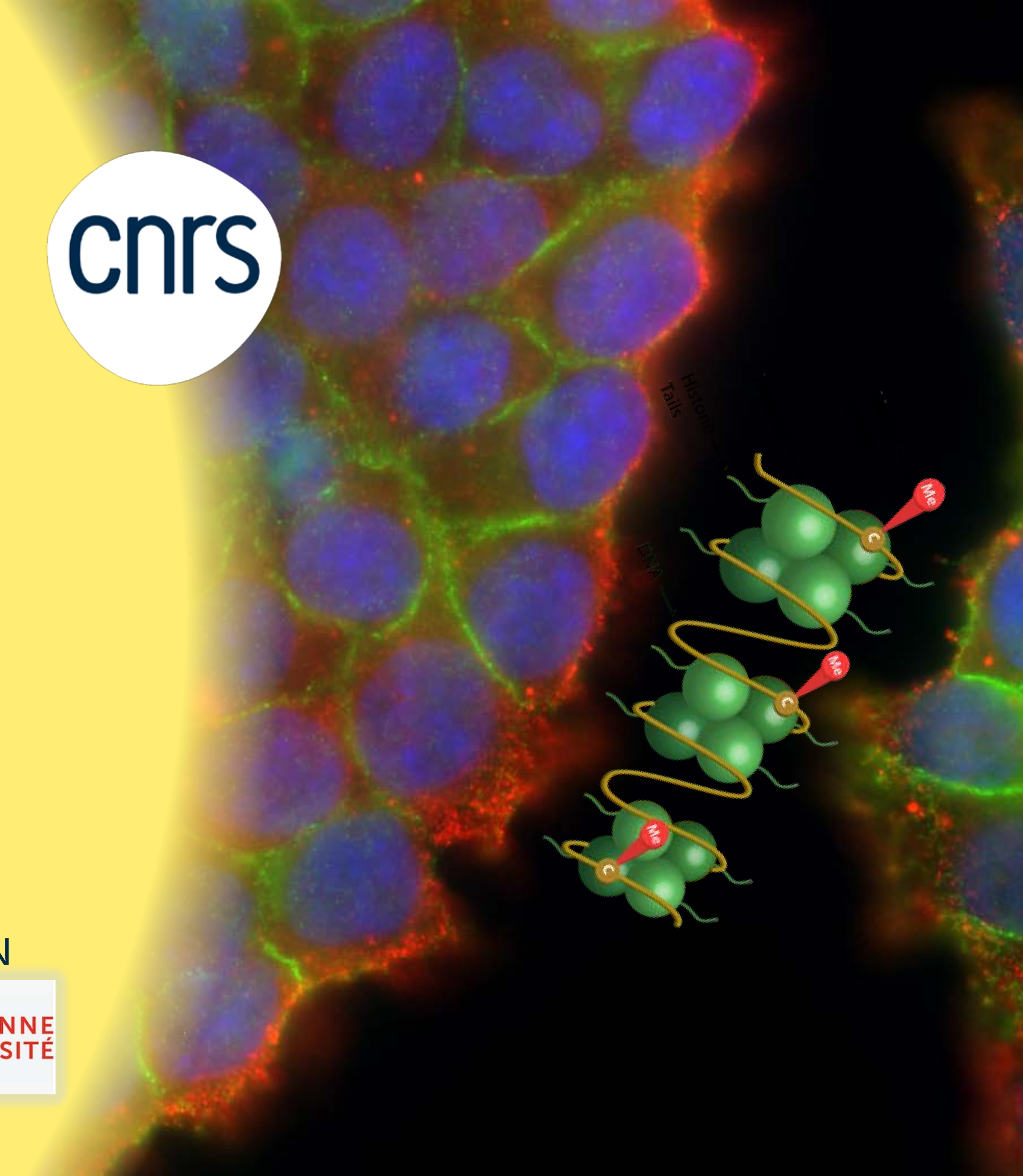
Epigenetics
&
RNA alternative splicing regulation



cnrs

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dans les maladies humaines.

INSTITUT DE BIOLOGIE PARIS SEINE
Comprendre le vivant pour agir

SORBONNE UNIVERSITÉ

→ 10/10/2025

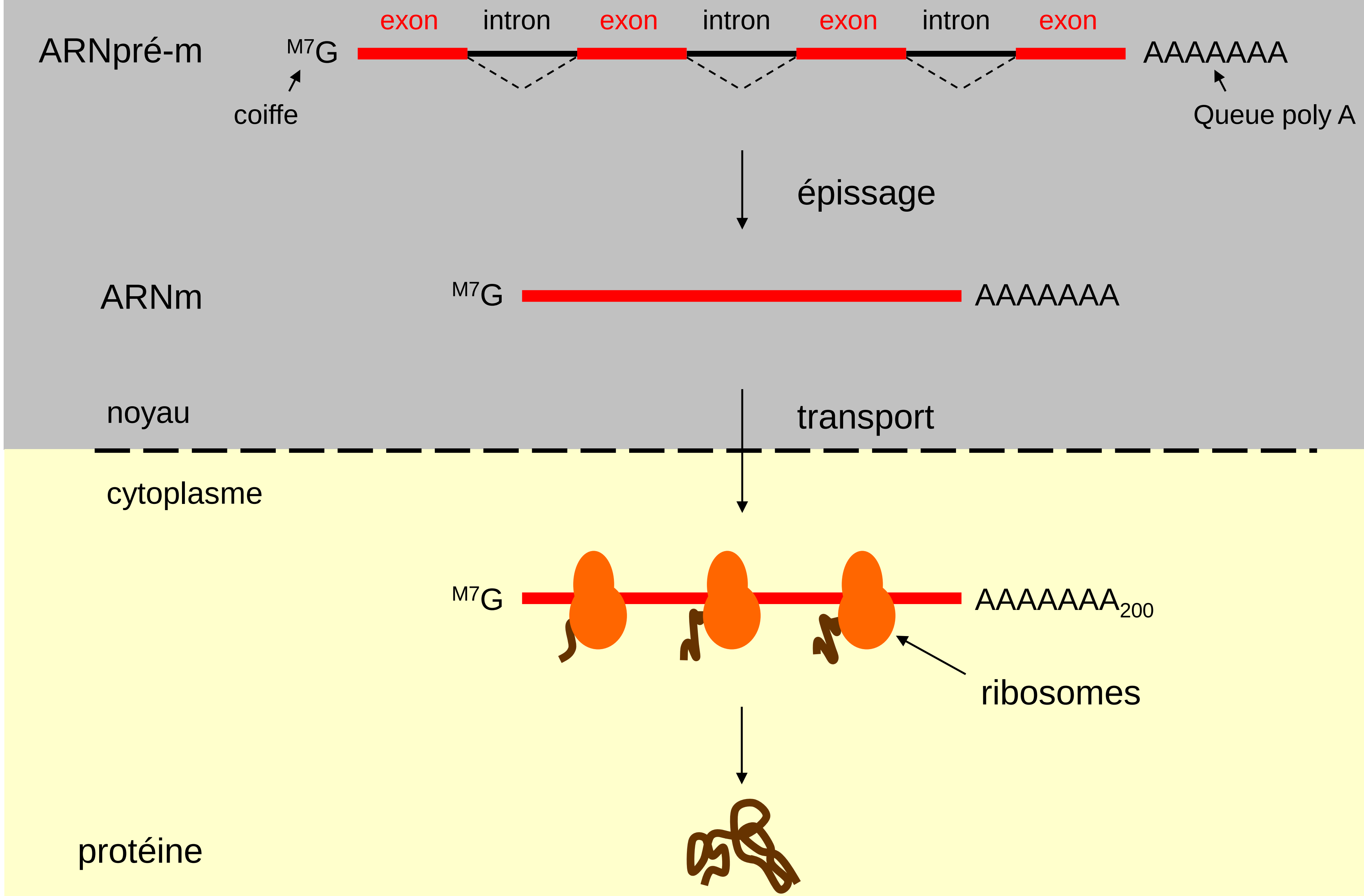
Sommaire

1 RNA splicing is cotranscriptional
2 The chromatine is organised around exons
3 Epigenetic regulations of exons
4 HP1γ an important reader for alter. splicing
5 DNA methylation
6 Nuclear RNA interference

