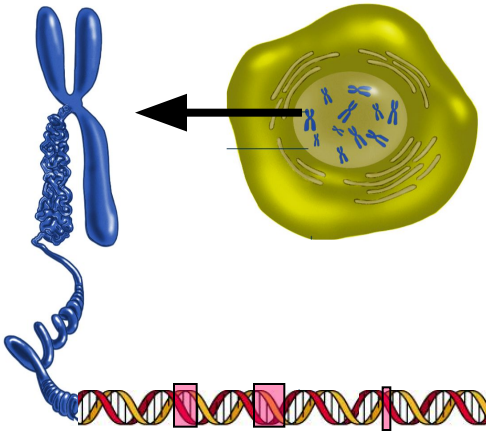
Los principales productos de los genes son proteínas

¿Cómo se fabrican?


transcripción
traducción
DNA
RNA
PROTEÍNA
retro-transcripción
duplicación

cromosoma

célula



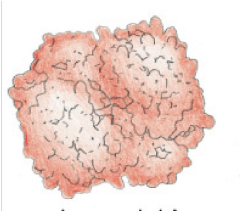
gen

ADN

TRANSCRIPCIÓN

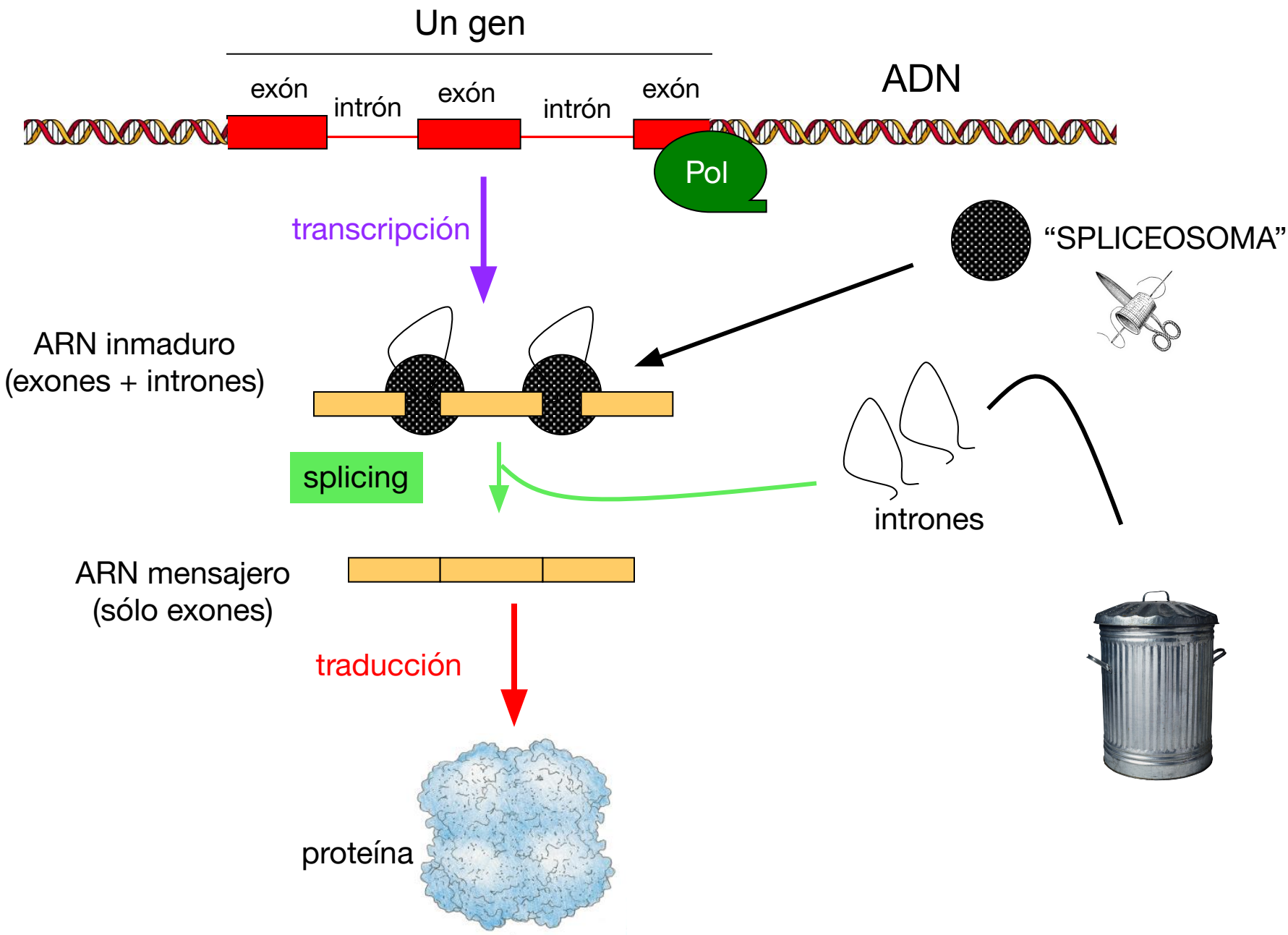
Ácido ribonucleico (ARN)

TRADUCCIÓN

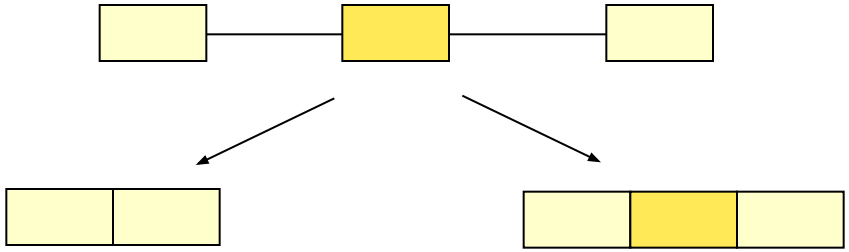


proteína

función


Un gen
exón
intrón
exón
intrón
exón
ADN
Pol
transcripción
"SPLICEOSOMA"
ARN inmaduro
(exones + intrones)
splicing
intrones
ARN mensajero
(sólo exones)
traducción
proteína

Splicing alternativo



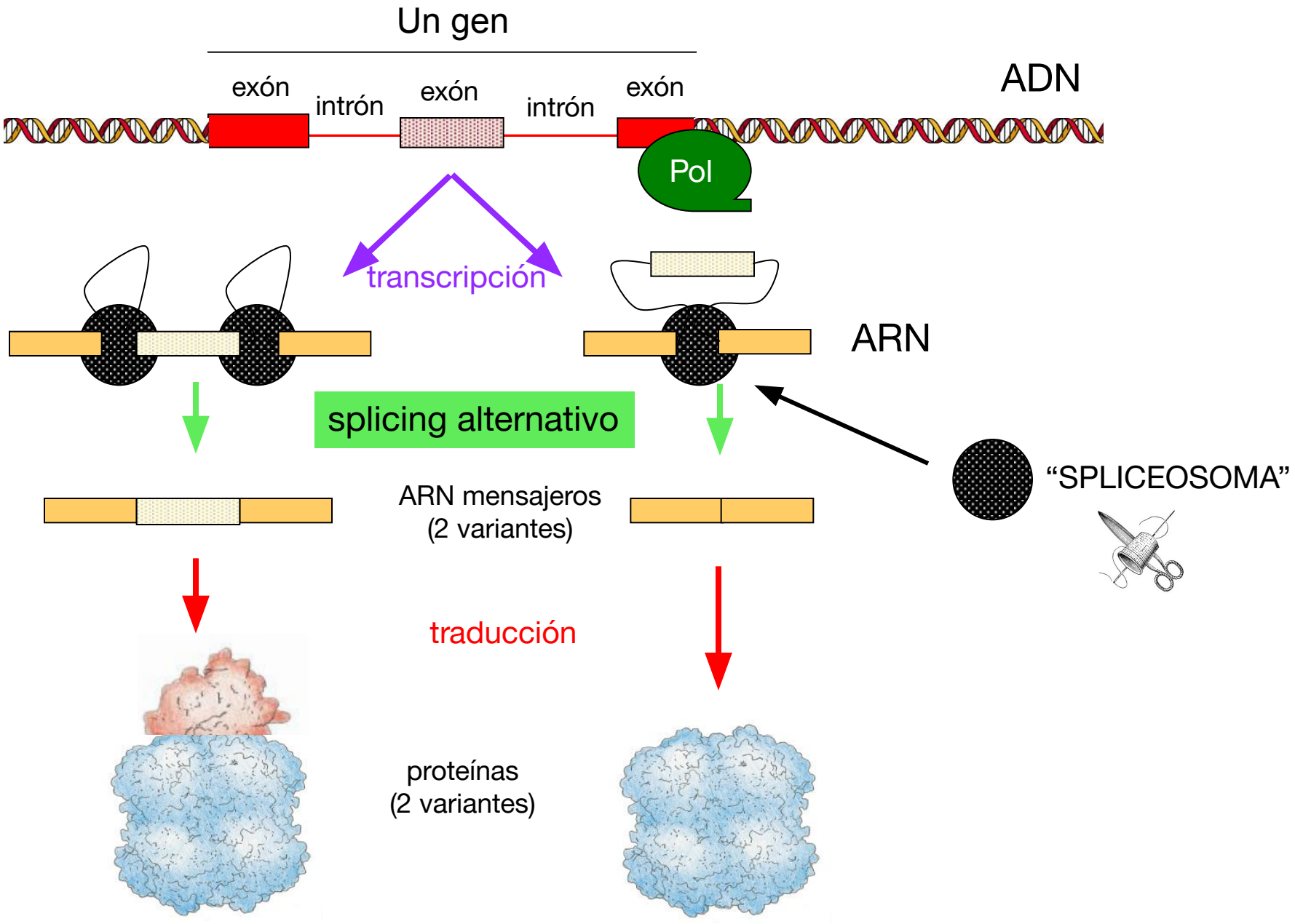
Alternative splicing

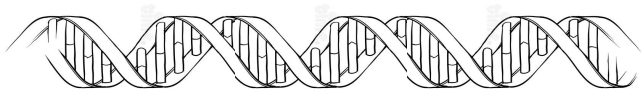
1. It is more a rule than an exception. It is estimated to affect the expression of nearly 95% of human genes.

2. It explains how a vast protein diversity is achieved with a limited number of genes.

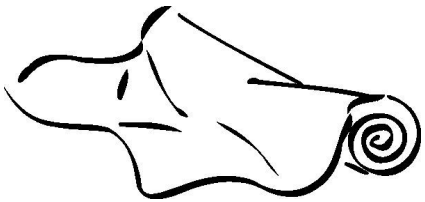
3. Mutations that affect alternative splicing regulatory sequences (splicing enhancers and silencers) are a widespread source of human disease.

4. Alternative splicing regulation not only depends on the interaction of splicing factors with their target sequences in the pre-mRNA but is coupled to RNA polymerase II (pol II) transcription.

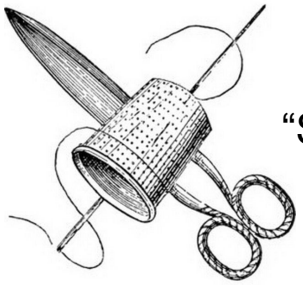

Un gen
exón
intrón
exón
intrón
exón
ADN
Pol
transcripción
ARN
splicing alternativo
"SPLICEOSOMA"
ARN mensajeros
(2 variantes)
traducción
proteínas
(2 variantes)



ADN (gen)



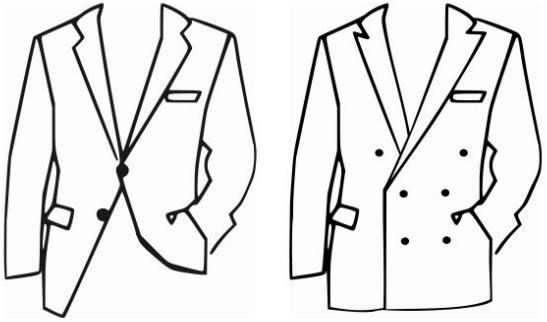
ARN mensajero precursor



“SPLICEOSOMA”

“SPLICING ALTERNATIVO”

NÚCLEO



2 ARN mensajeros diferentes

CITOPLASMA

2 proteínas diferentes

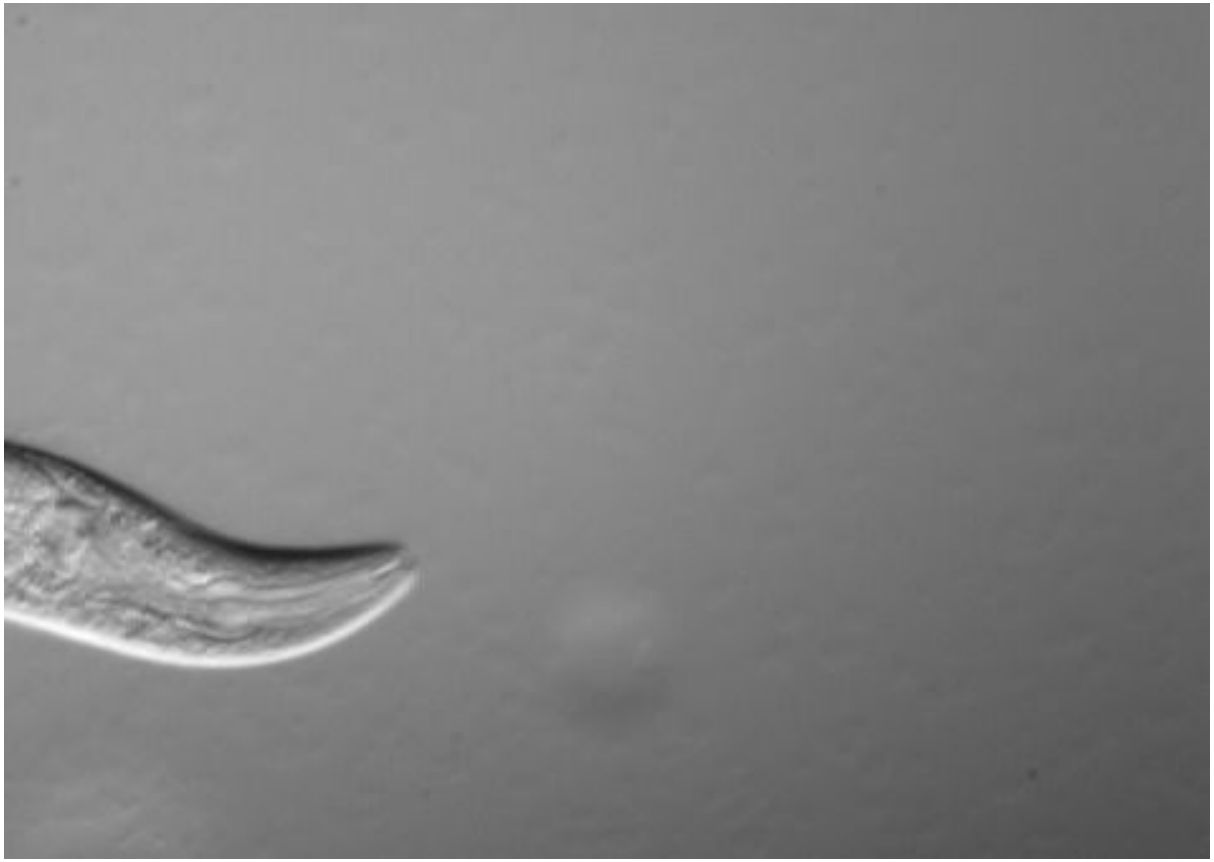
un gen → una proteína

un gen → muchas proteínas

El splicing alternativo parece ser la causa de la gran complejidad de los vertebrados (nosotros)

Gusano *Caenorhabditis elegans*

Invertebrado microscópico de 1 mm de largo formado por 1000 células

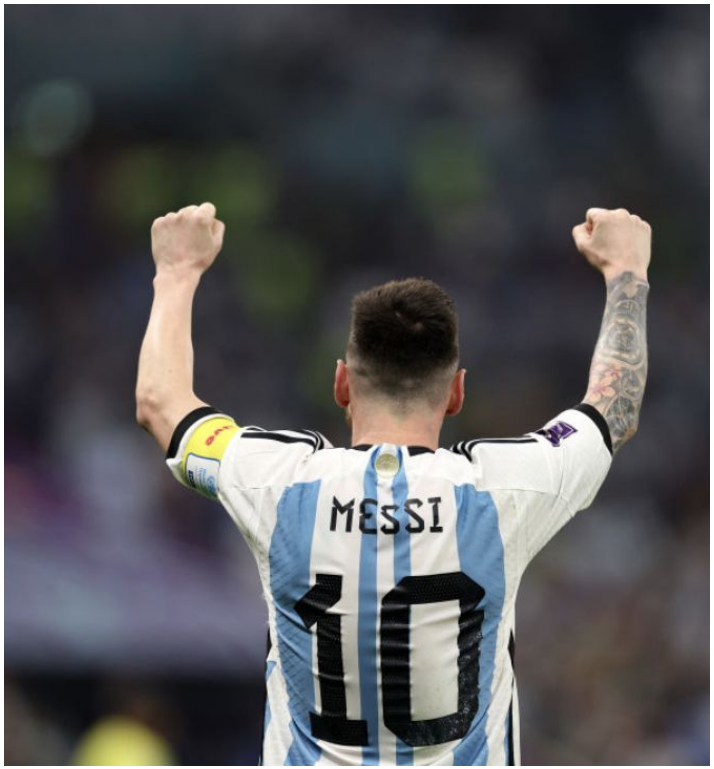


19.000 genes en cada célula

Fuente: http://www.bio.unc.edu/faculty/goldstein/lab/movies.html

Homo sapiens sapiens

Vertebrado macroscópico de casi 2 m de largo formado por 10^{13} células

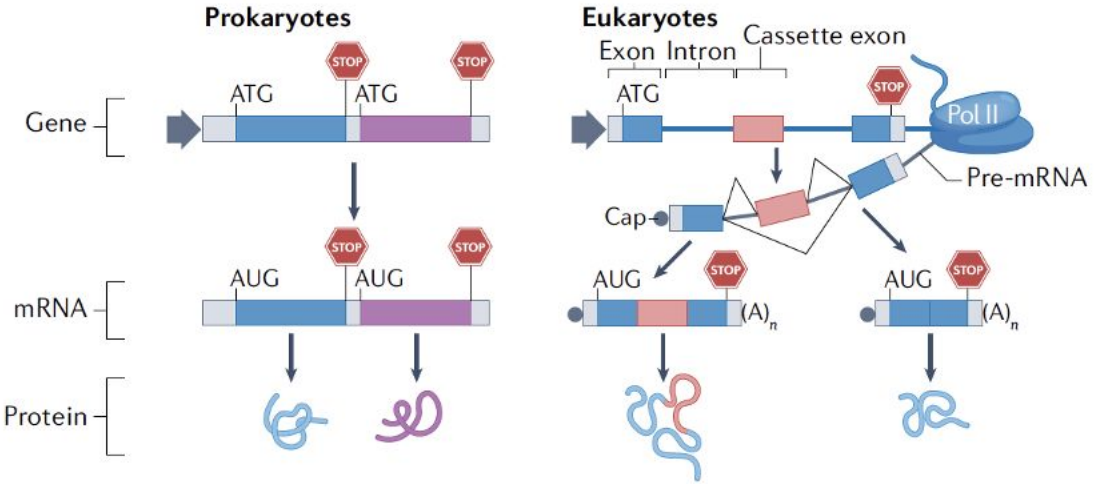


21.000 genes en cada célula

The physiology of alternative splicing

Luciano E. Marasco[1,2] *and Alberto R. Kornblihtt*[1] ✉

NATURE REVIEWS | MOLECULAR CELL BIOLOGY 24, 242–254 (2023)