A brief introduction

RNA splicing

Messenger RNAs are spliced



exon intron exon intron exon intron exon

ARNpré-m ^{M7}G AAAAAAA

coiffe

Queue poly A

épissage

ARNm ^{M7}G AAAAAAA

noyau

transport

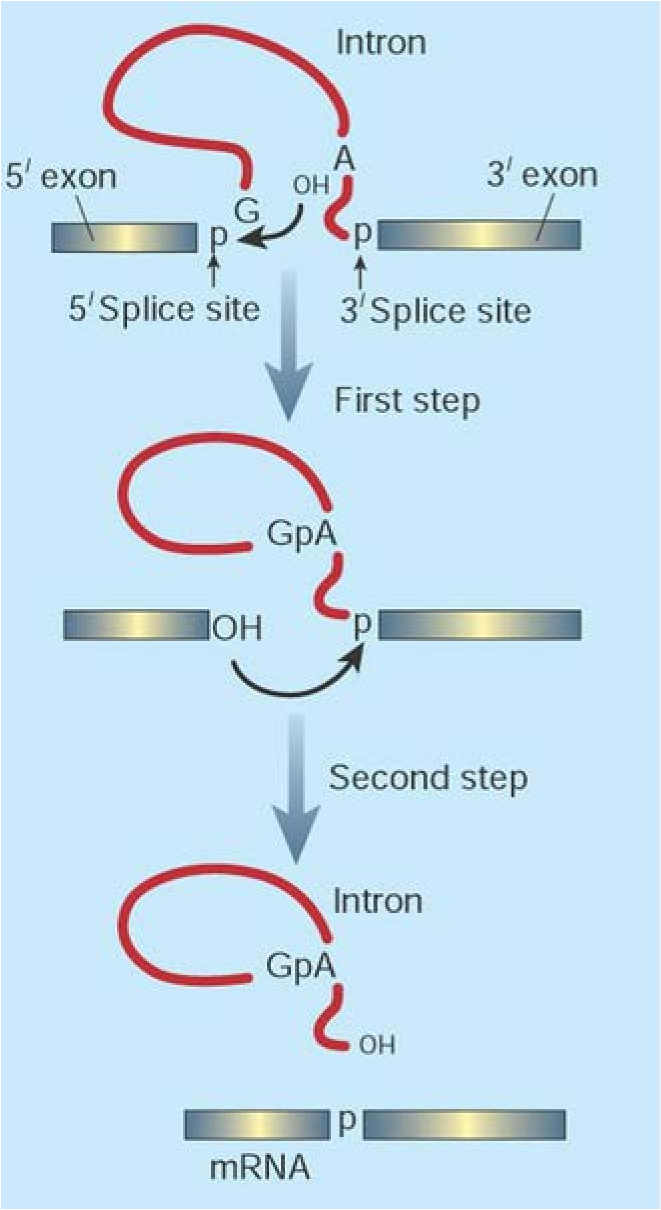
cytoplasme

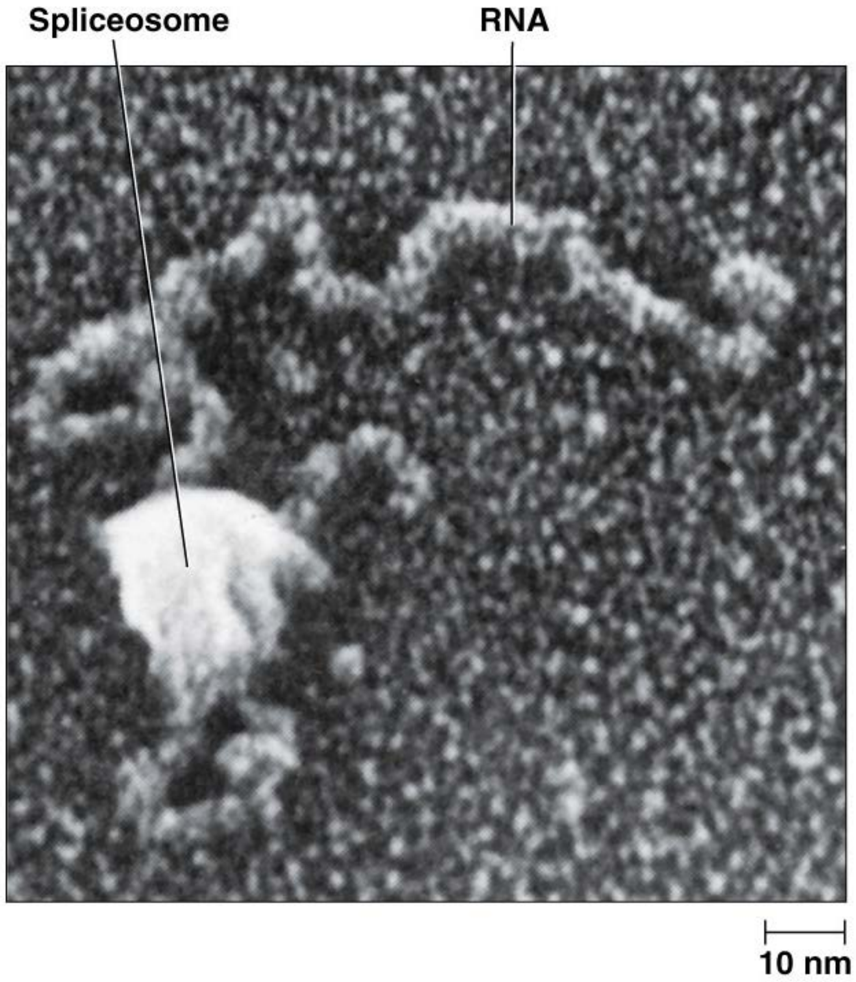
^{M7}G AAAAAAA$_{200}$

ribosomes

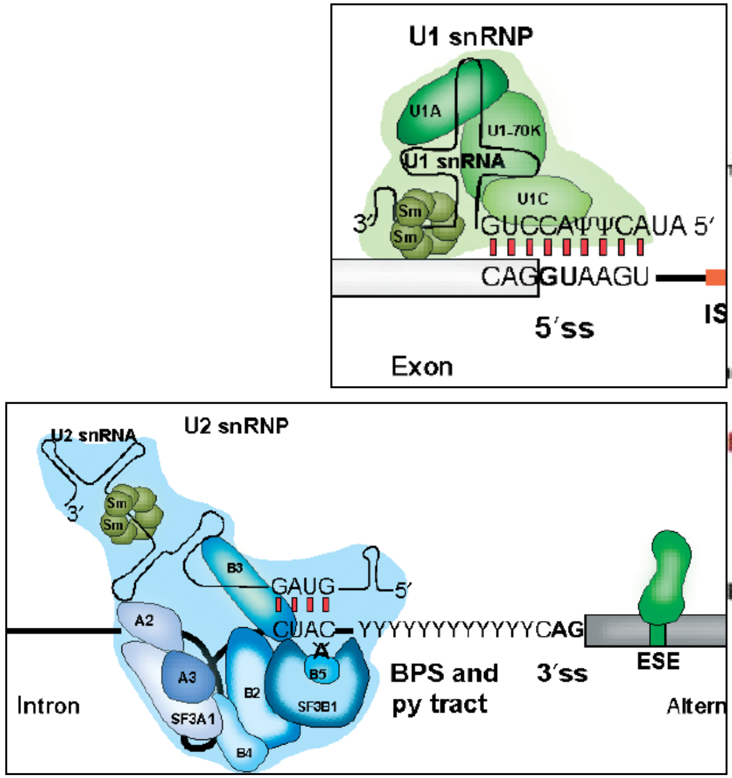
protéine

Le spliceosome

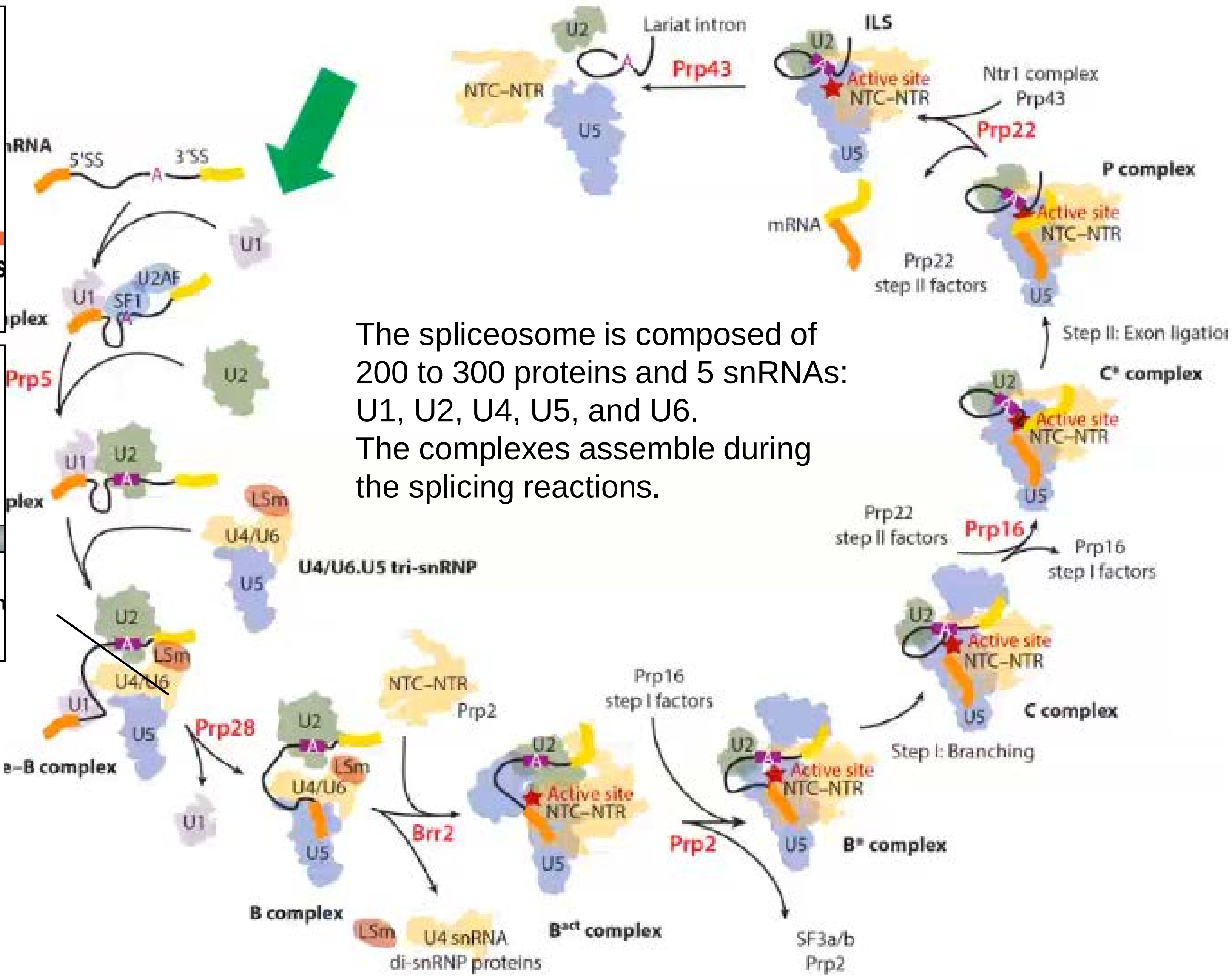




the spliceosome



(from Mallory et al. 2013
WIREs RNA)

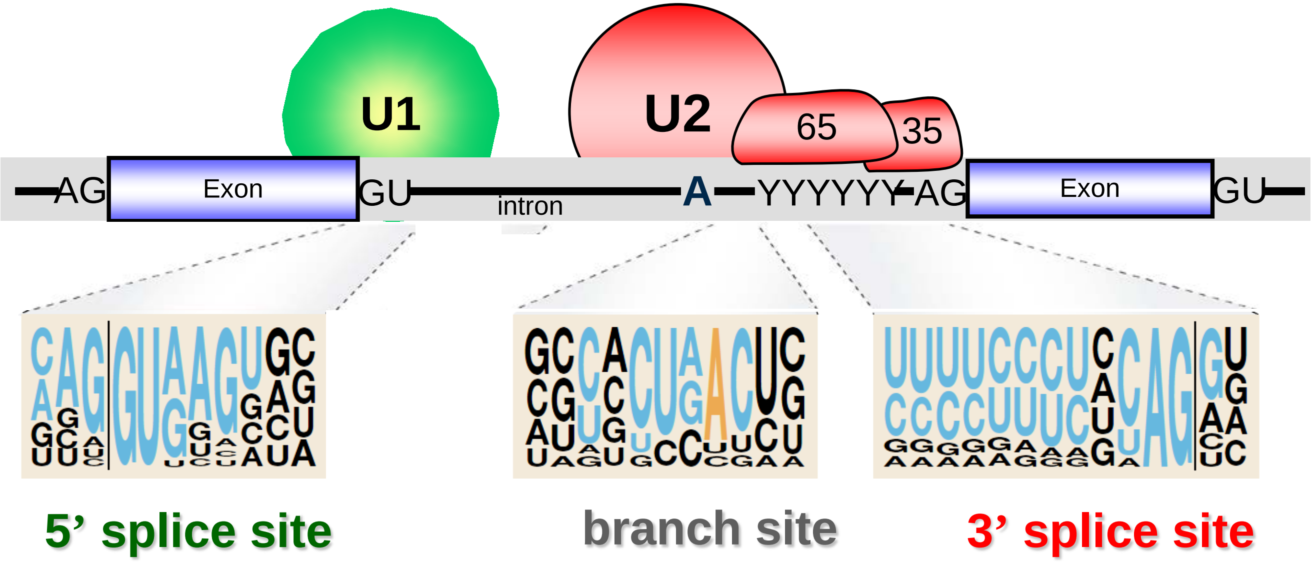


The spliceosome is composed of
200 to 300 proteins and 5 snRNAs:
U1, U2, U4, U5, and U6.
The complexes assemble during
the splicing reactions.