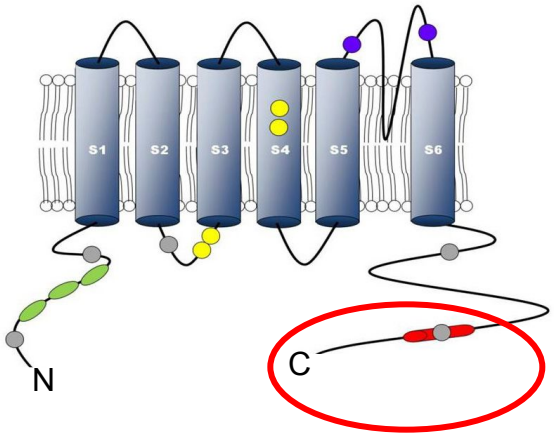


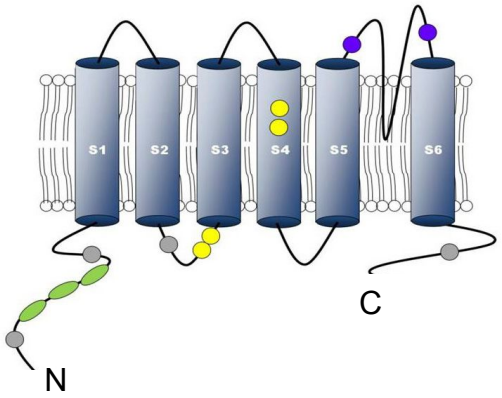
Fibronectina humana

1981-1984
Francisco « Tito » Baralle
Oxford, UK

Kornblihtt et al., PNAS 1983
Kornblihtt et al., EMBO J 1984
Vibe Pedersen et al., EMBO J. 1984
Kornblihtt et al., EMBO J. 1985
Gutman et al., FEBS Lett. 1986
Gutman et al., PNAS 1987

TRPV1 (CANAL IÓNICO DE NEURONAS)





Ganglios de la raíz dorsal

>43 ºC

CALOR NOCIVO

DOLOR

Ganglios del trigémino

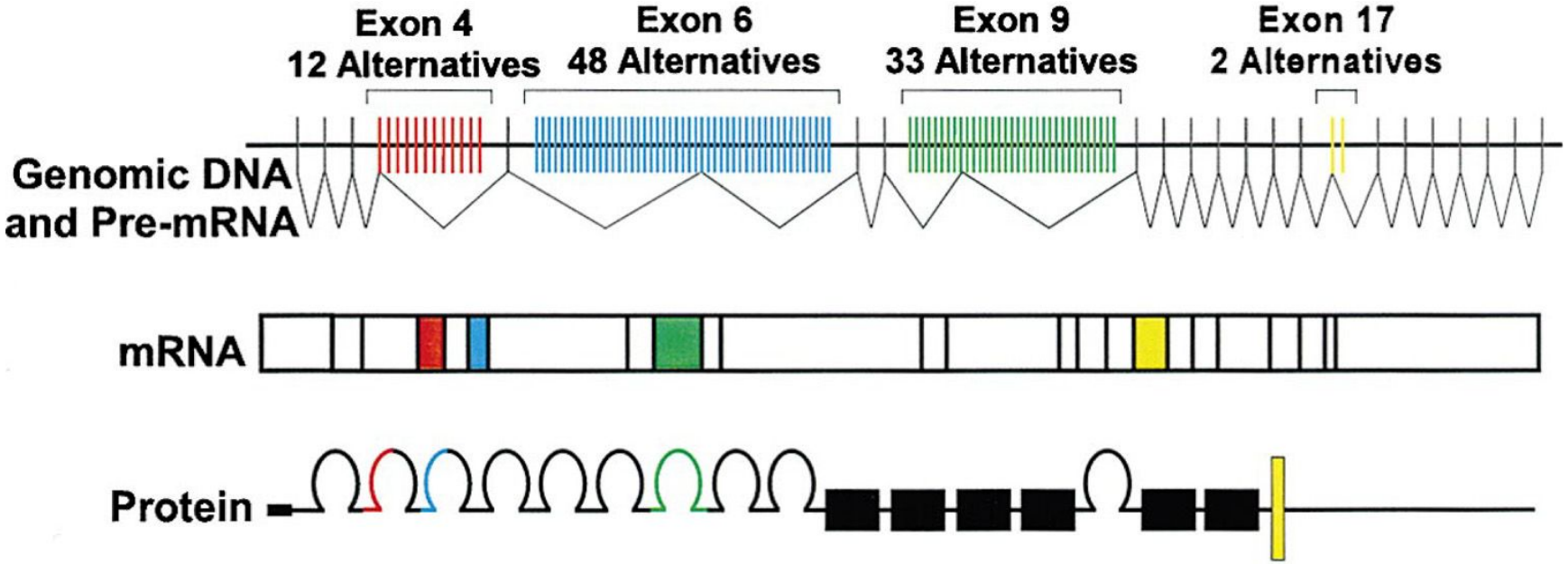
>30 ºC

SENSADO DE INFRARROJO

ALIMENTACIÓN

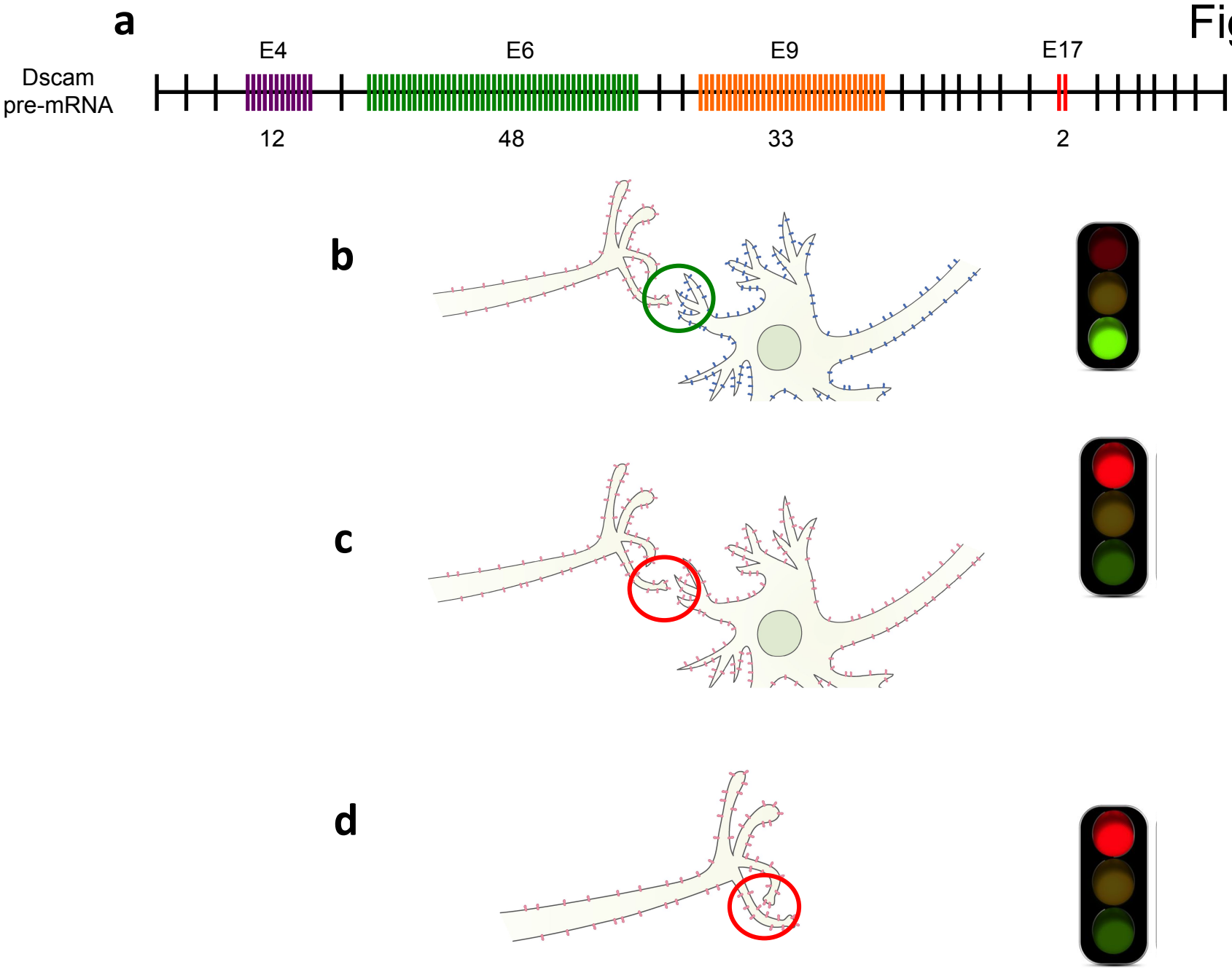
Julius group: Gracheva et al. *Nature* 2011

Gene de la mosca DSCAM
38.016 variantes de mRNA

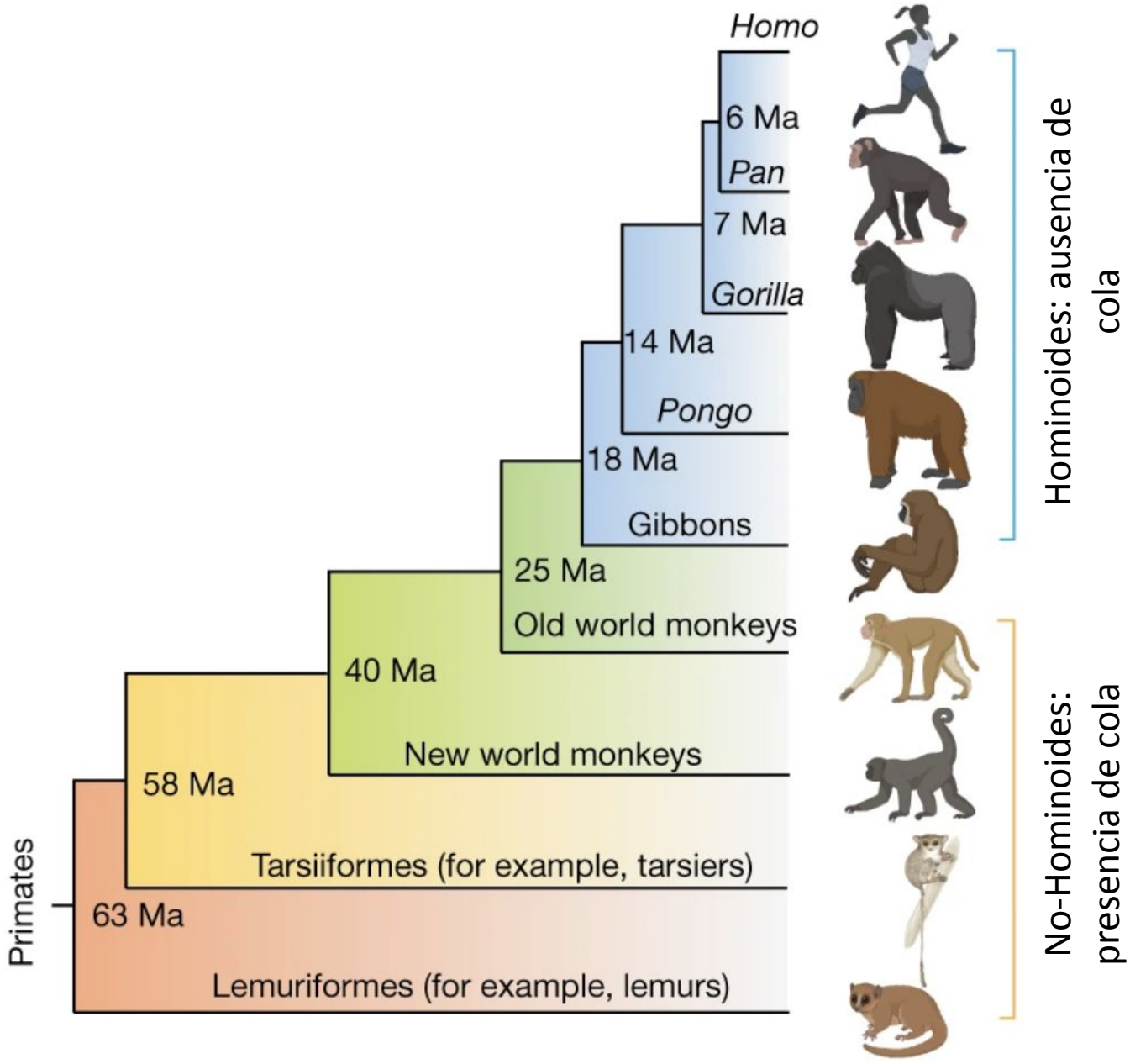


Schmucker et al. *Cell* 101, 671-684 (2000)

Fig. 3



El splicing alternativo es la causa de la pérdida de la cola en los primates hominoides (gibones, orangutanes, gorilas, chimpancés y humanos). Nature, febrero 2024.


Homo
6 Ma
Pan
7 Ma
Gorilla
14 Ma
Pongo
18 Ma
Gibbons
25 Ma
Old world monkeys
40 Ma
New world monkeys
58 Ma
Tarsiiformes (for example, tarsiers)
63 Ma
Primates
Lemuriformes (for example, lemurs)
Hominoides: ausencia de cola
No-Hominoides: presencia de cola

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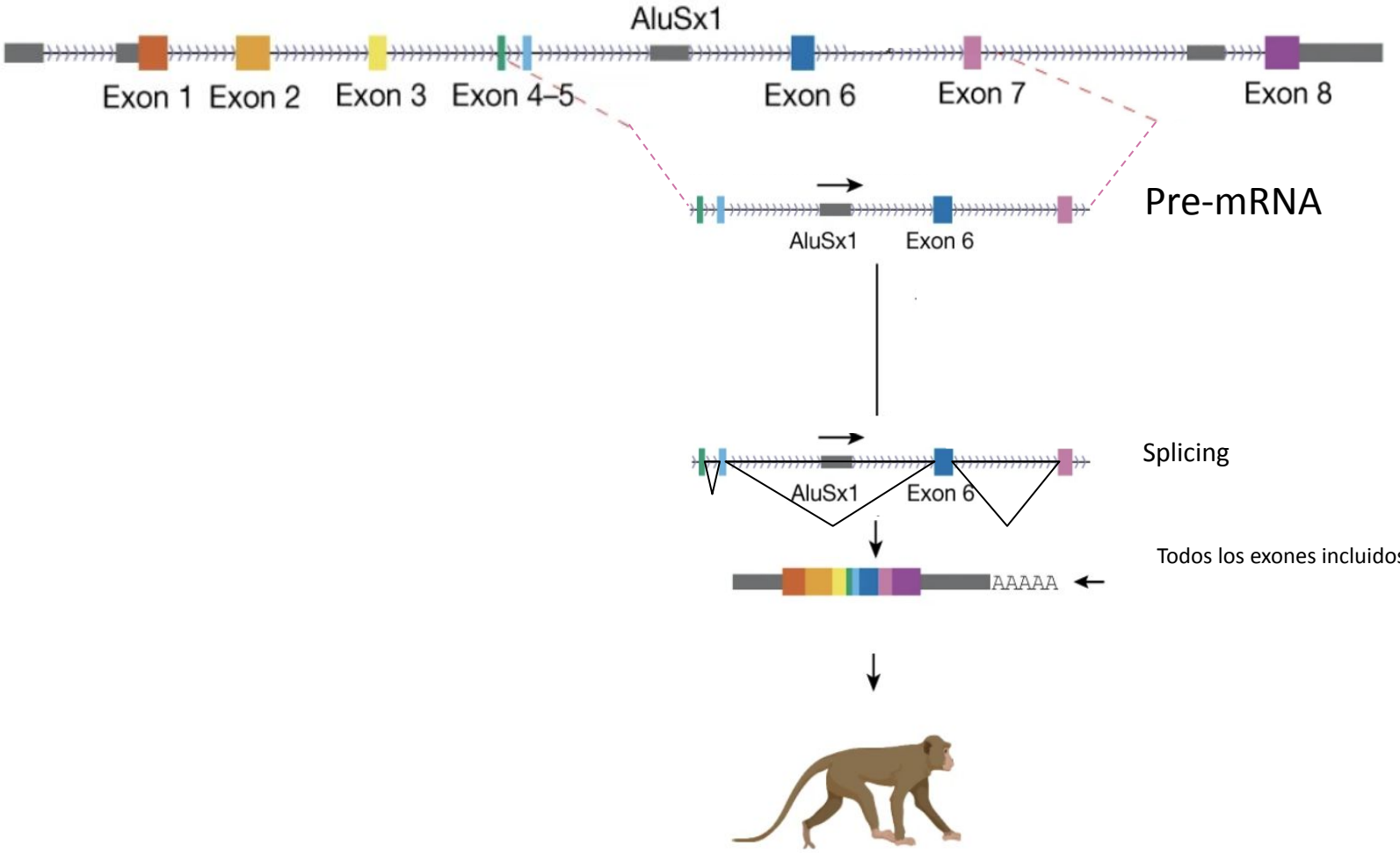
Article | Open access | Published: 28 February 2024