(from Will & Lürhman 2011 in CSH Perspect Biol)

The Splicing factors recognize RNA binding sites

General and specific splicing factors recognize numerous regulatory sequences, which are often degenerate in higher eukaryotes



Mammalian Consensus 5’-AG/**GU**AAGU-intron-YNCURAC-Y$_n$N**AG**/G-3’

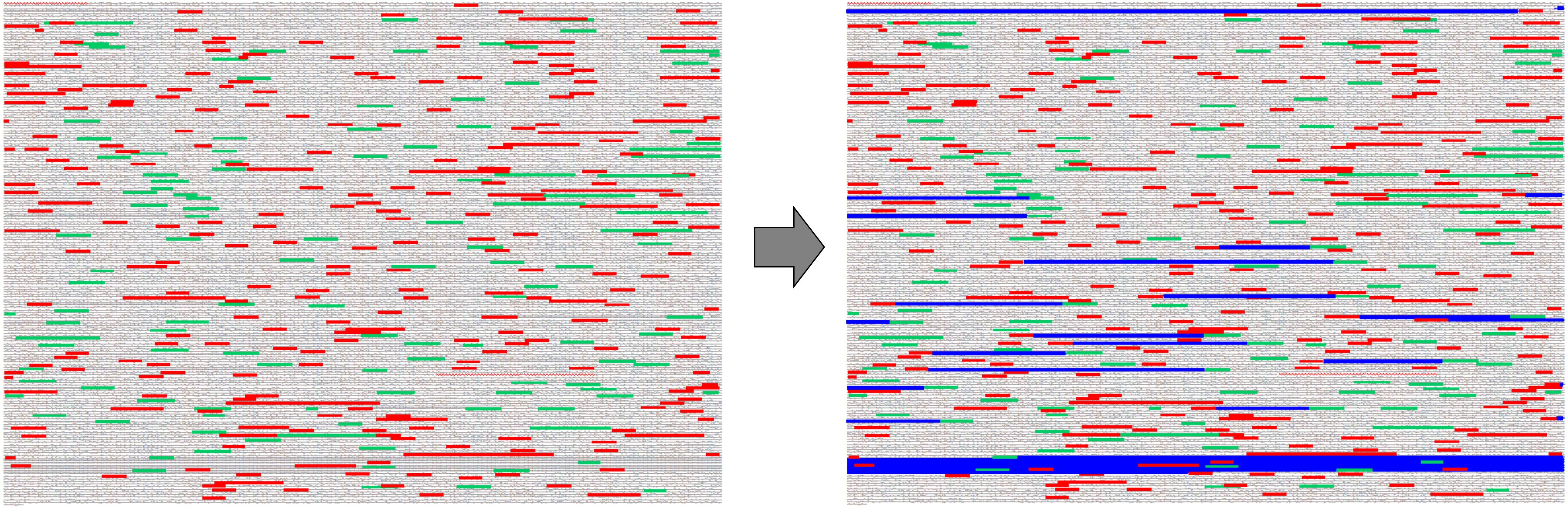
Yeast Consensus 5’-/GUAUGU-intron-UACUAAC-YAG/-3’

Splicing site consensus sequences are more degenerate in mammals.
This allows the creation of more alternative exons. (Ast 2004).

Cartegni et al. 2002 in Nat. Rev.

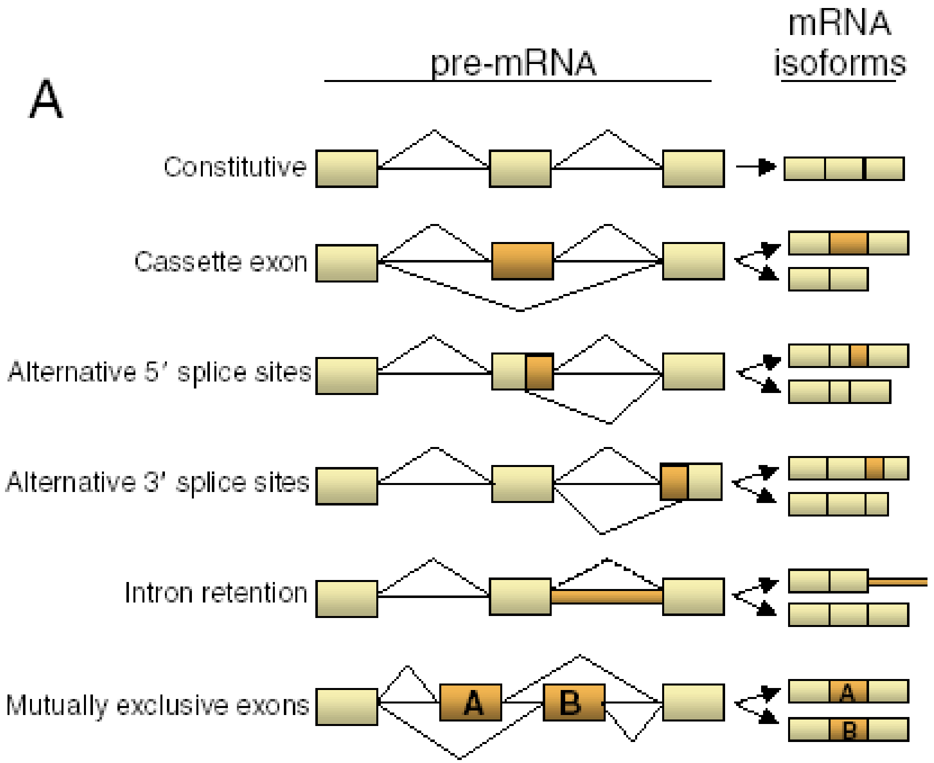
Recognition of splice sites 5’ and 3’

Only few splicing sites allow to define the exons in a gene



The splicing is highly regulated and variable

All multi-exonic genes have at least one alternative splicing event



- Biological prevalence
  - 95% of human genes have at least two splice variants
  - Depends on development stage *(sex in Drosophila)*
  - Cell-type specific
  - Regulated by signaling pathways
- Numerous diseases are associated with splicing defects
  - Cancers
  - Neurological disorders
  - Immunological disorders
  - …

Mutations in Splicing factors
- ***Spinal Muscular Atrophy** (Smn2)*
- ***Retinitis Pigmentosa** (PRP31)*
- ***Myotonic Dystrophy** (MBNL1)*
- …

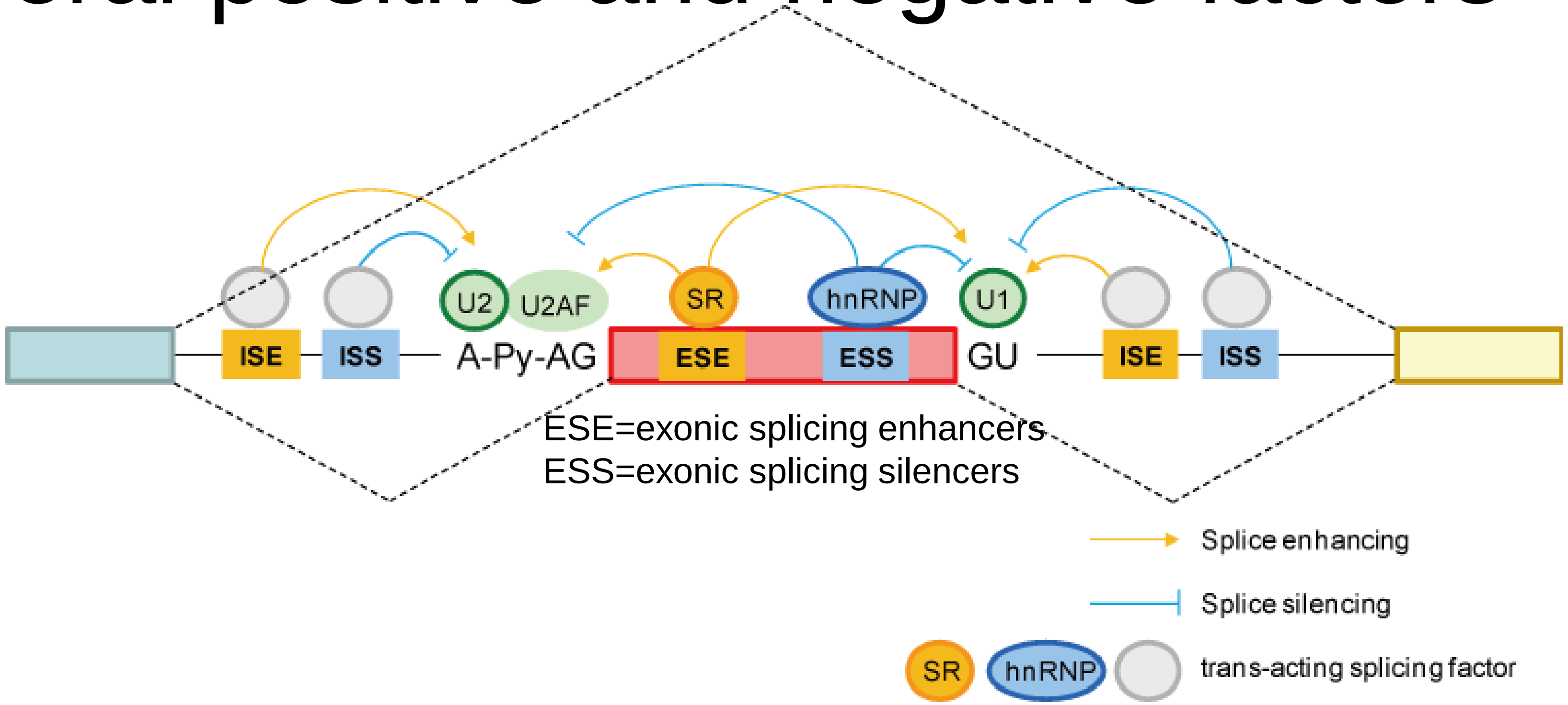
Mutations affecting one specific mRNA
- ***β-thalassemia** (β-globin)*
- ***Duchenne Muscular Dystrophy** (DMD)*
- ***Cystic Fibrosis** (CFTR)*
- ***Frasier Syndrome** (Wilm's tumor 1 gene)*
- ***Frontotemporal Dementia** & Parkinson (PARK2)*
- …

- Genetic alterations often affect splicing

~1/3 of inherited diseases are thought to involve a splicing component.

- Alternative splicing can influence the efficacy and safety of drugs:
  - as some treatments may only affect specific splice variants

The alternative splicing is regulated by several positive and negative factors



ESE=exonic splicing enhancers
ESS=exonic splicing silencers

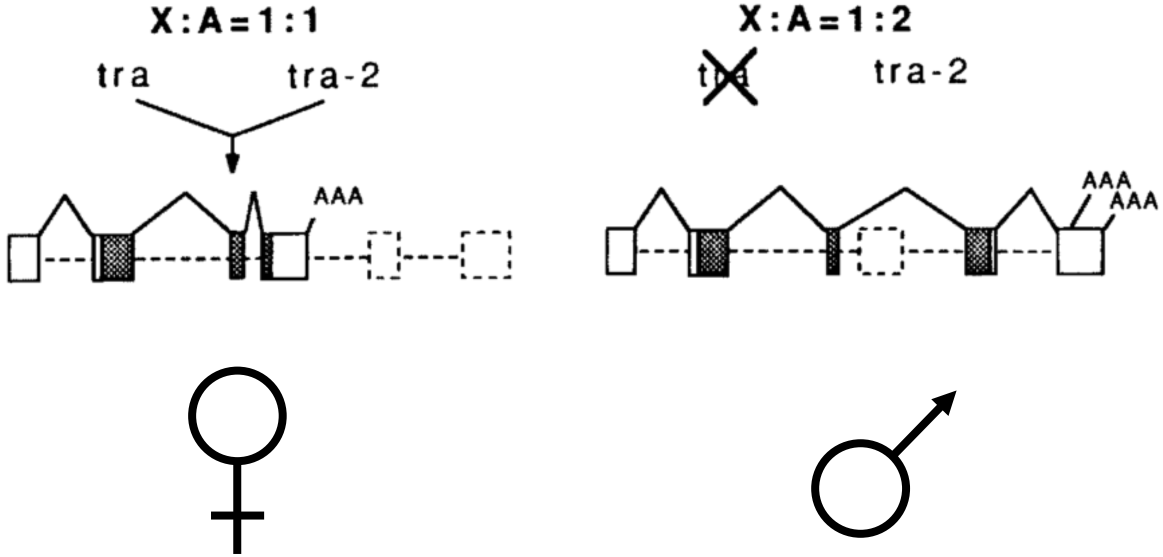
- For long introns, spliceosomal first assemble around exons, not introns
- U1 recognizes downstream 5'-splice site, U2AF recognizes upstream 3'-splice site
- SR proteins form cross-exon interactions, hnRNP antagonizes SR effects
- These cross-exon interactions are later replaced by cross-intron interactions in a series of events that are not well understood

- Alternative splicing affects ~95% of humans

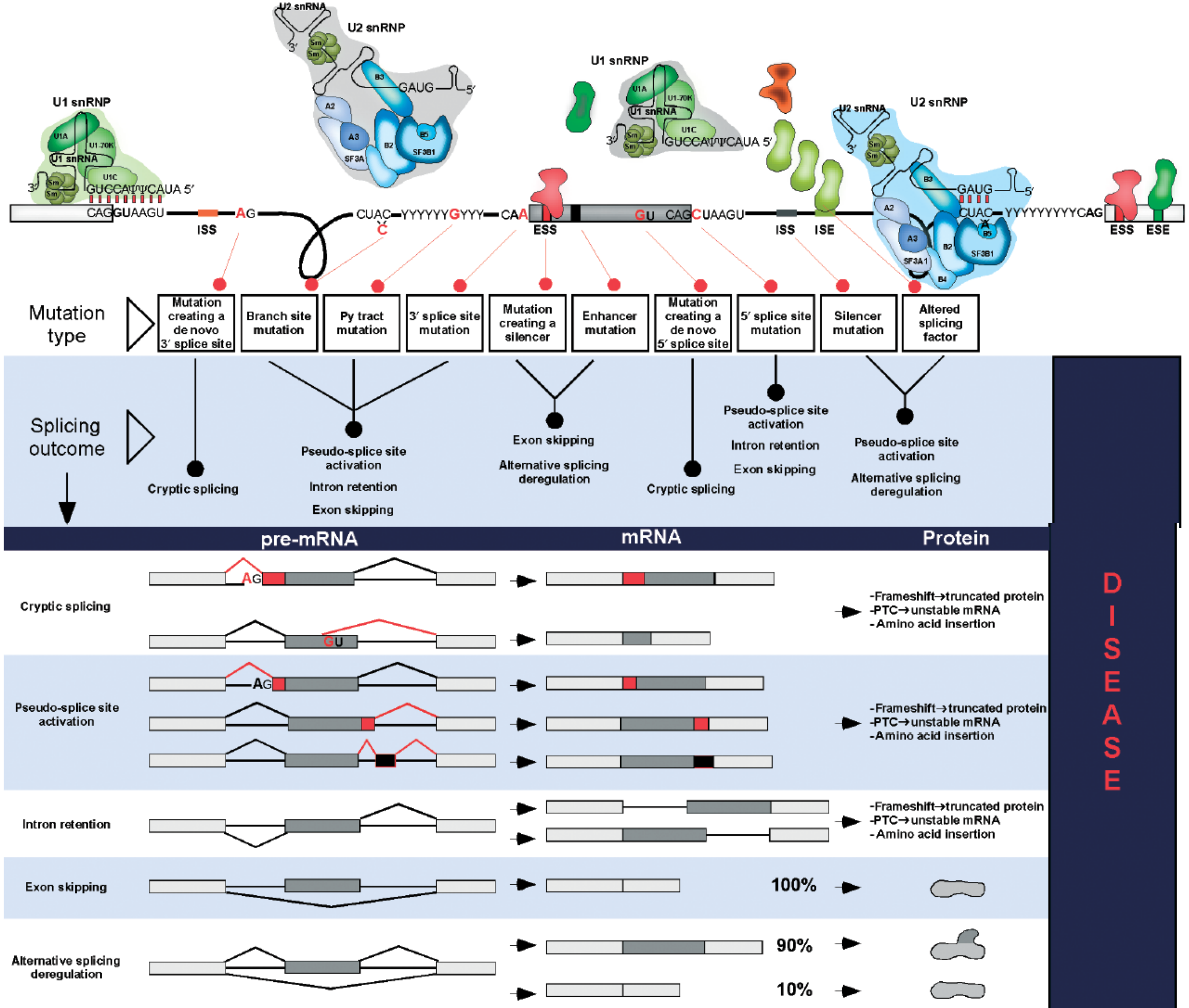
The dogma of ‘one gene for one protein’ is not generally valid.