On the genetic basis of tail-loss evolution in humans and apes

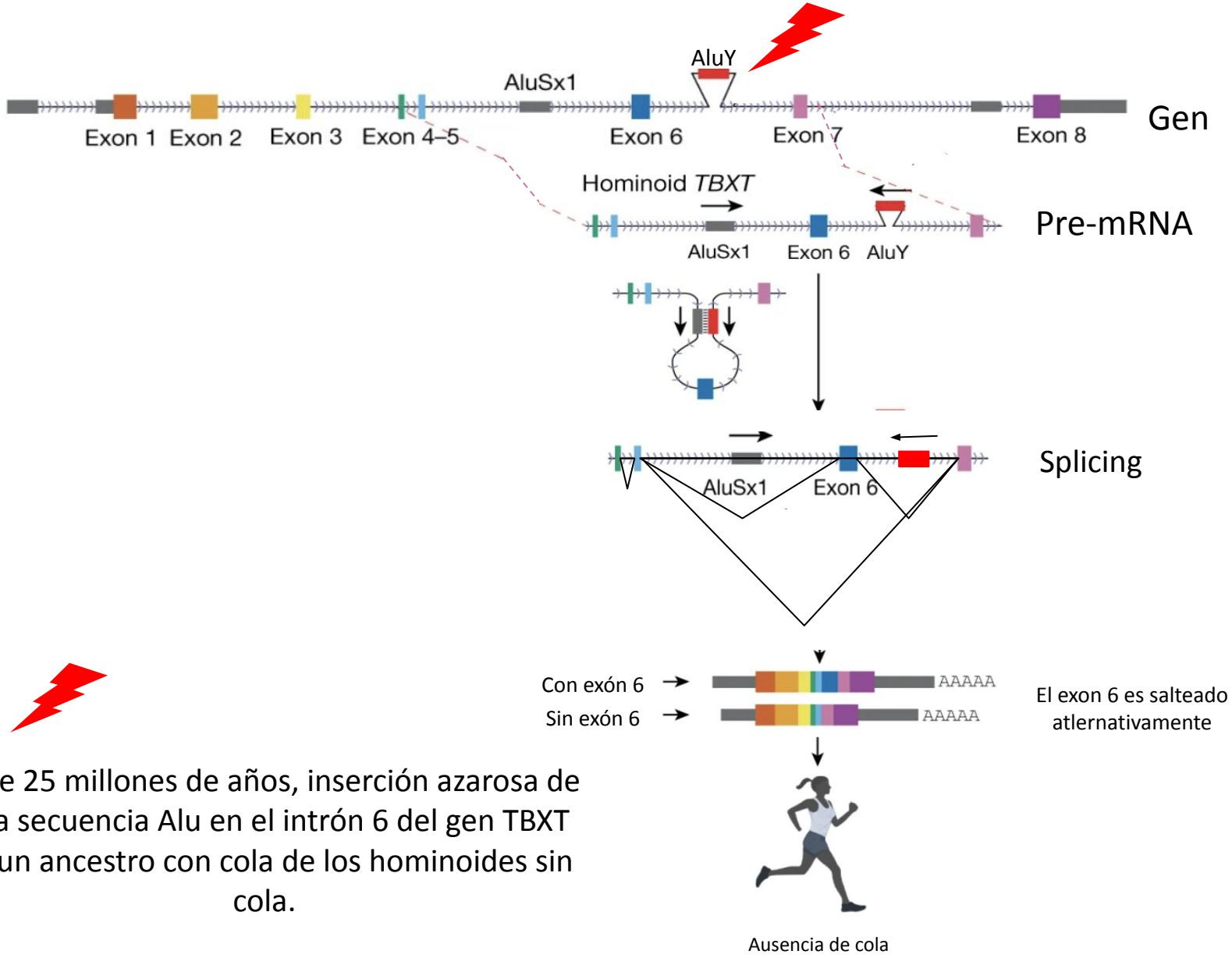
Bo Xia, Weimin Zhang, Guisheng Zhao, Xinru Zhang, Jiangshan Bai, Ran Brosh, Aleksandra Wudzinska, Emily Huang, Hannah Ashe, Gwen Ellis, Maayan Pour, Yu Zhao, Camila Coelho, Yinan Zhu, Alexander Miller, Jeremy S. Dasen, Matthew T. Maurano, Sang Y. Kim, Jef D. Boeke & Itai Yanai

Nature **626**, 1042–1048 (2024) | Cite this article

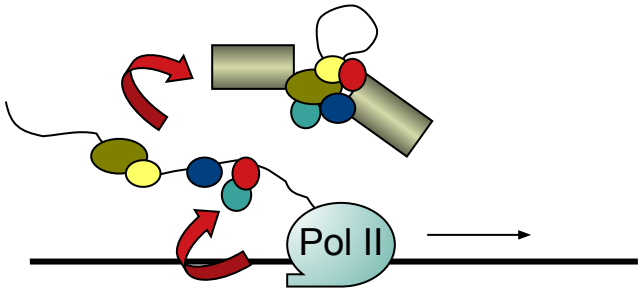
Gen TBXT en primates no hominoides



Gen TBXT en primates hominoides



Hace 25 millones de años, inserción azarosa de una secuencia Alu en el intrón 6 del gen TBXT en un ancestro con cola de los hominoides sin cola.



Splicing and alternative splicing are coupled to RNA polymerase II transcription

Cramer et al. PNAS 1997
Cramer et al. Molecular Cell 1999

Modes of coupling

changes in pol II elongation rate

(kinetic coupling)

and/or

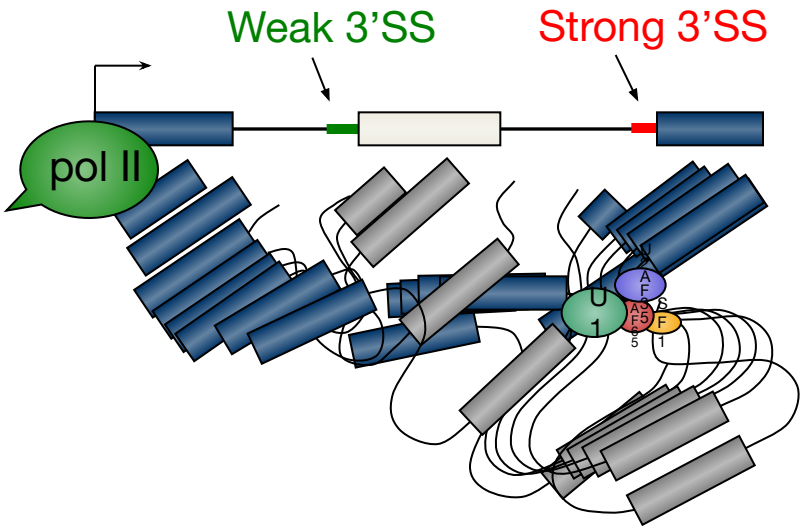
recruitment of processing factors to RNA polymerase II (CTD), chromatin or nascent RNA

(recruitment coupling)

Slow elongation, higher exon inclusion

First come, first served

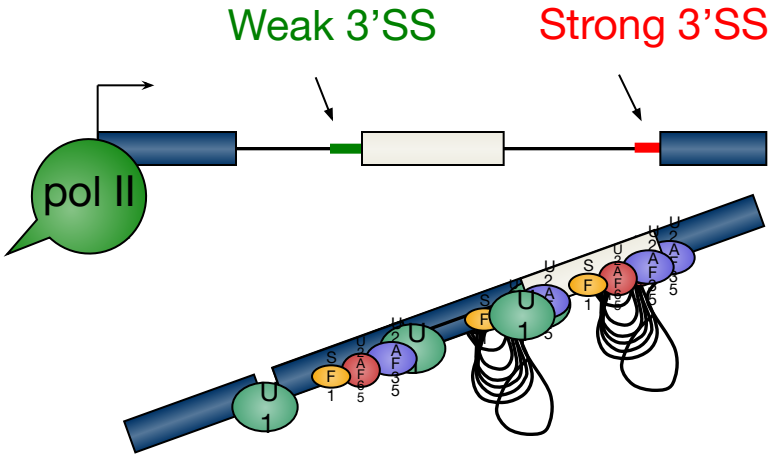
Fast elongation/no pauses



Exclusion

Kadener *et al.* EMBO J. 2001
Nogués *et al.* JBC 2002
de la Mata *et al.* Mol. Cell 2003
Fededa *et al*. Mol. Cell 2005
Alló *et al.* NSMB 2009
Muñoz *et al.* Cell 2009
de la Mata *et al.* RNA 2010
Dujardin *et al.* Mol. Cell 2014

Slow elongation/ with pauses



Inclusion

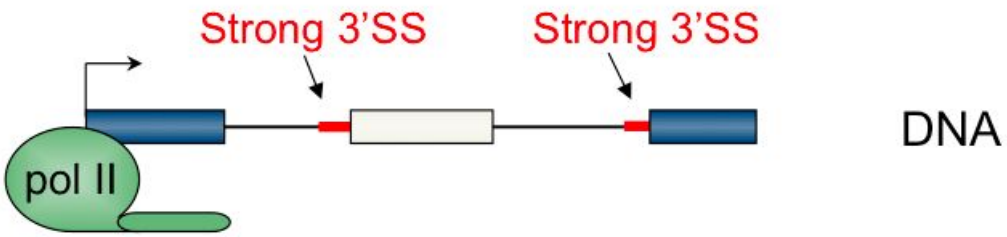
Slow elongation, higher exon inclusion

50-80% of elongation-sensitive alternative splicing events

Slow elongation, higher exon skipping

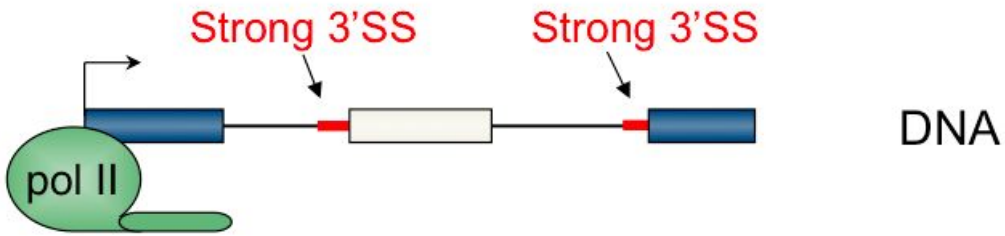
20-50% of elongation-sensitive alternative splicing events

Fast elongation



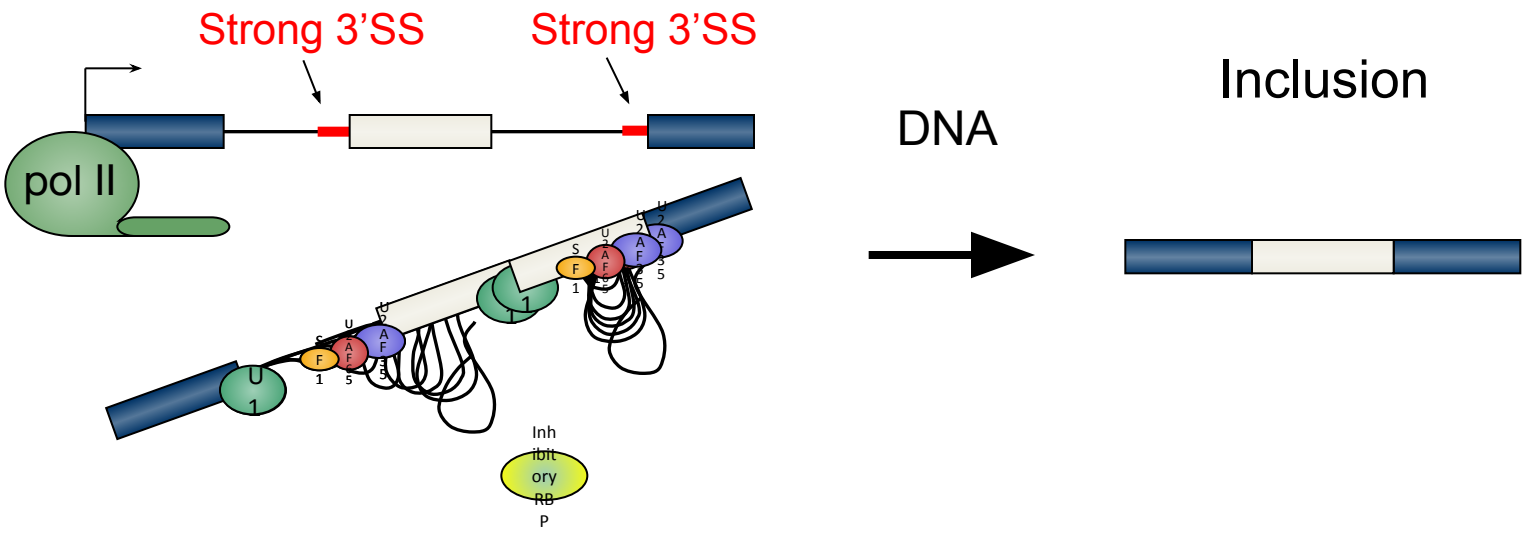
ETR-3

Slow elongation/pauses

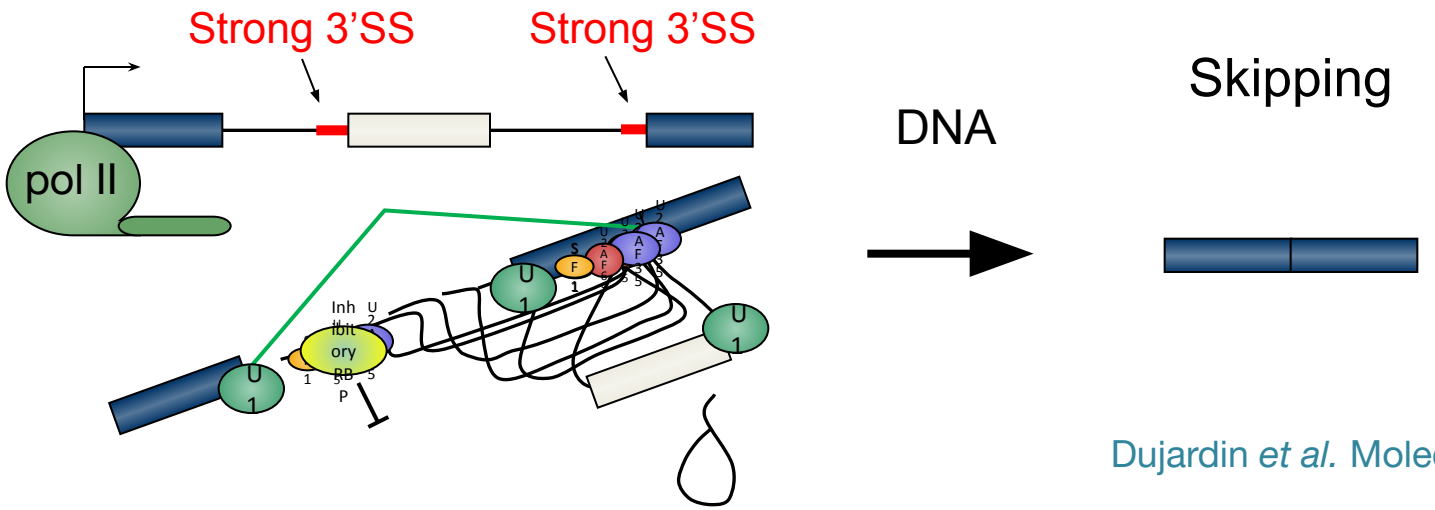


Dujardin et al. Molecular Cell 2014

Fast elongation



Slow elongation/pauses

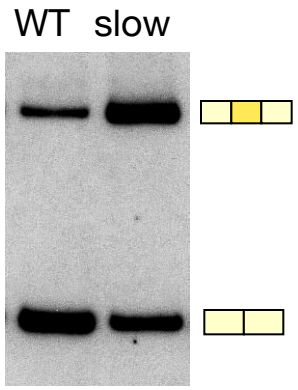


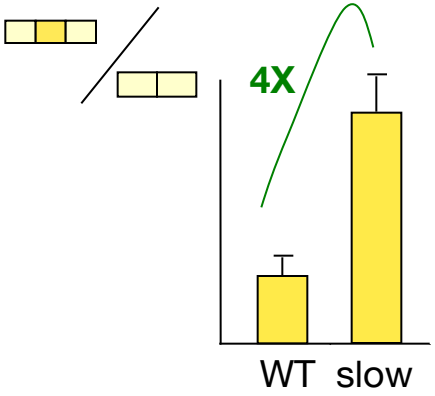
Dujardin *et al.* Molecular Cell 2014

Slow Pol II

FN E33 (EDI)

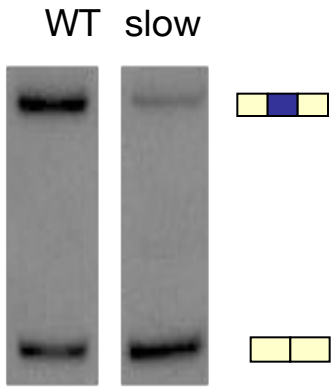
RT-PCR

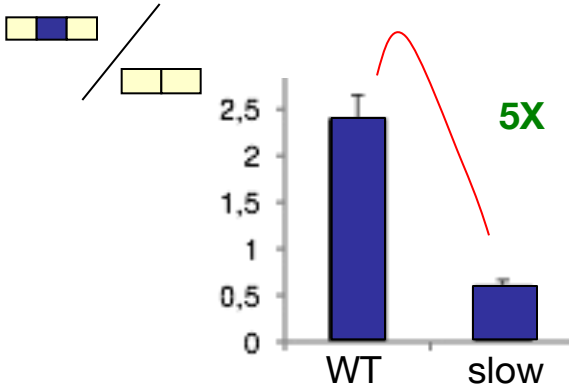




de la Mata et al. Molecular Cell 2009

CFTR E9

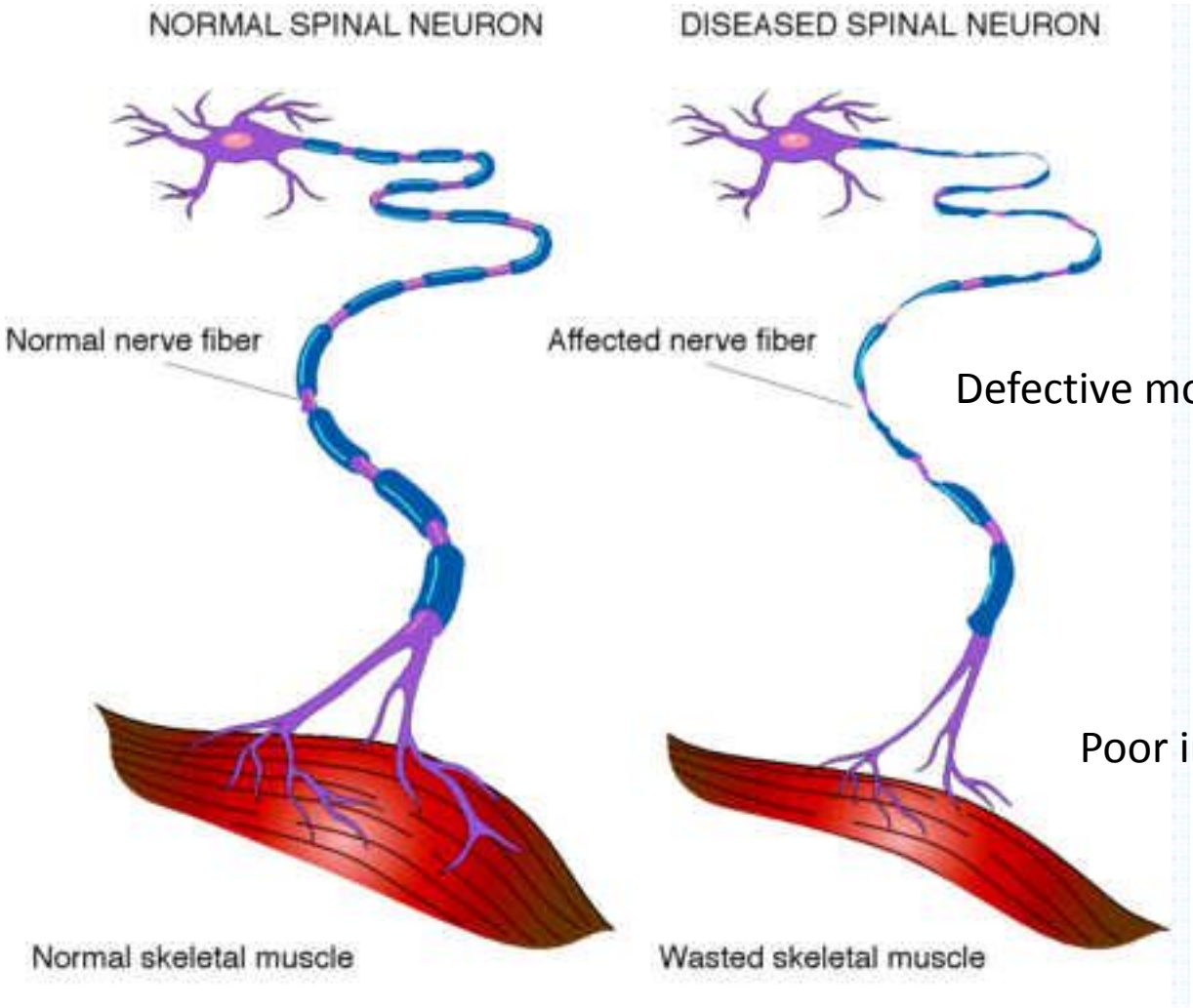




Dujardin et al. Molecular Cell 2014

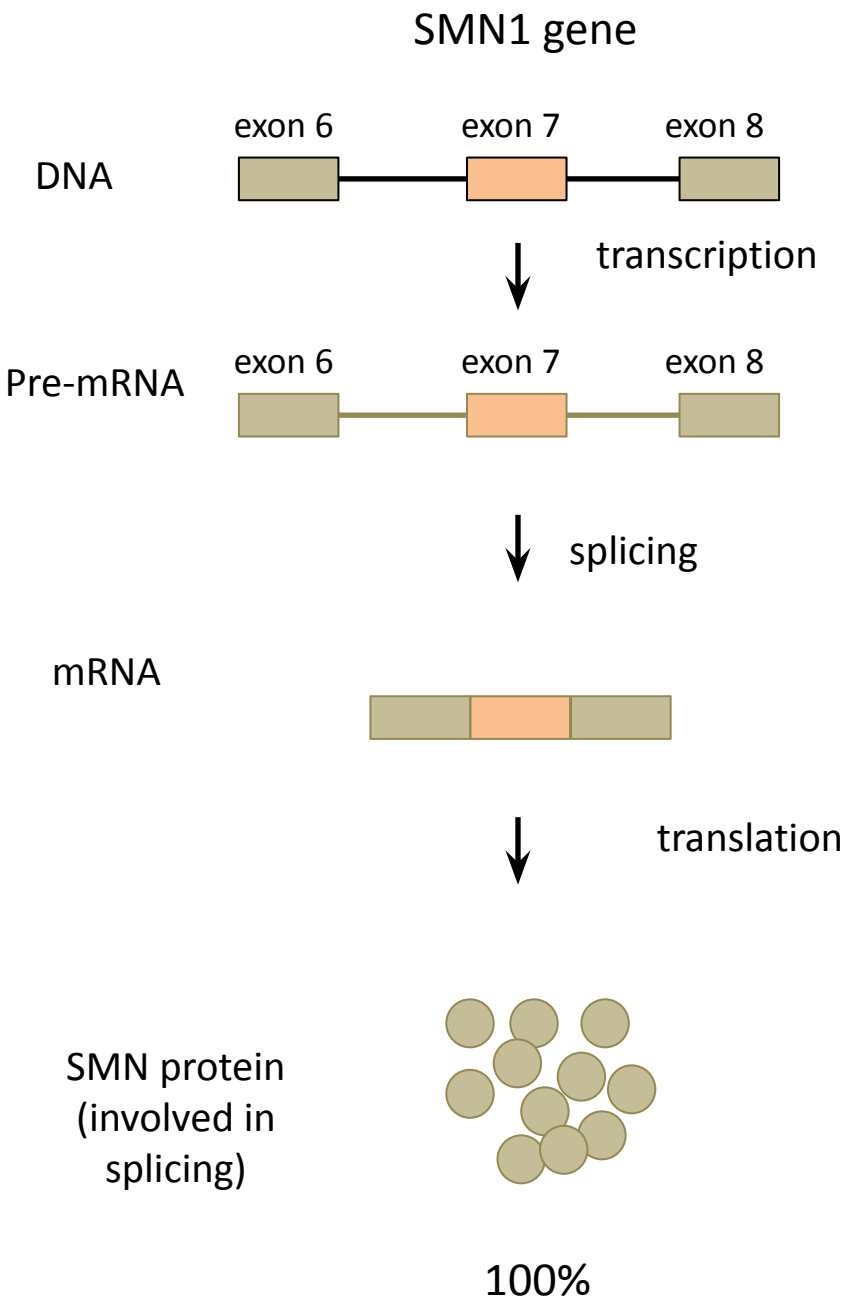
SMA = Spinal muscular atrophy

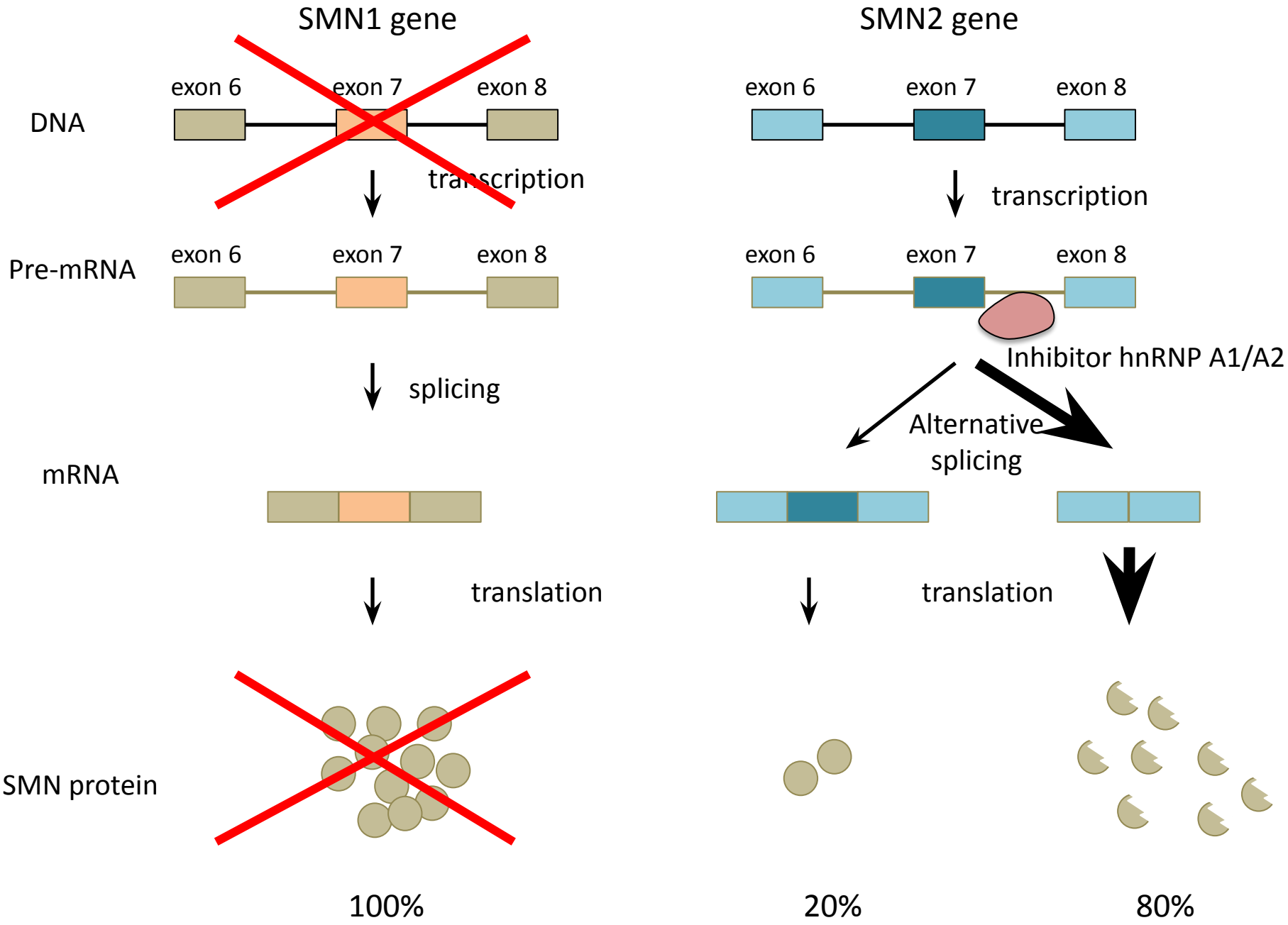
SPINAL MUSCULAR ATROPHY (SMA)
(autosomal recessive)



Defective motor neurons

Poor innervation

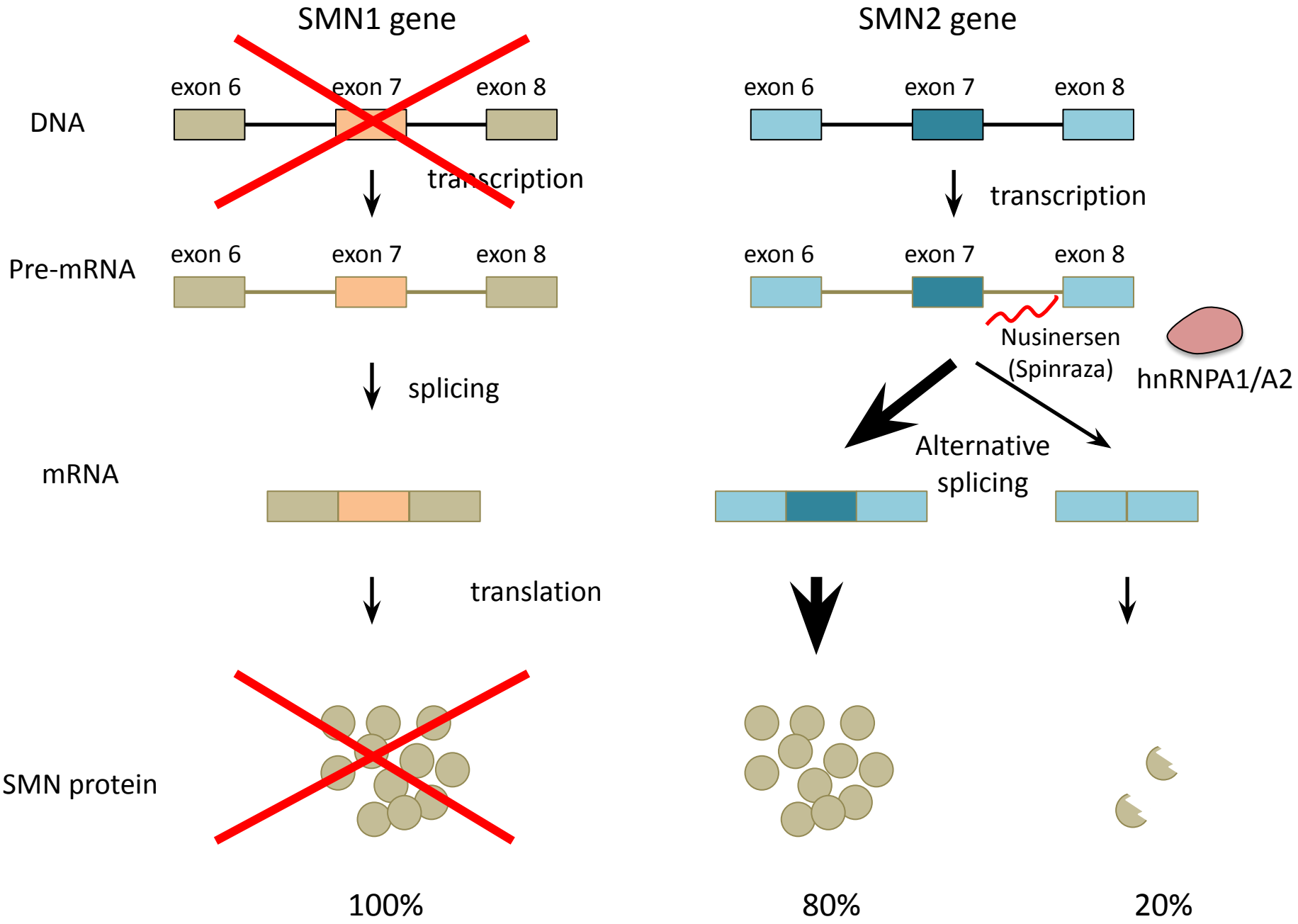

SMN1 gene
exon 6
exon 7
exon 8
DNA
transcription
Pre-mRNA
exon 6
exon 7
exon 8
splicing
mRNA
translation
SMN protein
(involved in
splicing)
100%

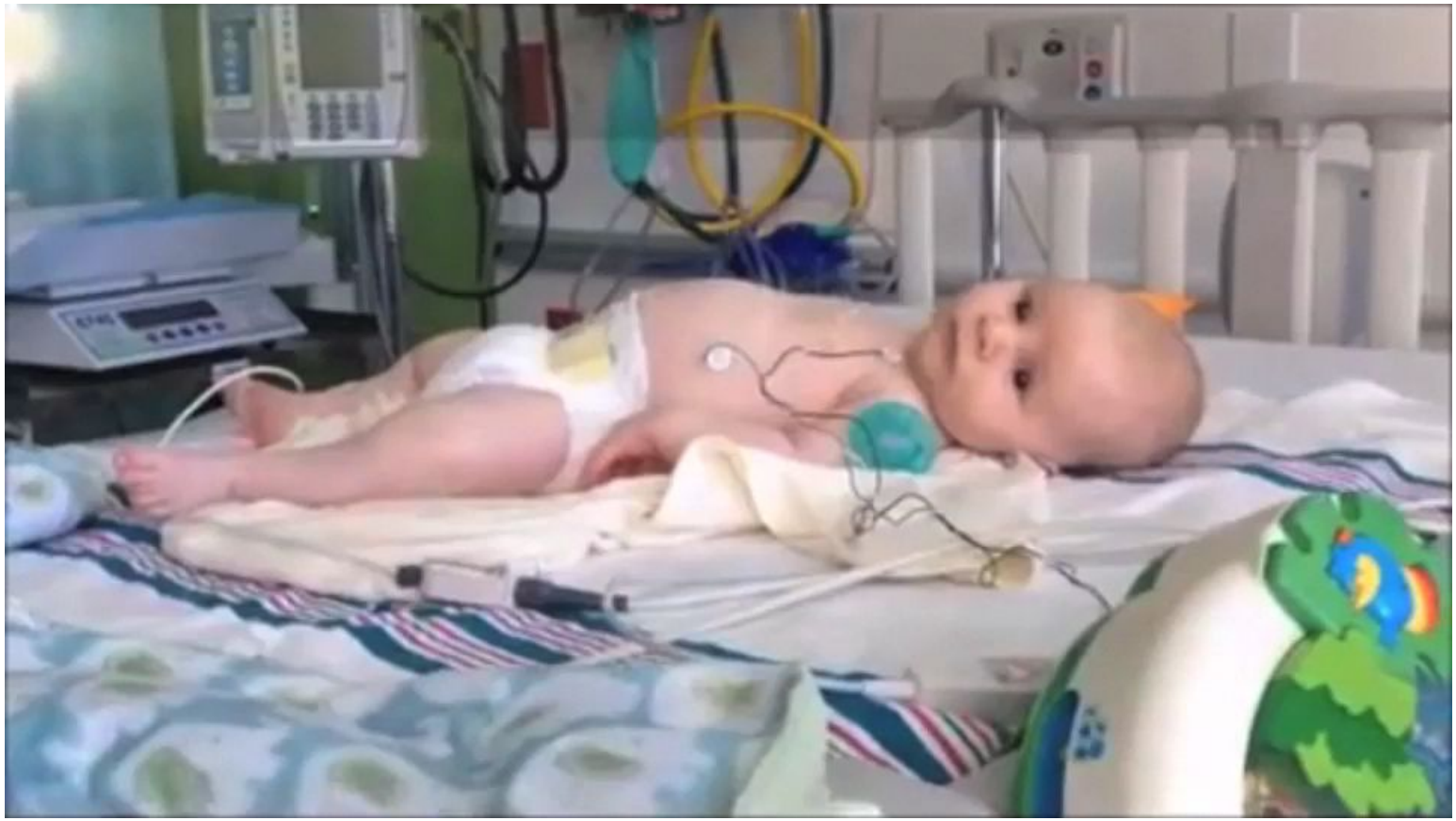

SMN1 gene
SMN2 gene
DNA
exon 6
exon 7
exon 8
transcription
Pre-mRNA
splicing
Inhibitor hnRNP A1/A2
Alternative
splicing
mRNA
translation
SMN protein
100%
20%
80%

Adrian Krainer
CSHL, New York

Spinraza (nusinersen) is an antisense oligonucleotide (ASO)

2'O (2-methoxyethyl) phosphorothyonate antisense oligonucleotide


SMN1 gene
SMN2 gene
DNA
exon 6
exon 7
exon 8
transcription
Pre-mRNA
splicing
Nusinersen
(Spinraza)
hnRNPA1/A2
Alternative
splicing
mRNA
translation
SMN protein
100%
80%
20%



Adrian Krainer
CSHL, New York

3 years old, riding a tricycle
1 year old, holding a heavier toy

Is SMN2 E7 alternative splicing controlled by Pol II elongation?