Alternative splicing regulates essential functions

Sex-specific splicing of the doublesex (dsx) pre-messenger RNA controls sex determination in Drosophila. In females, a proximal terminal exon 3' called exon f is used. The use of this exon requires the activity of the transformer (tra) and transformer 2 (tra-2) genes. In males, a distal terminal exon called exon m is used. The use of this exon is independent of the activity of the transformer (tra) and transformer 2 (tra-2) genes.

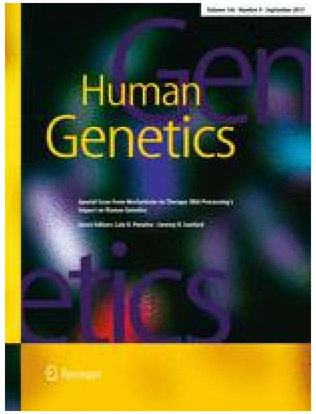


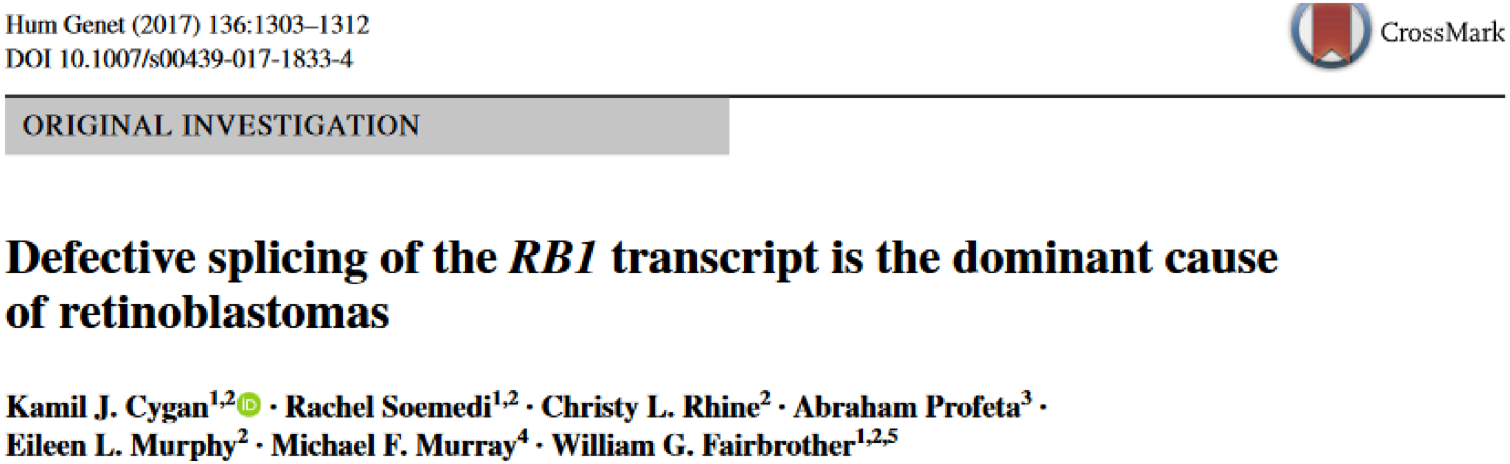
> 35% of genetic diseases are associated with splicing defects.



Mallory et al. 2013
WIREs RNA

Alternative splicing is an important process in the regulation of gene expression.




Hum Genet (2017) 136:1303–1312
DOI 10.1007/s00439-017-1833-4

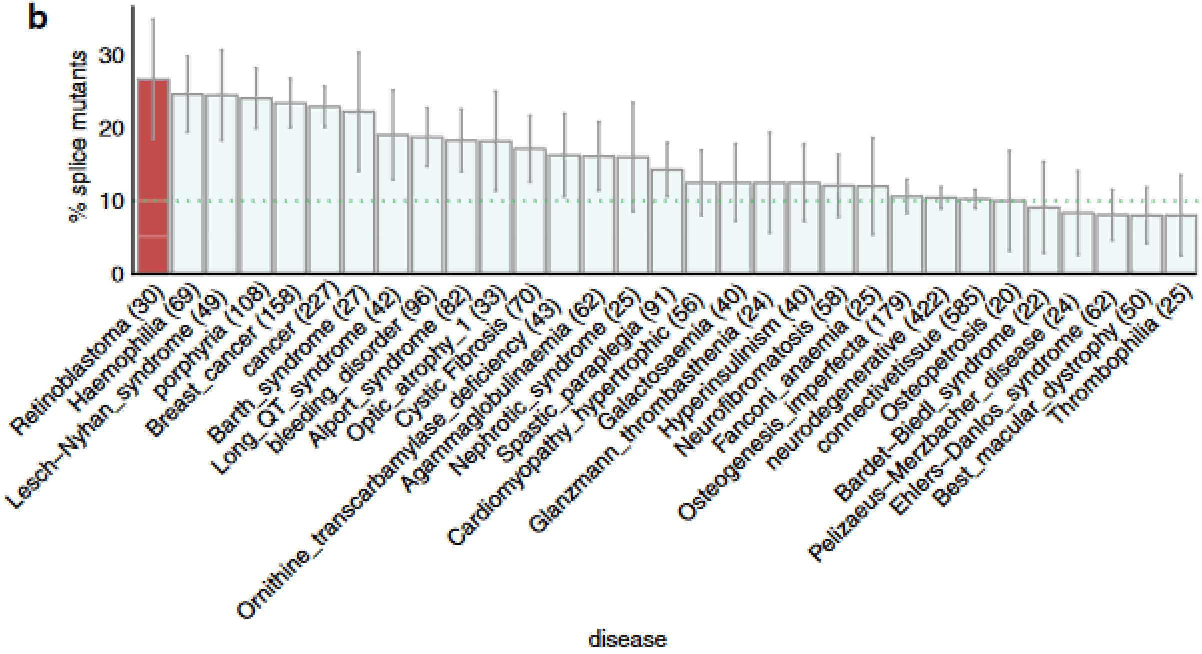
CrossMark

ORIGINAL INVESTIGATION

Defective splicing of the *RB1* transcript is the dominant cause of retinoblastomas

Kamil J. Cygan[1,2] · Rachel Soemedi[1,2] · Christy L. Rhine[2] · Abraham Profeta[3] · Eileen L. Murphy[2] · Michael F. Murray[4] · William G. Fairbrother[1,2,5]

“there is a great excess (46%; ~threefold) of hereditary disease alleles that map to splice sites in *RB1* that cause retinoblastoma.
Also, […] 27% of *RB1*-coding mutations tested also disrupt splicing.”