Spinraza = nusinersen

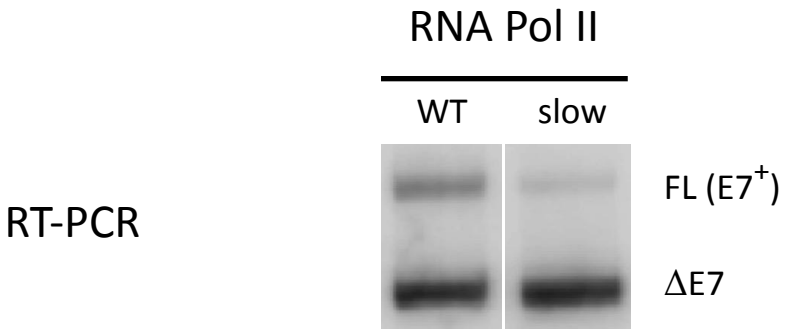
ccagcattattgaaagtga

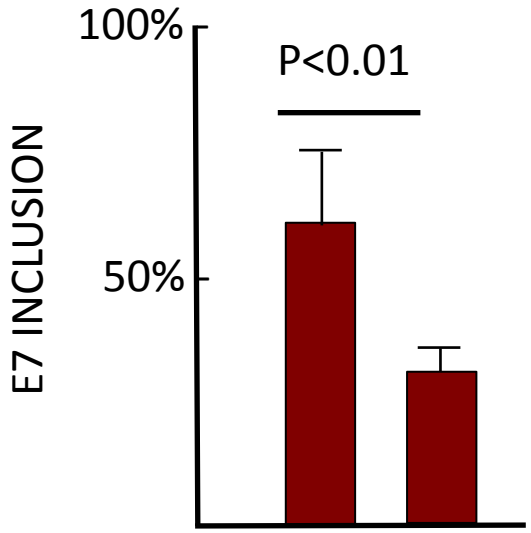
ASO1

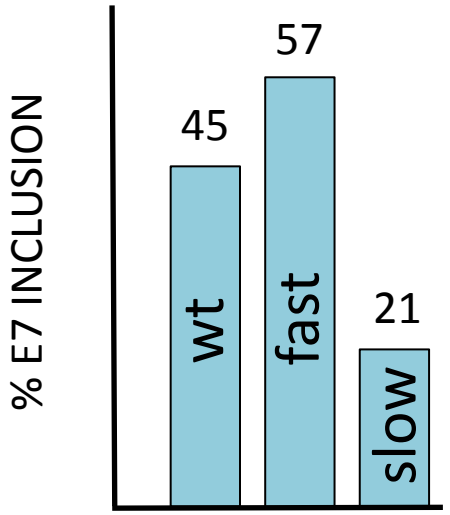
ccagcattattgaaagtgaat

SMN2 exon 7 is skipped when elongation is slow

HEK293 cells







Bentley lab: Fong et al. 2014

SMN2 exon 7 is a class II exon

Marasco *et al.*, Cell 2022

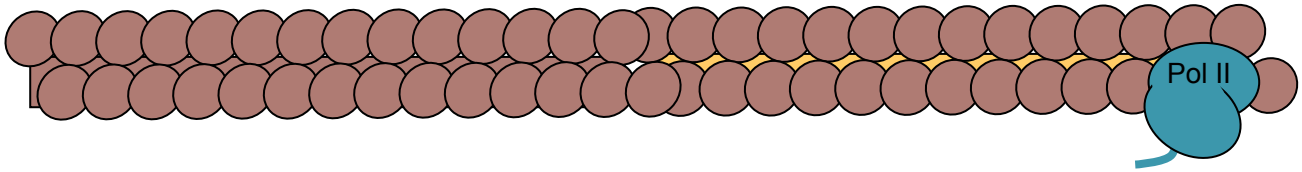
SMN2 E7 is a type II exon

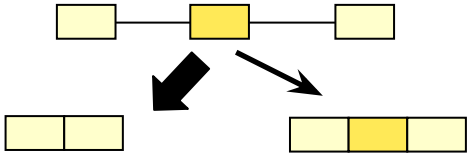
Slow elongation, higher exon skipping

Marasco *et al.*, Cell 2022

Chromatin opening should increase SMN2 E7 inclusion by promoting intragenic Pol II elongation: use of histone deacetylase inhibitors trichostatin A (TSA) or valproic acid (VPA) that, by promoting histone acetylation, will open the chromatin

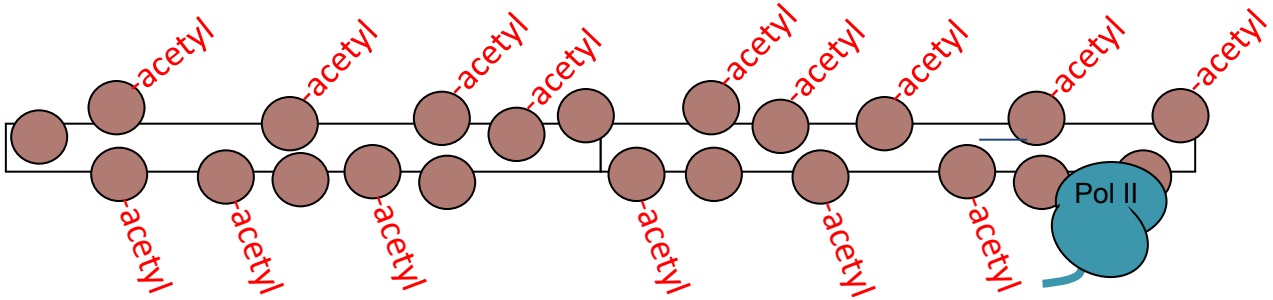
COMPACT CHROMATIN (CLOSED)

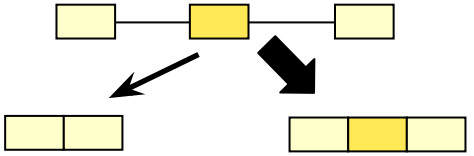




Valproic acid (VPA)
"Chromatin opener"

RELAXED CHROMATIN (OPEN)



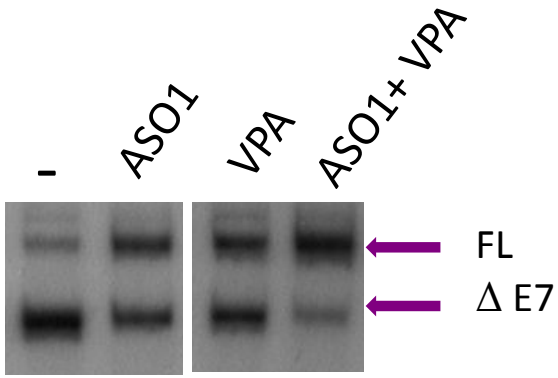


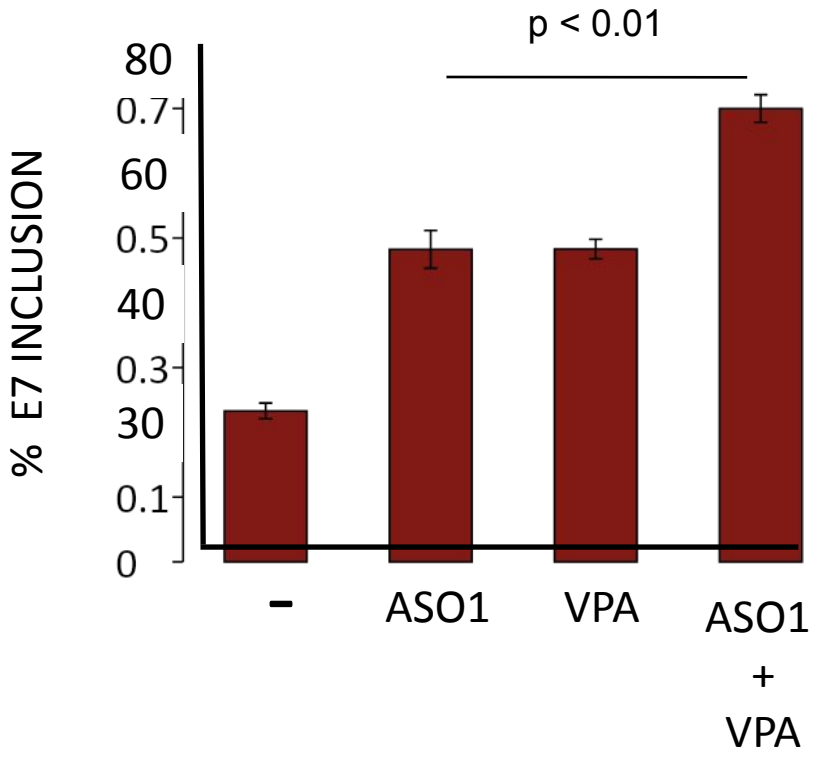
Kadener *et al.*, EMBO J. 2001
Nogués *et al.*, J. Biol. Chem. 2002

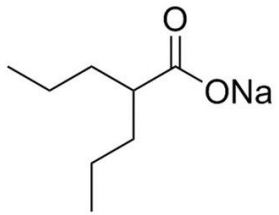
ASO1 (Spinraza) and VPA act cooperatively to promote SMN2 E7 inclusion

HEK293 cells

RT-PCR





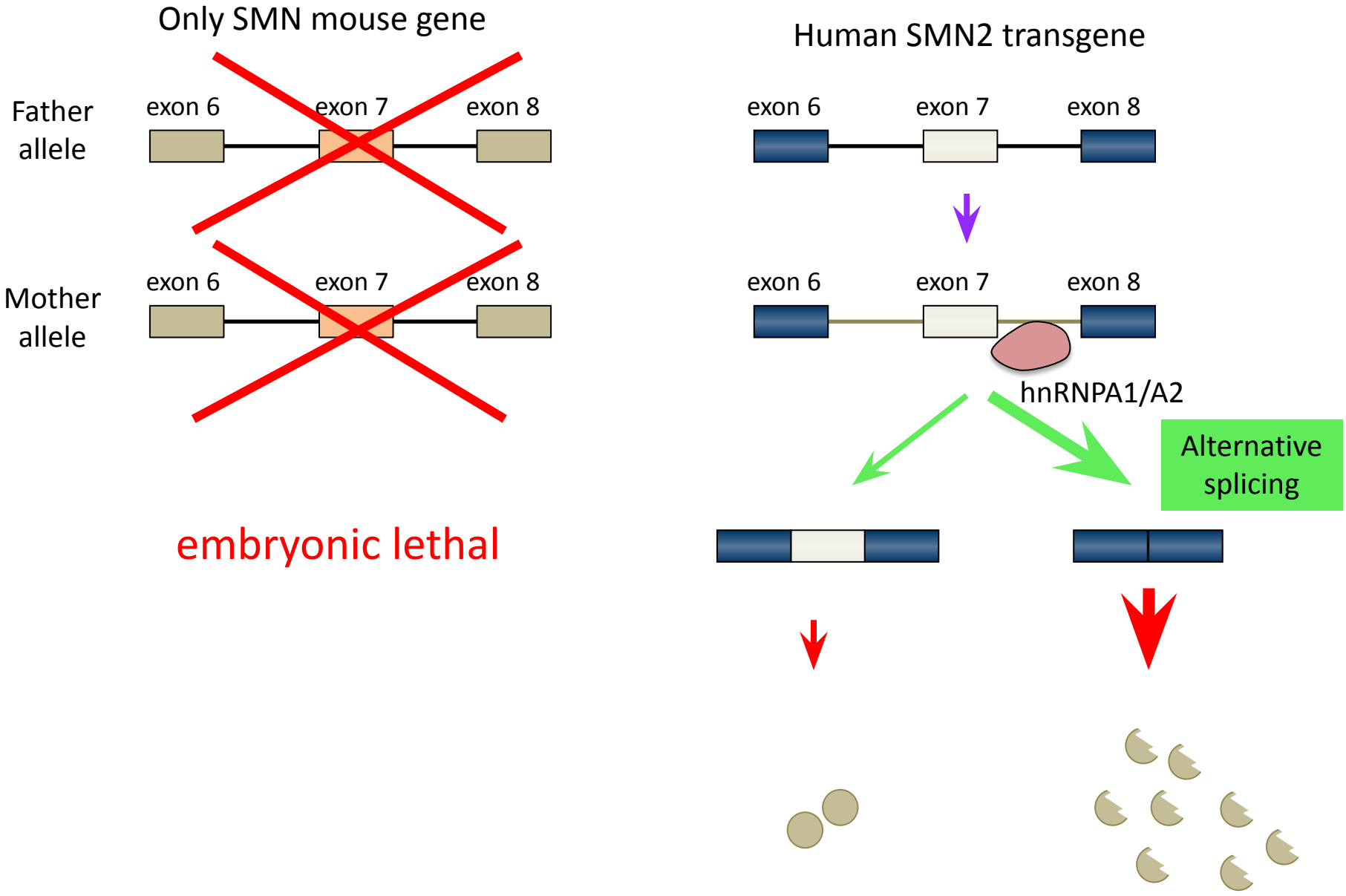


Valproic acid (VPA)
Class I and II HDACs
FDA approved
Similar results with TSA and SAHA and in patient fibroblasts

Marasco *et al.*, Cell 2022

Experiments with an SMA mouse model

(Taiwan strain)

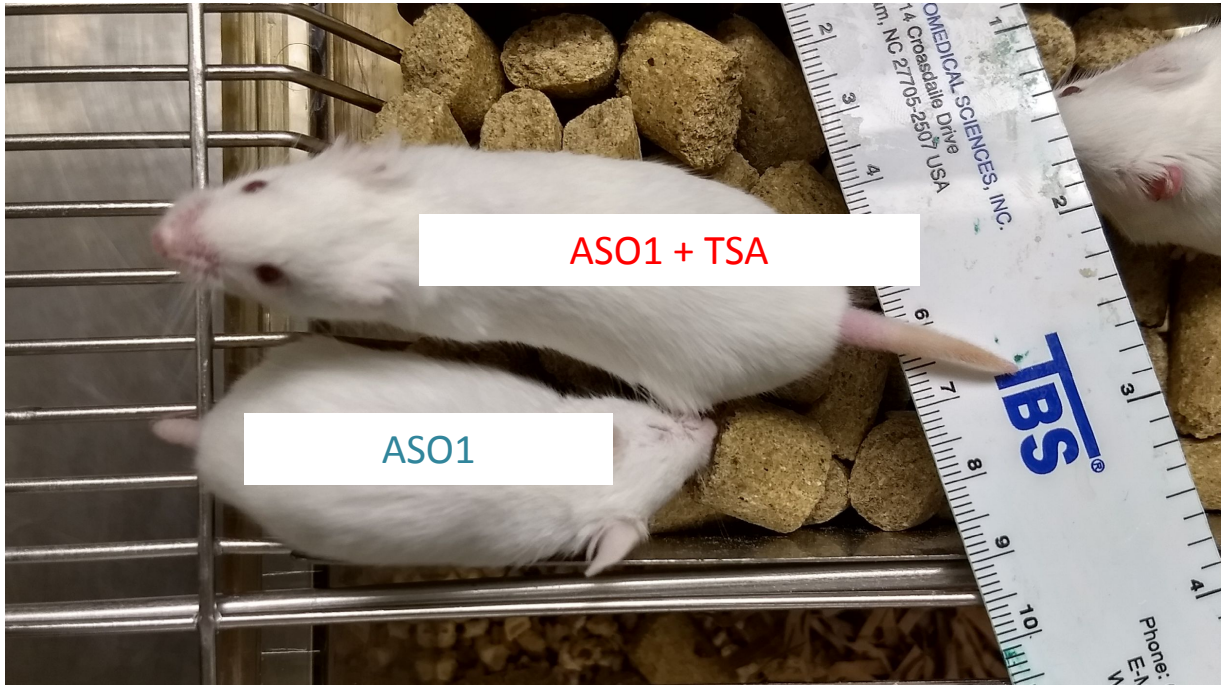


Hsieh-Li, H.M. et al. A mouse model for spinal muscular atrophy. *Nat. Genet.* **24**, 66-70 (2000).