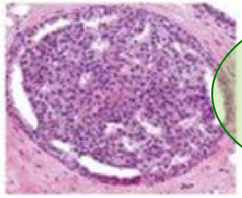


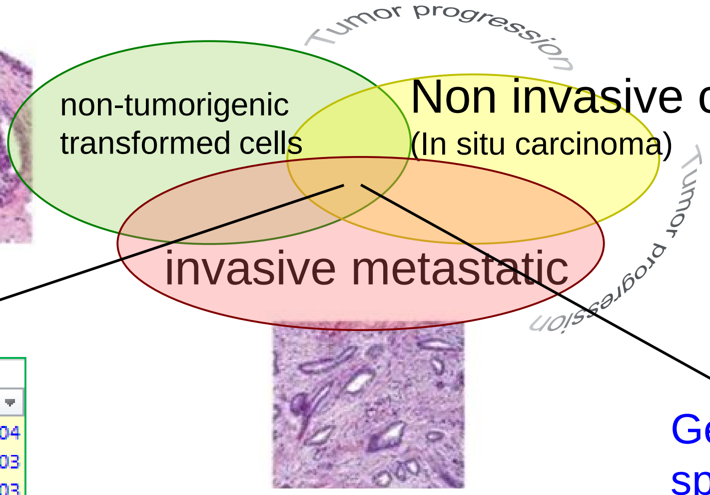
Gene expression & alternative splicing regulation define different pathways of breast cancer progression

MCF10A epithelial breast cells :

Normal ductal lumen

+RAS





Tumor progression

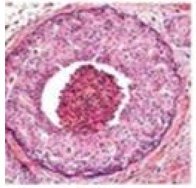
non-tumorigenic transformed cells

Non invasive carcinoma

(In situ carcinoma)

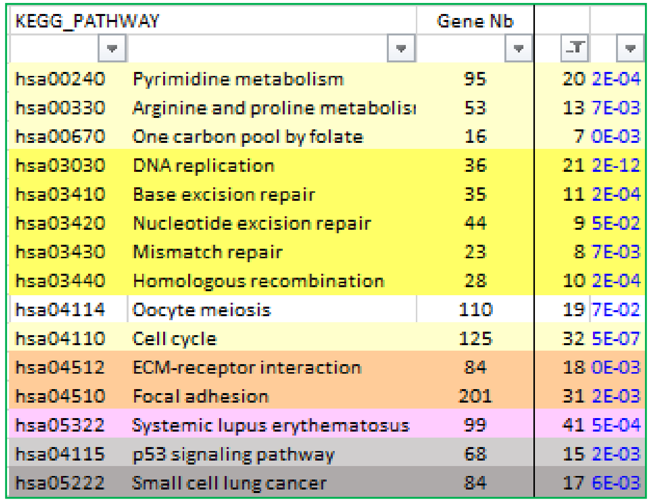
invasive metastatic

Tumor progression



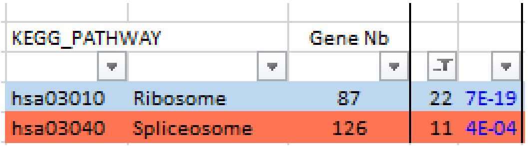
Comparison vs MCF10A

Regulated Genes



KEGG_PATHWAY		Gene Nb		
hsa00240	Pyrimidine metabolism	95	20	2E-04
hsa00330	Arginine and proline metabolisı	53	13	7E-03
hsa00670	One carbon pool by folate	16	7	0E-03
hsa03030	DNA replication	36	21	2E-12
hsa03410	Base excision repair	35	11	2E-04
hsa03420	Nucleotide excision repair	44	9	5E-02
hsa03430	Mismatch repair	23	8	7E-03
hsa03440	Homologous recombination	28	10	2E-04
hsa04114	Oocyte meiosis	110	19	7E-02
hsa04110	Cell cycle	125	32	5E-07
hsa04512	ECM-receptor interaction	84	18	0E-03
hsa04510	Focal adhesion	201	31	2E-03
hsa05322	Systemic lupus erythematosus	99	41	5E-04
hsa04115	p53 signaling pathway	68	15	2E-03
hsa05222	Small cell lung cancer	84	17	6E-03

Genes with differential splicing events



KEGG_PATHWAY		Gene Nb		
hsa03010	Ribosome	87	22	7E-19
hsa03040	Spliceosome	126	11	4E-04

almost no genes alternatively spliced in transcriptionnaly regulated genes

=> splicing unrelated to transcriptional levels

=> Expression and Splicing analysis give rise to different output

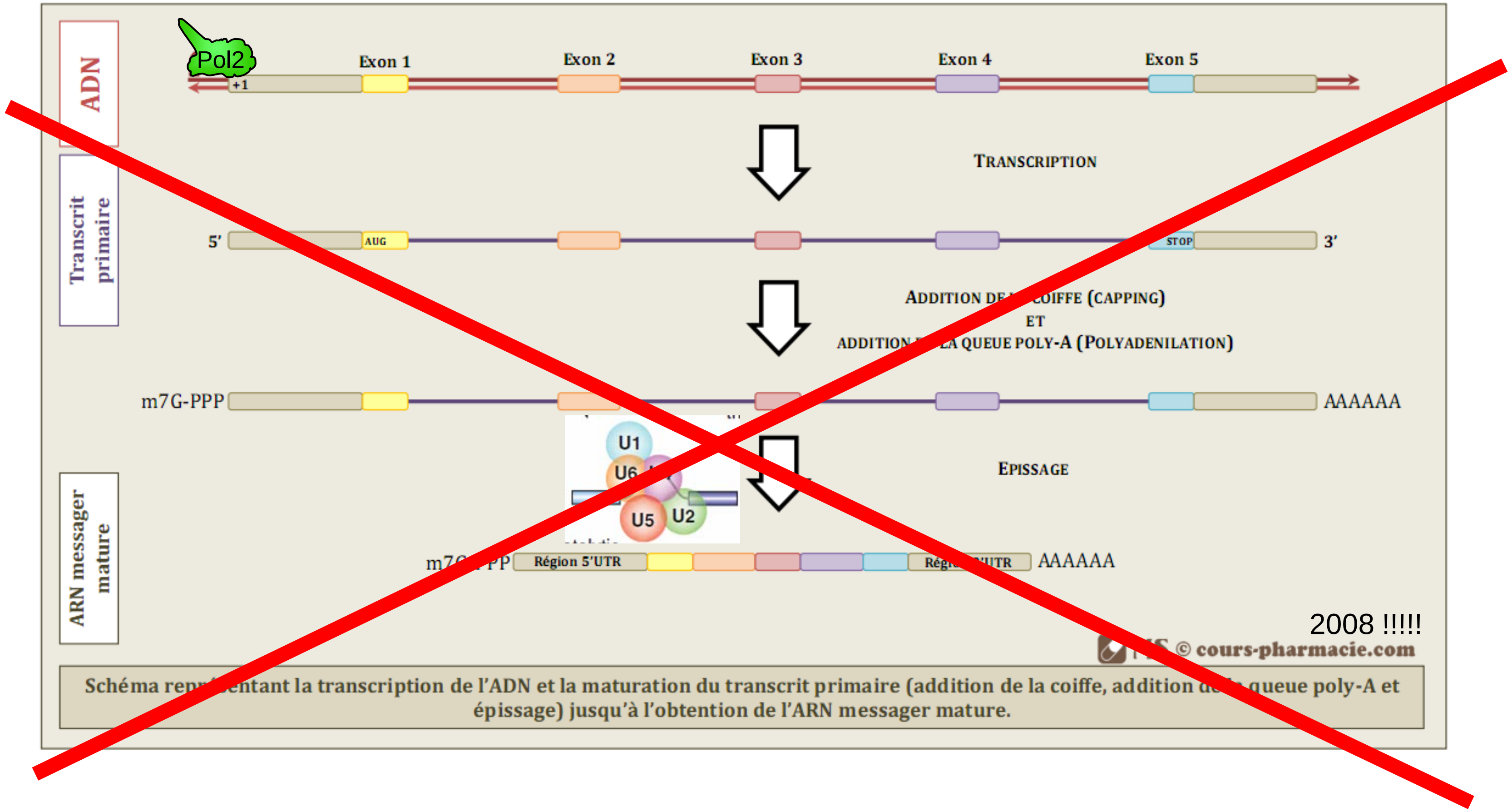
… a brief introduction

RNA splicing is coupled with transcription

Transcriptional Elongation : RNA synthesis

RNAs undergoing synthesis undergo several stages of maturation, including the removal of introns to form messenger RNAs (mRNAs).