Single subcutaneous injection of nusinersen-like ASO1 (18 µg/g) and/or HDAC inhibitor (10 µg/g) 1-2 days after birth

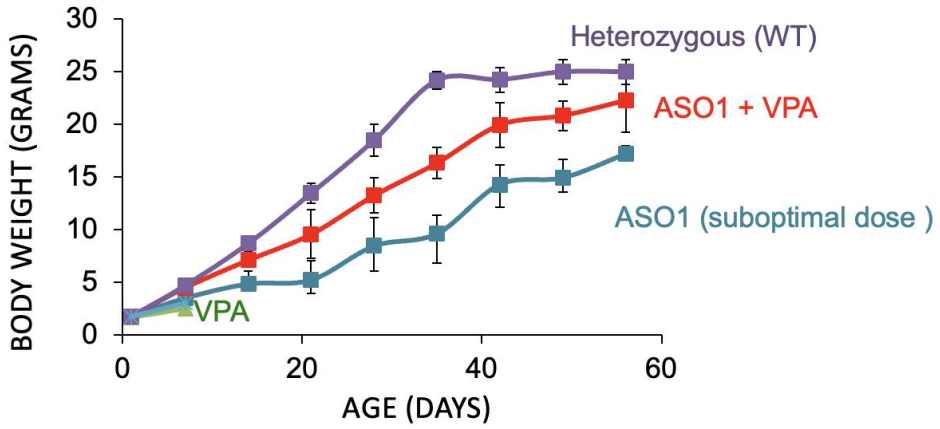
Spinraza (ASO1) and HDACs inhibitor act cooperatively in SMA mice model

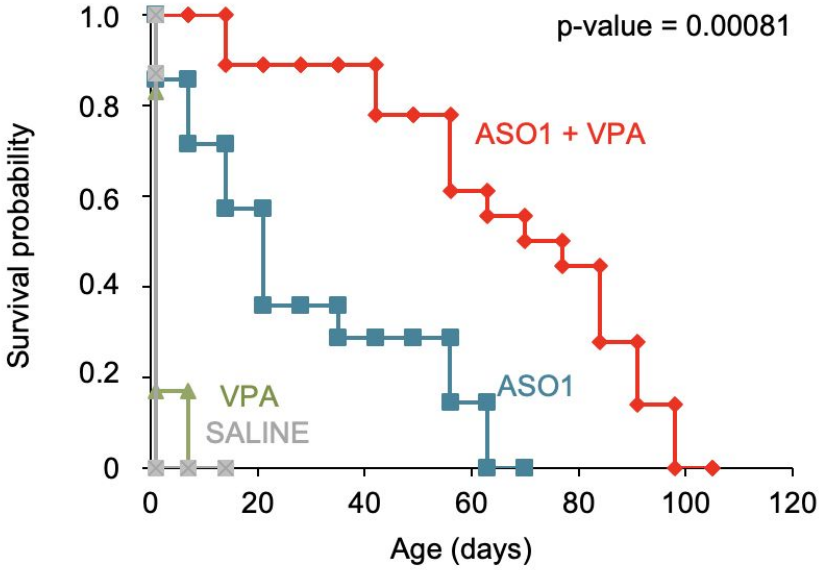
42 days old



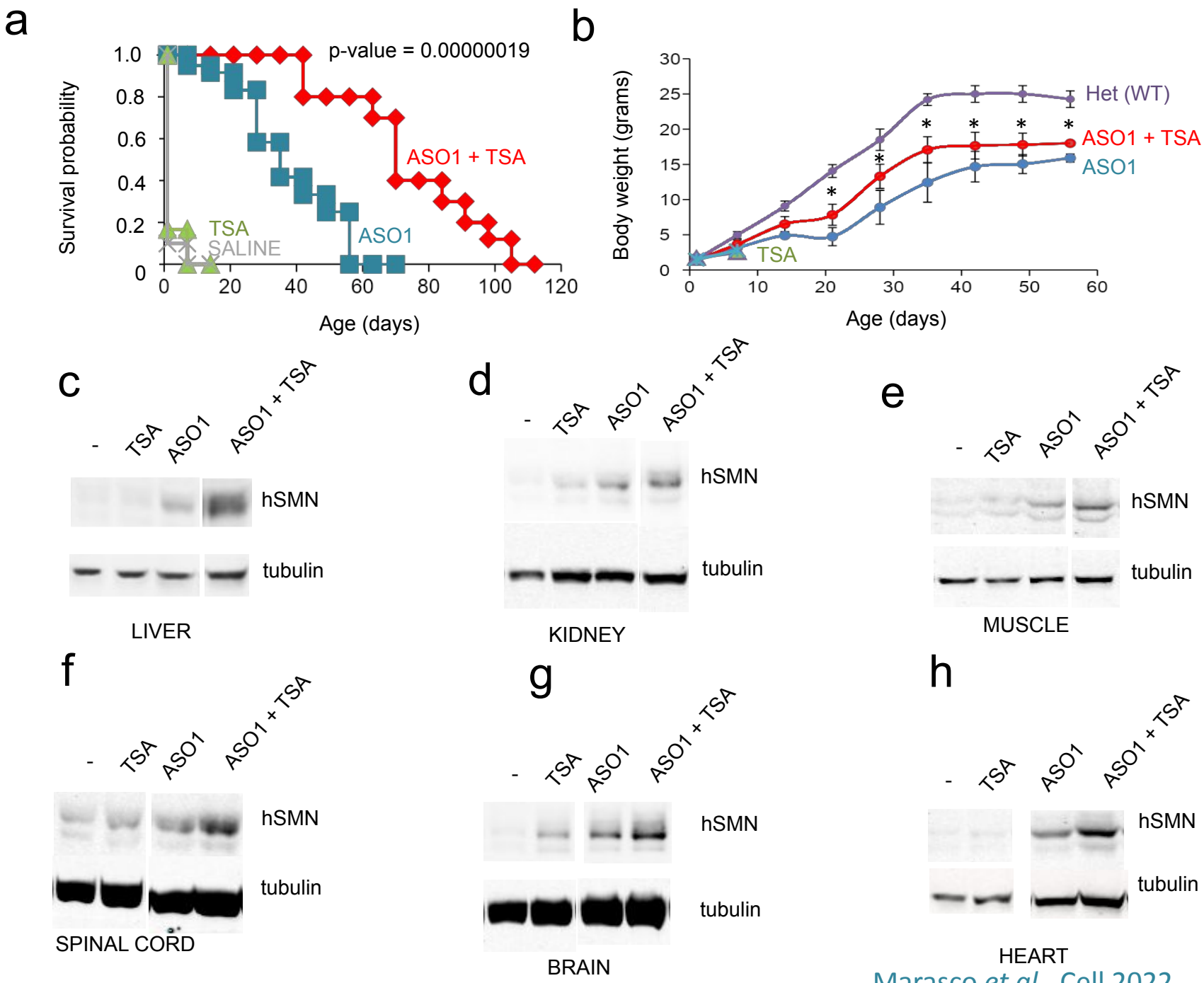
Marasco *et al.*, Cell 2022

ASO1 and VPA act cooperatively in SMA mice model





Marasco *et al.*, Cell 2022



Marasco *et al.*, Cell 2022

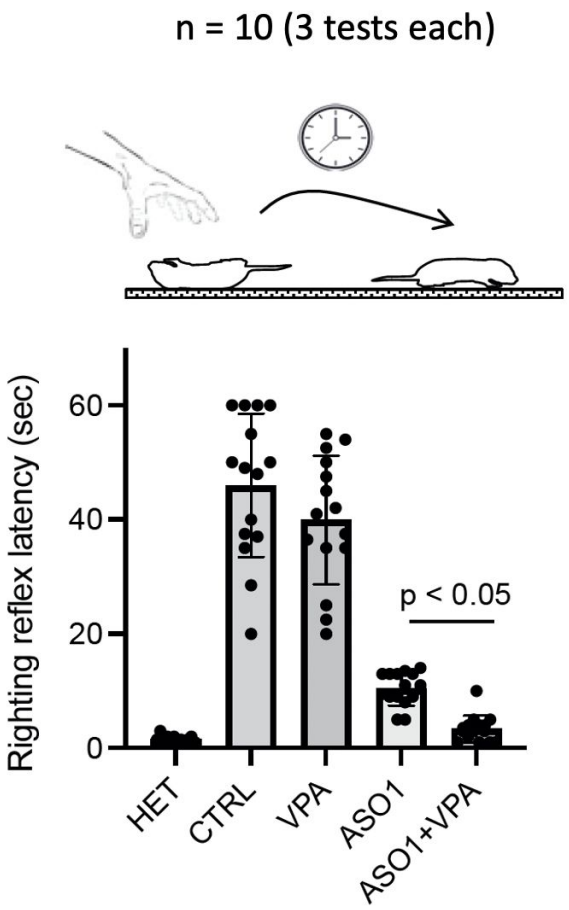
Neuromuscular function tests

Surface righting

11 days old

ASO1

ASO1 + VPA



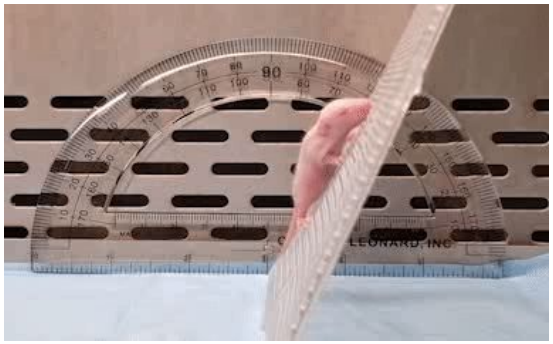
Marasco *et al.*, Cell 2022

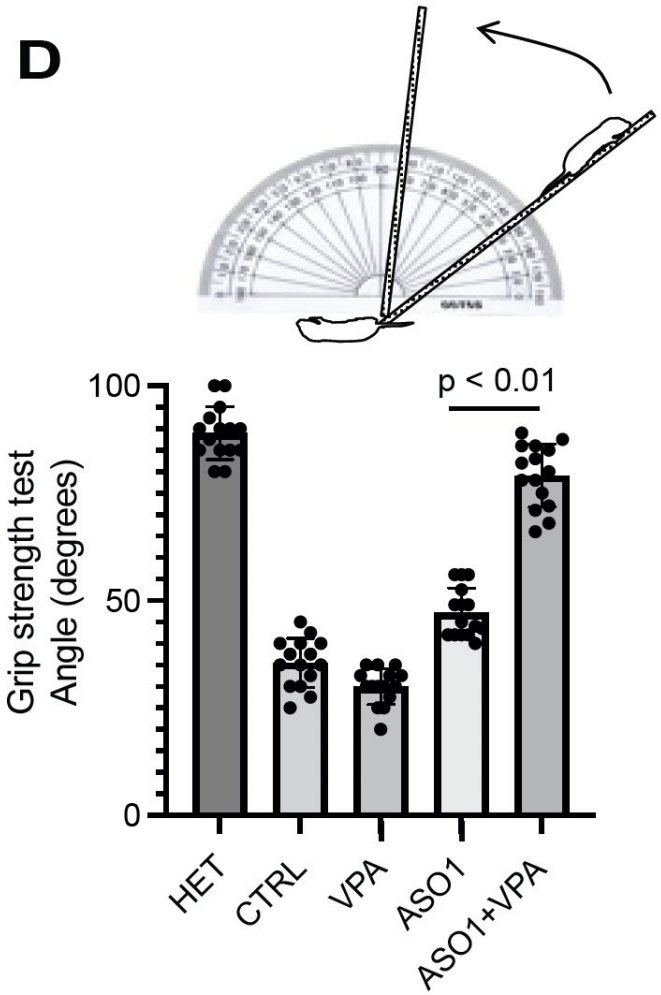
Grip strength

P7

ASO1

ASO1 + VPA



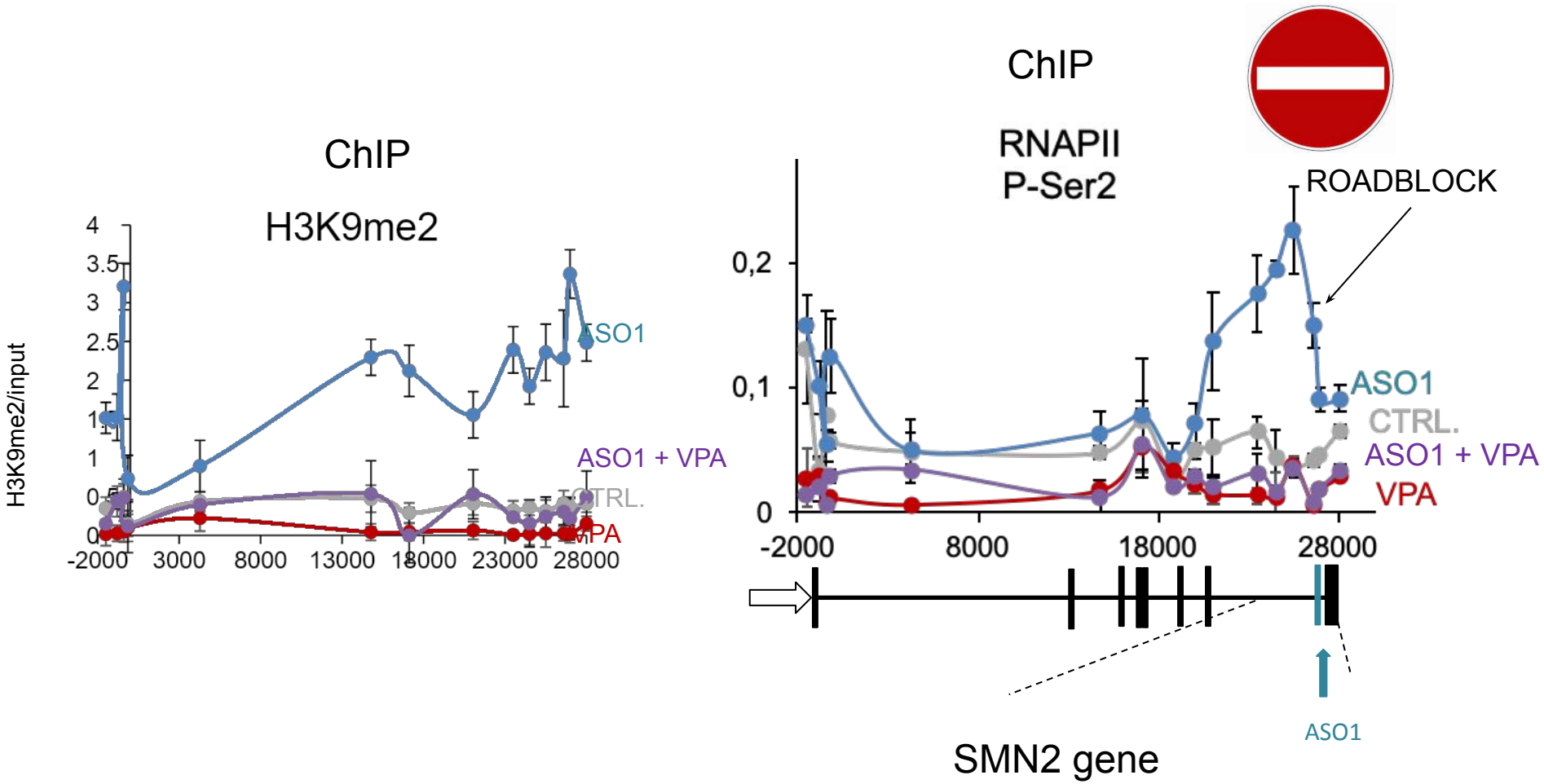


Marasco *et al.*, Cell 2022

Chromatin effects of the ASO

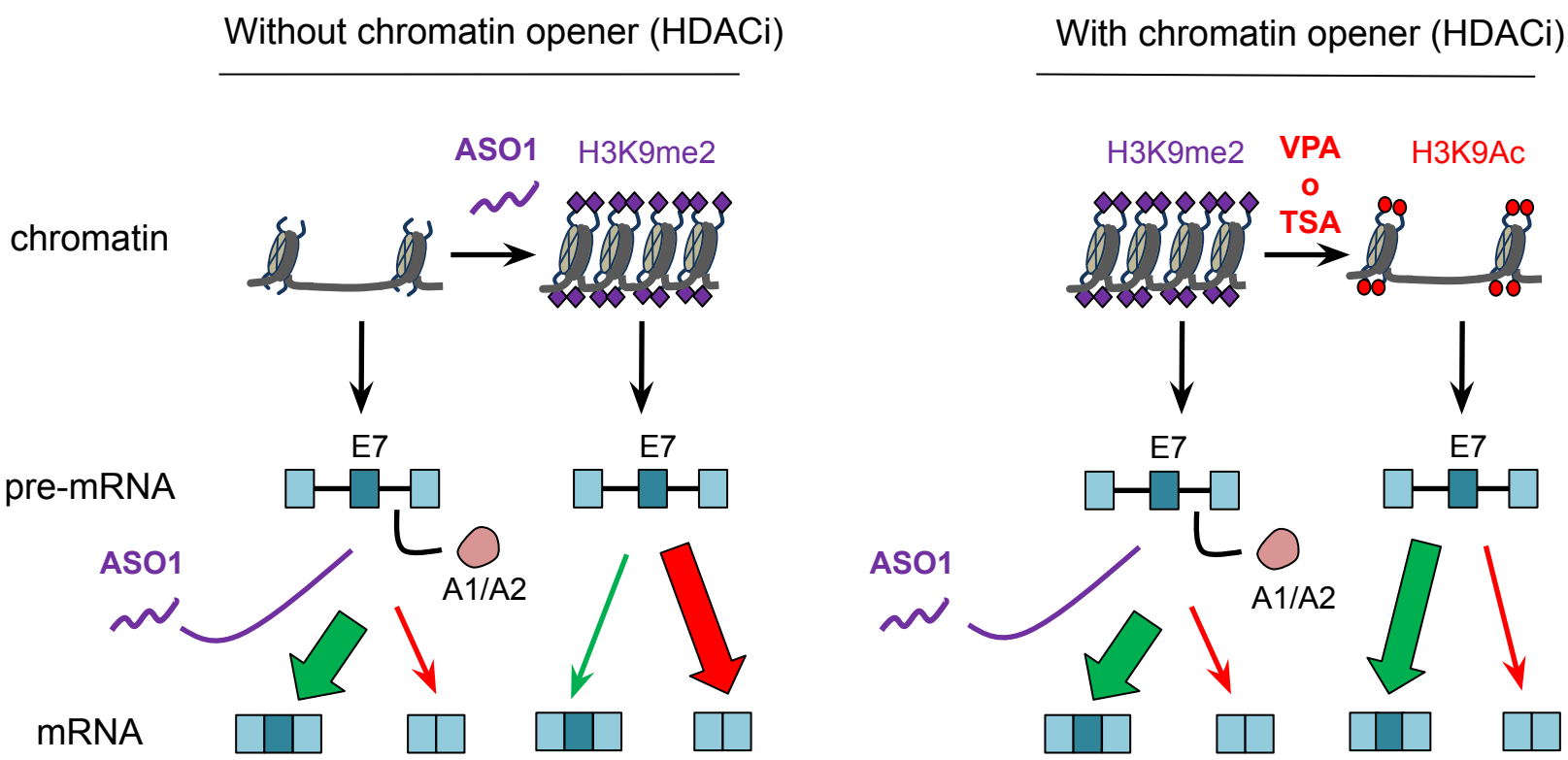
ASO1 creates a roadblock to elongation

ASO1 (nusinersen-like) creates a roadblock to PolII elongation



Marasco *et al.*, Cell 2022

ASO1 has two opposite effects

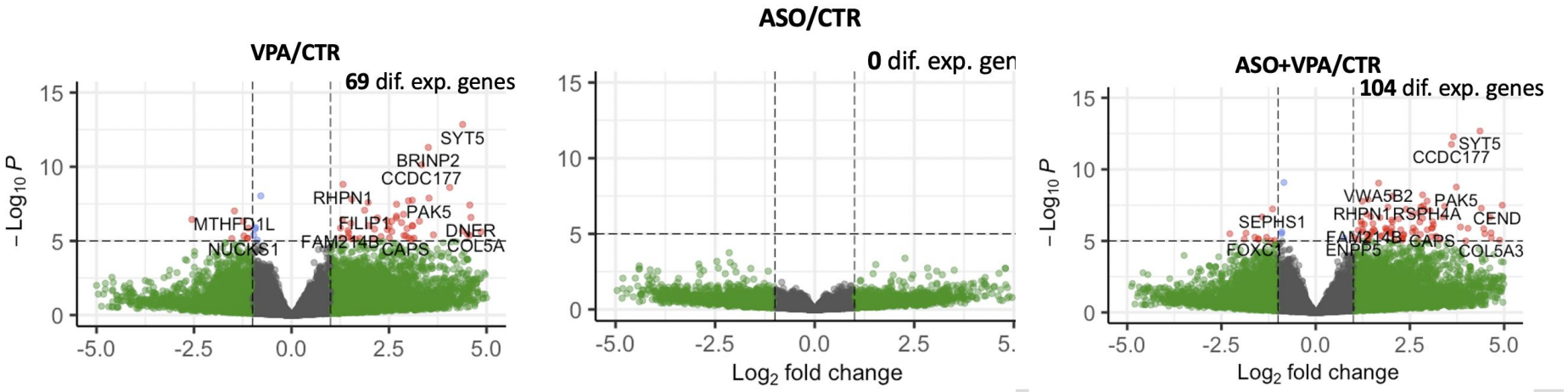


Marasco *et al.*, Cell 2022

How pleiotropic is VPA?

RNA-seq in HEK293 cells

RNA-seq HEK (gene expression)



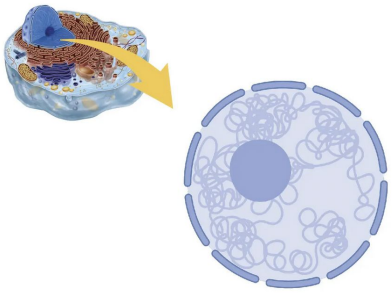
Marasco *et al.*, Cell 2022

Anyway and just in case:

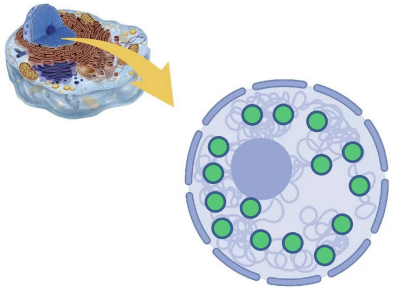
Can we target H3K9 acetylation just to the SMN2 gene?

New strategy

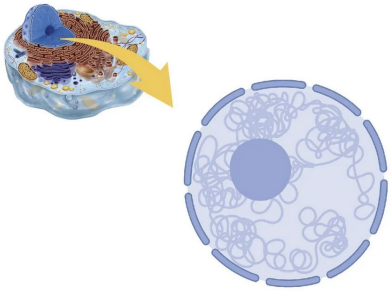
ACETYLATION = OPEN CHROMATIN



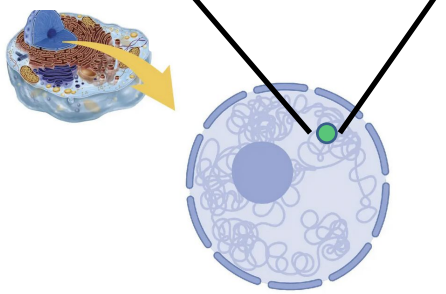
VPA



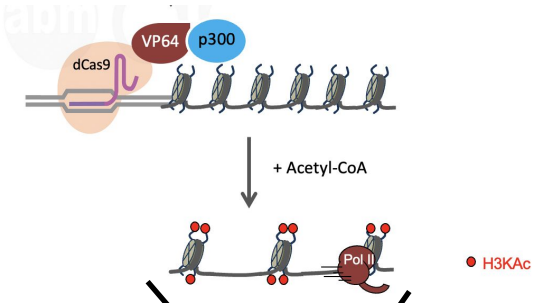
ALL GENES



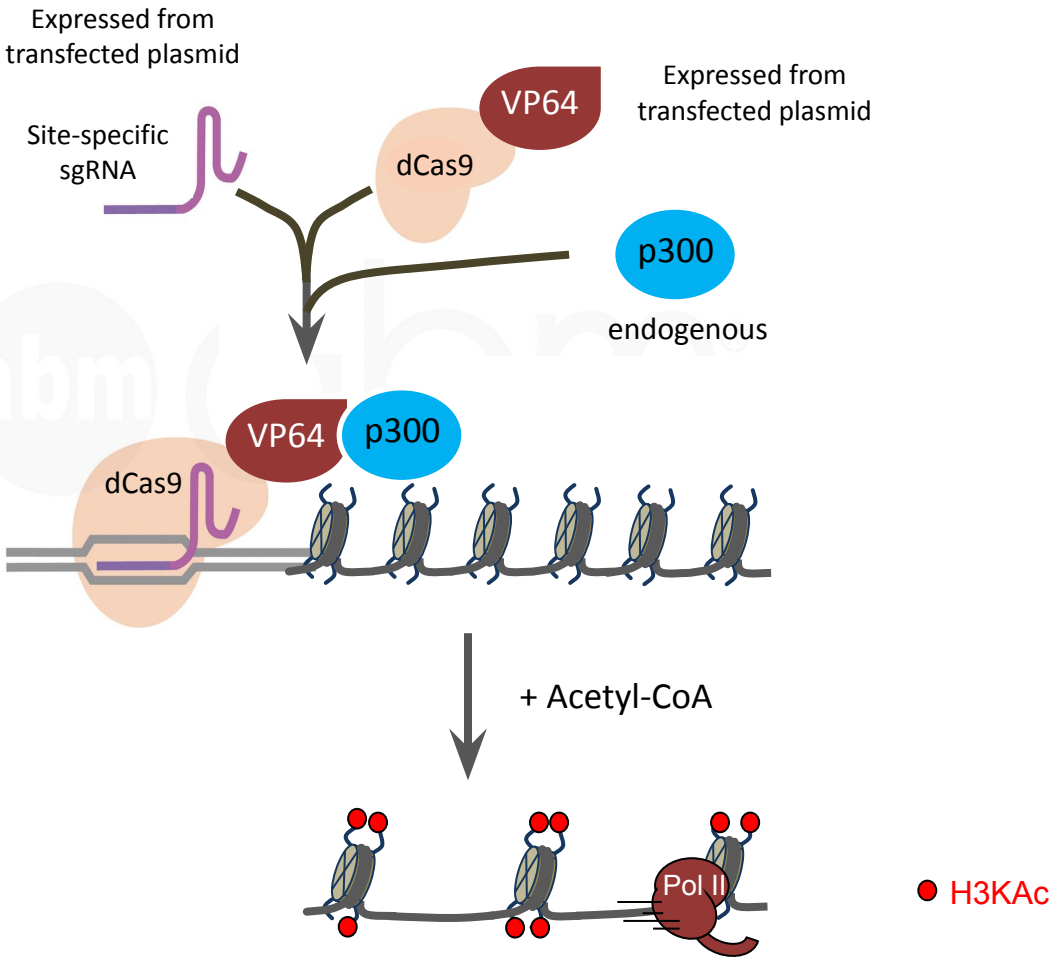
NEW CRISPR-BASED STRATEGY



ONLY *SMN1/2*

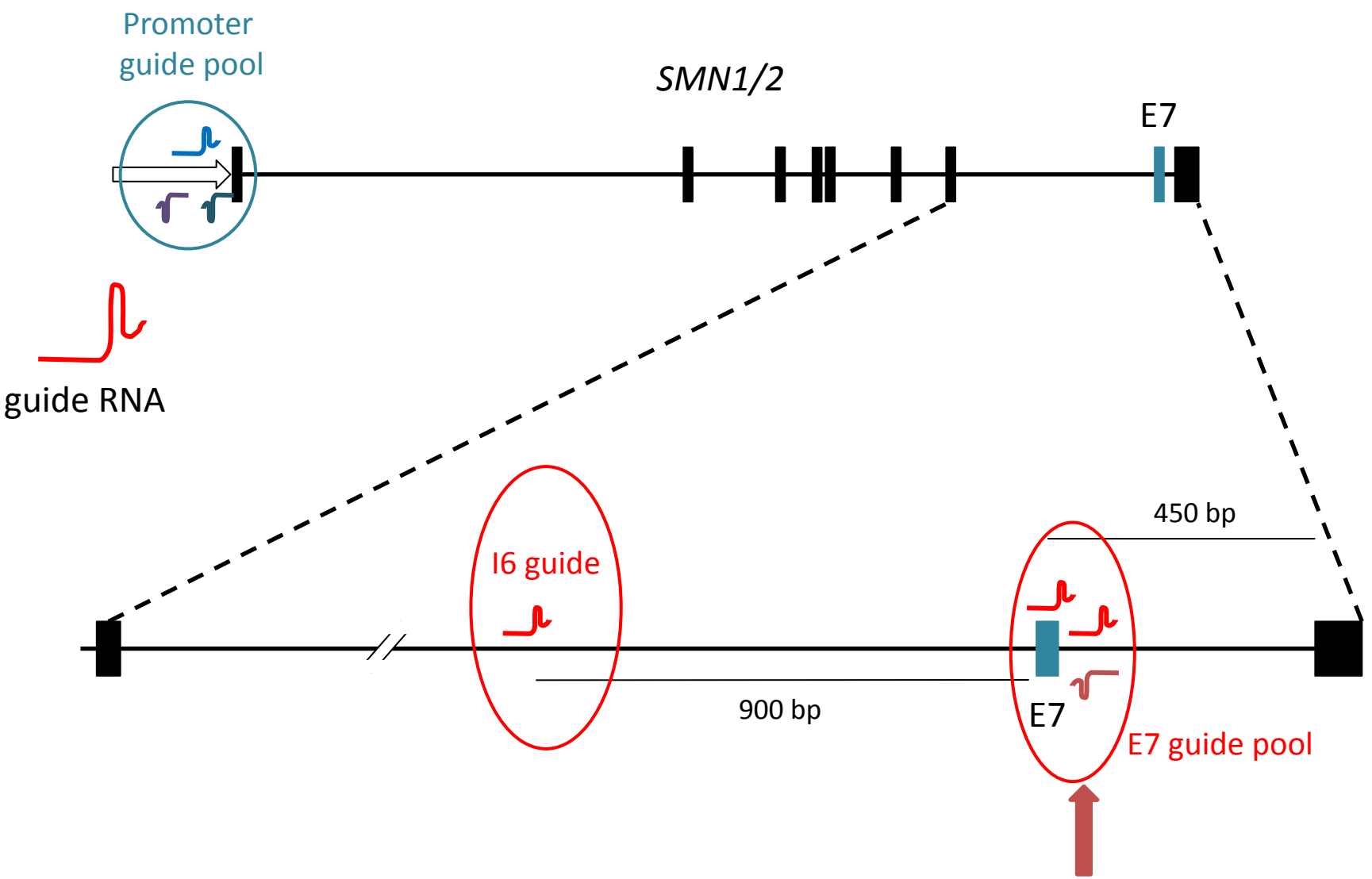


Dead Cas9 (dCas9) strategy



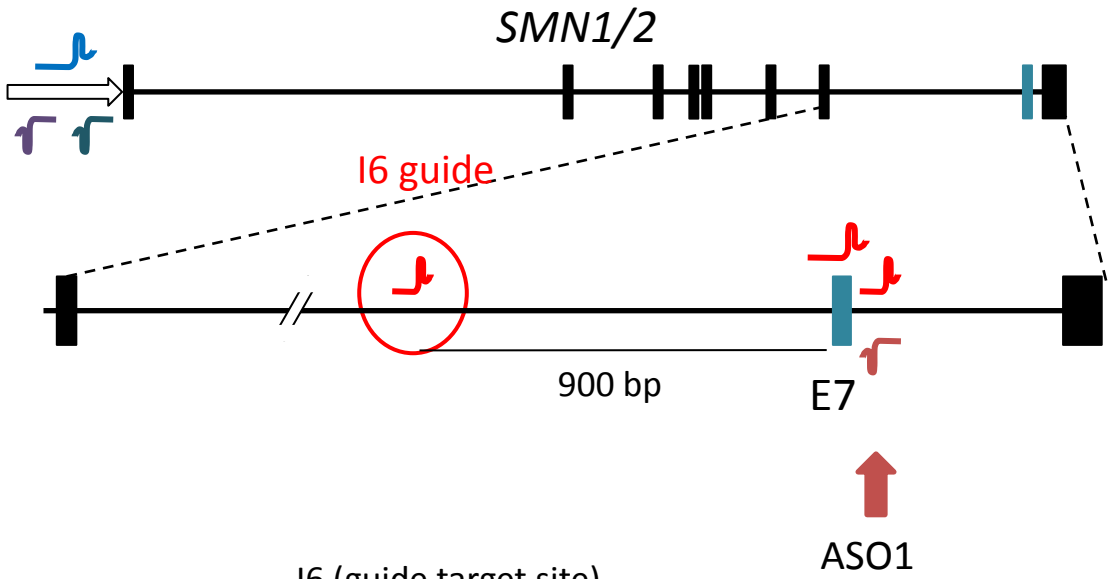
Stigliano*, Haberfeld* *et al.*, unpublished

Dead Cas9 (dCas9) strategy

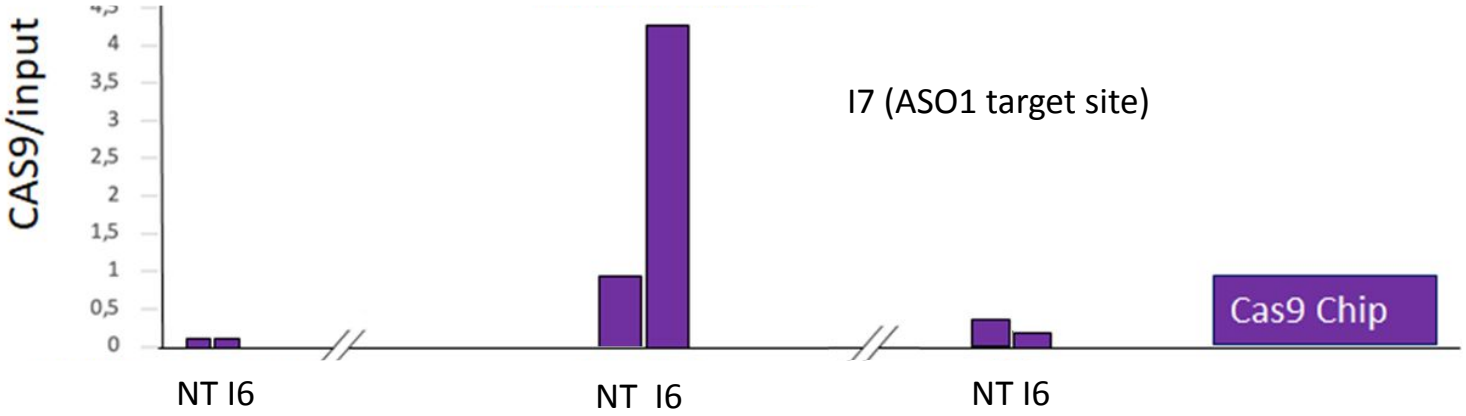


Stigliano*, Haberfeld* *et al.*, unpublished

dCAs9-VP64 is specifically recruited to the guide target site



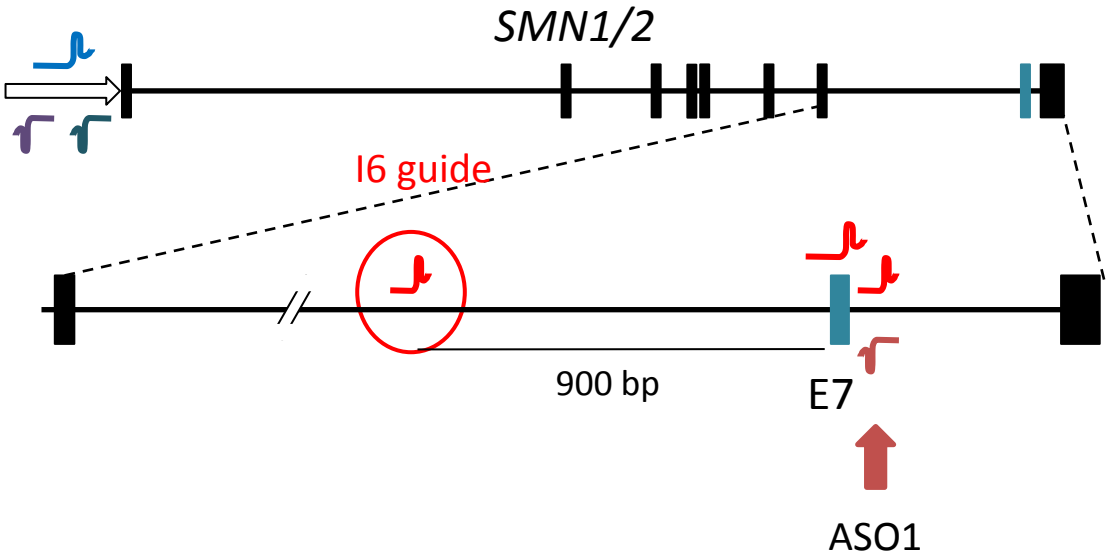
I6 (guide target site)

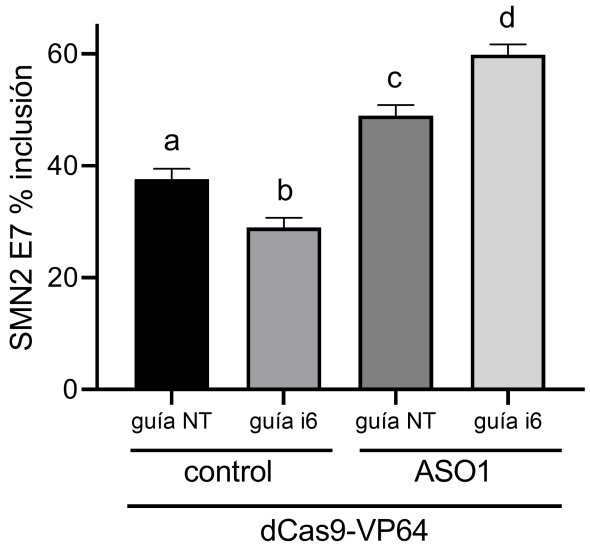


promoter

Stigliano*, Haberfeld* *et al.*, unpublished

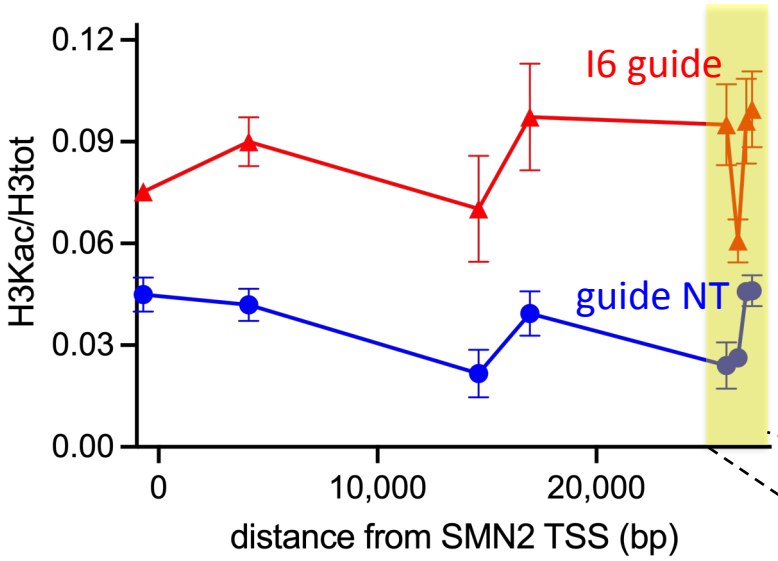
dCas9-VP64 with I6 guide cooperate with Spinraza (ASO1)

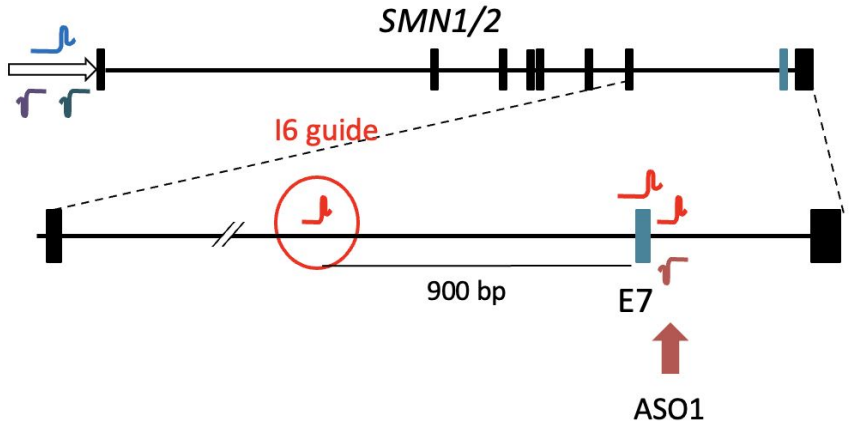




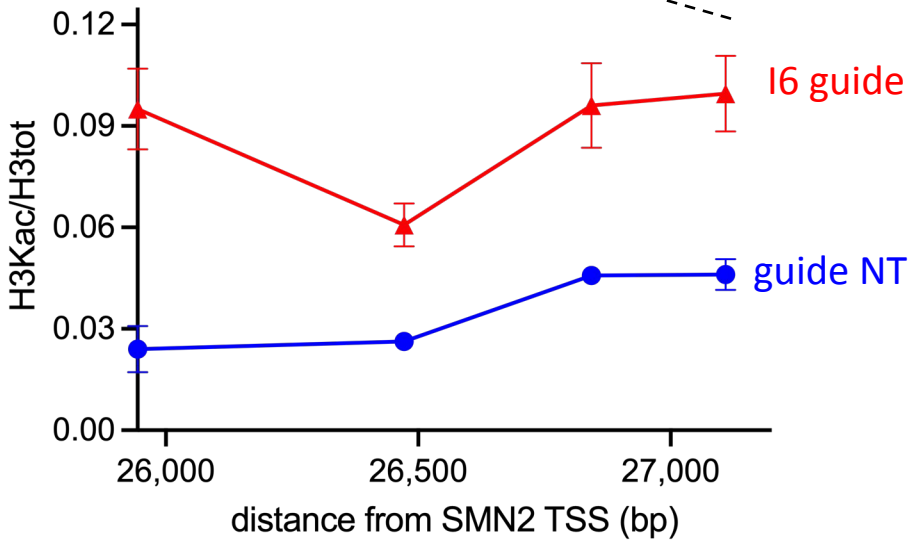
Stigliano*, Haberfeld* *et al.*, unpublished

Intron 6 guide promotes histone acetylation along the entire SMN1/2 gene



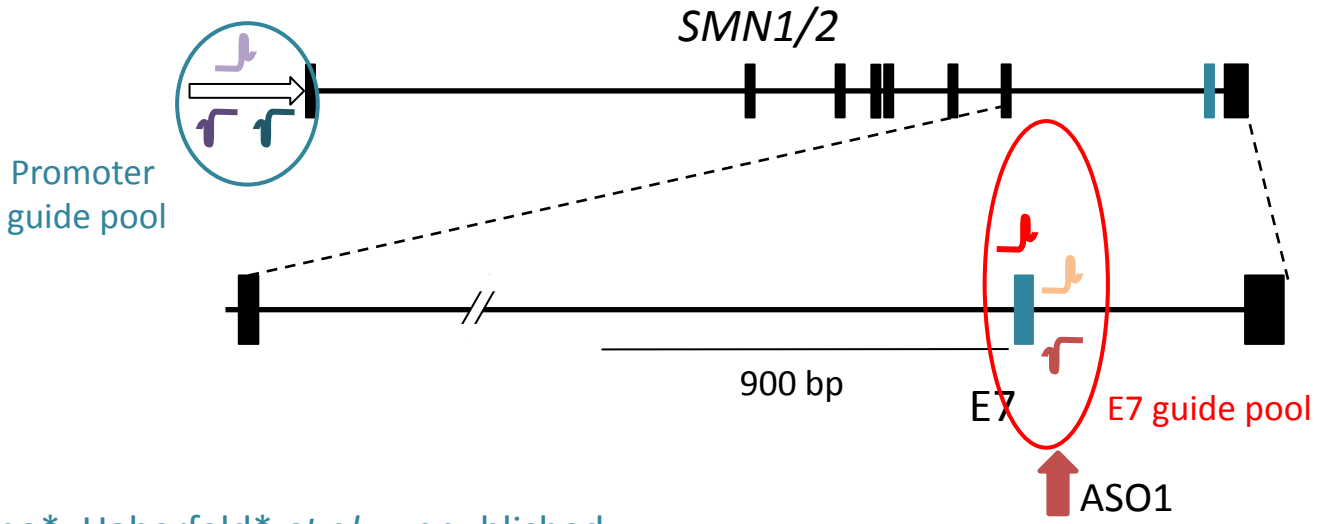


ChIP
H3Kac



Stigliano*, Haberfeld* *et al.*, unpublished

SMN2-specific acetylation cooperates with Spinraza (ASO1)

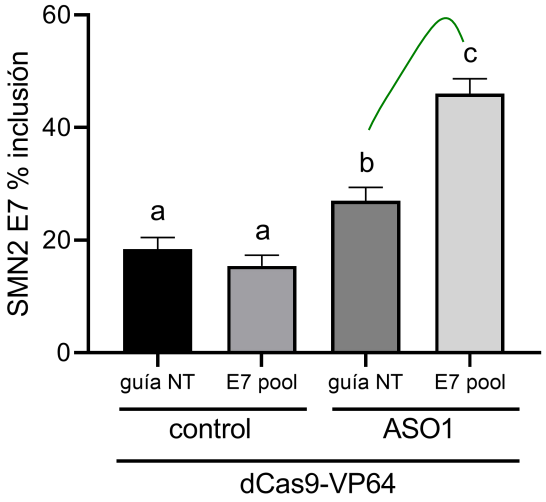


Stigliano*, Haberfeld* *et al.*, unpublished

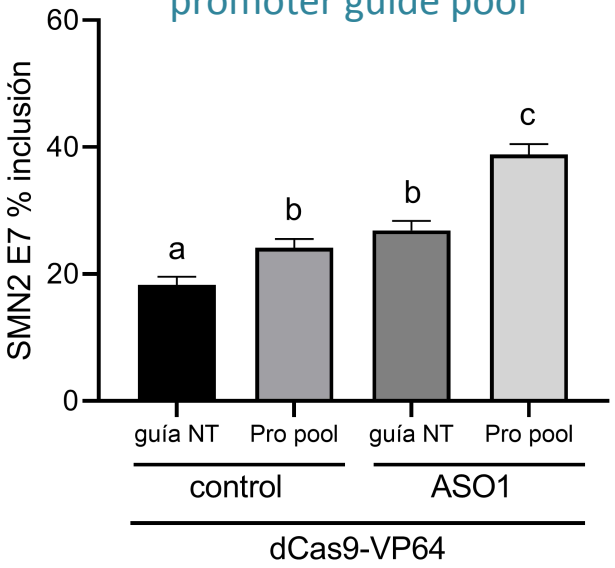
SMN2 E7 % splicing

RT-PCR

E7 guide pool



promoter guide pool



Conclusions

SMN2 E7 is a type II exon: Slow transcript elongation promotes its skipping while fast elongation promotes its inclusion.

Combined therapy for Spinal muscular atrophy: Spinraza (ASO1) and chromatin opening with histone deacetylase inhibitors (VPA).

Mechanism: Spinraza (ASO1) has two opposite effects. The negative effect is counteracted by opening the chromatin with histone deacetylase inhibitors.

Trageting histone acetylation specifically to *SMN1/2* also enhances the effect of Spinraza.

Designed by Luciana Giono, inspired in Alexander Calder mobiles

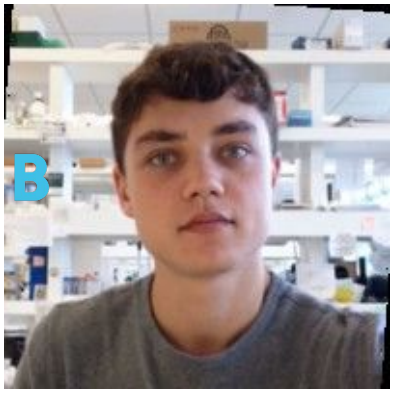
Marasco, L. E.*, Dujardin, G.*, Sousa Luís, R., Hsiu Liu, Y., Stigliano, J., Nomakuchi, T., Proudfoot, N. J., Krainer, A. R. & Kornblihtt. A. R. Counteracting chromatin effects of a splicing-correcting antisense oligonucleotide improves its therapeutic efficacy in spinal muscular atrophy. ***Cell*** 185, 2057-2070 (2022).

Adrián Krainer

Luciano Marasco

Gwendal Dujardin

Nick Proudfoot



Jose Stigliano

Emilia Haberfeld

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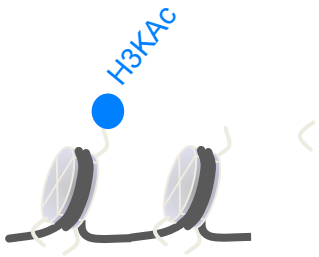

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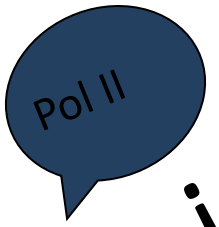
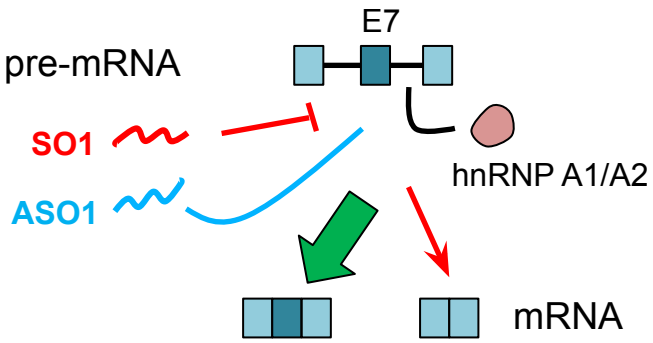
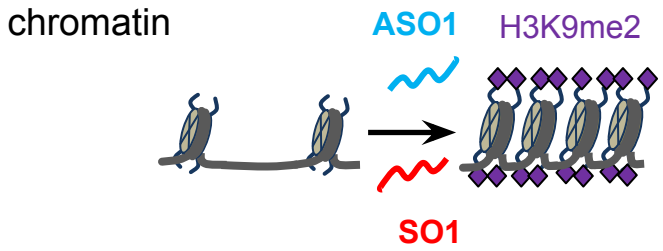


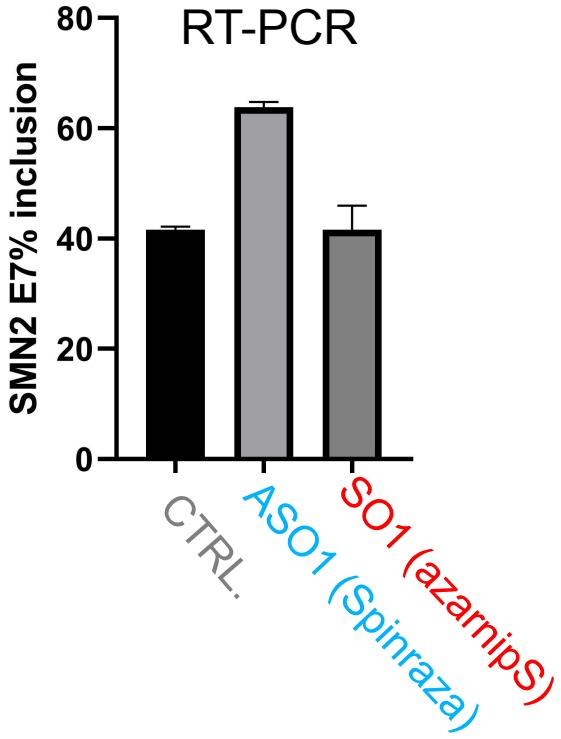
¡Gracias!

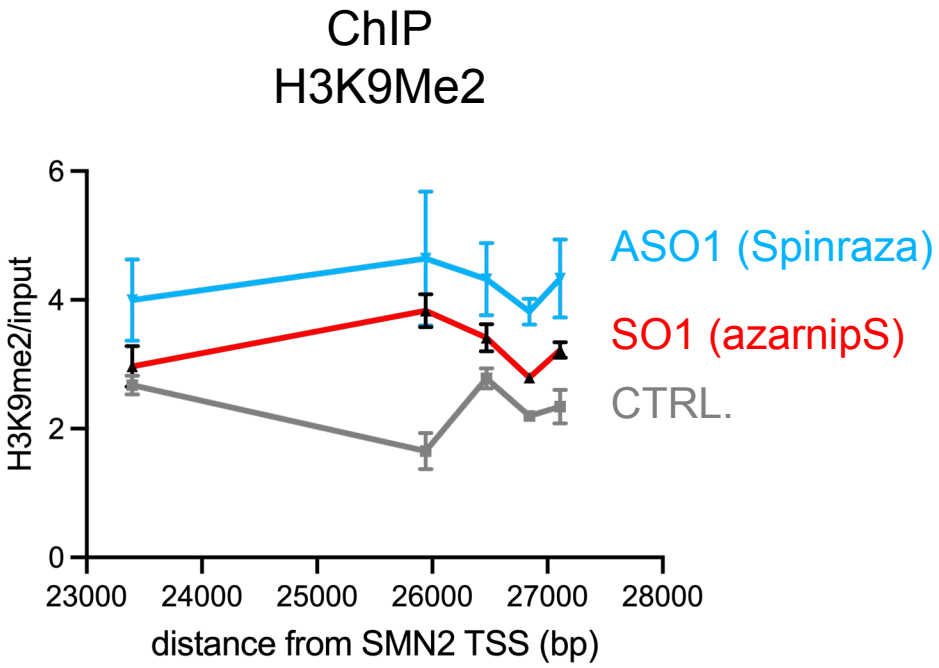
Chromatin effect of ASOs: DNA or RNA?

The chromatin effect seems to be due to interaction of the ASO with DNA









Stigliano *et al.*, unpublished