Gene expression at school

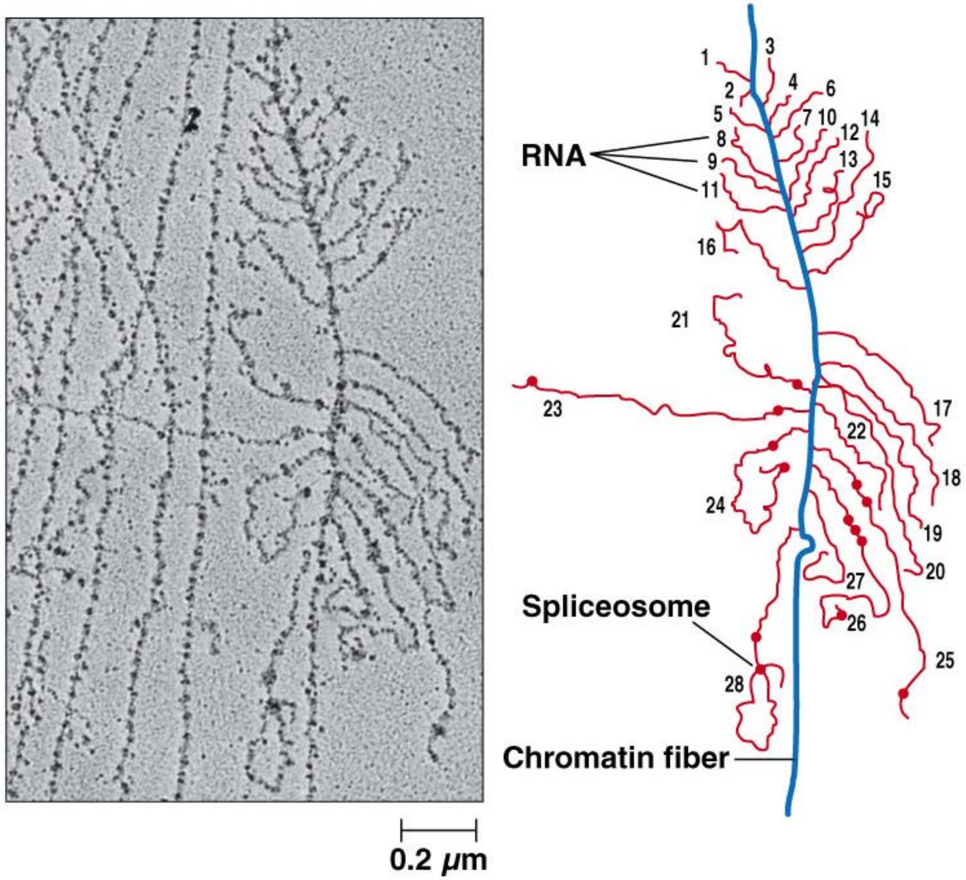


2008 !!!!!

Schéma représentant la transcription de l'ADN et la maturation du transcrit primaire (addition de la coiffe, addition de la queue poly-A et épissage) jusqu'à l'obtention de l'ARN messager mature.

Transcriptional Elongation : RNA synthesis

RNA synthesis and splicing occur simultaneously and in a coordinated manner



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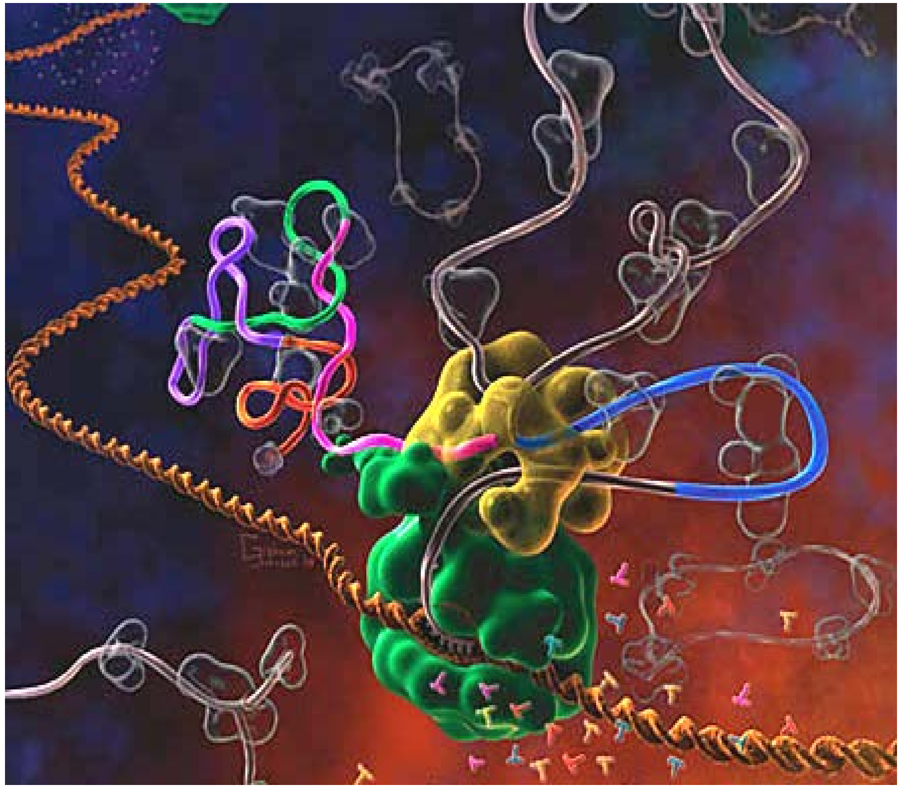
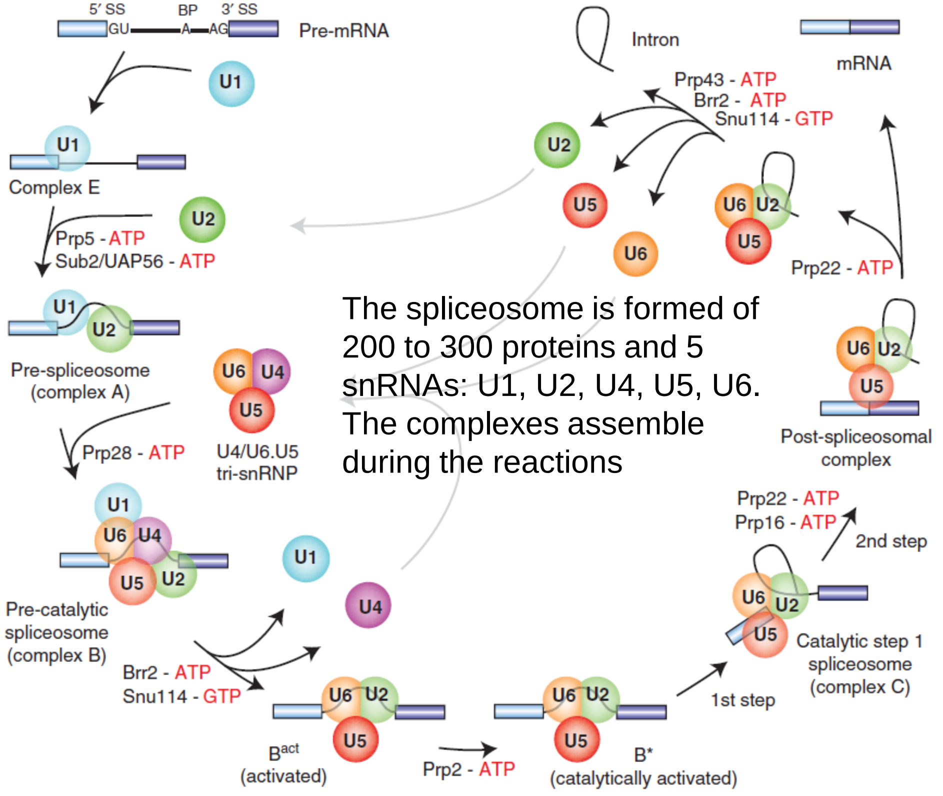


Splicing factors and the spliceosome interact with RNA polymerase 2

Cotranscriptionality of RNA splicing

Concordance and coupling.

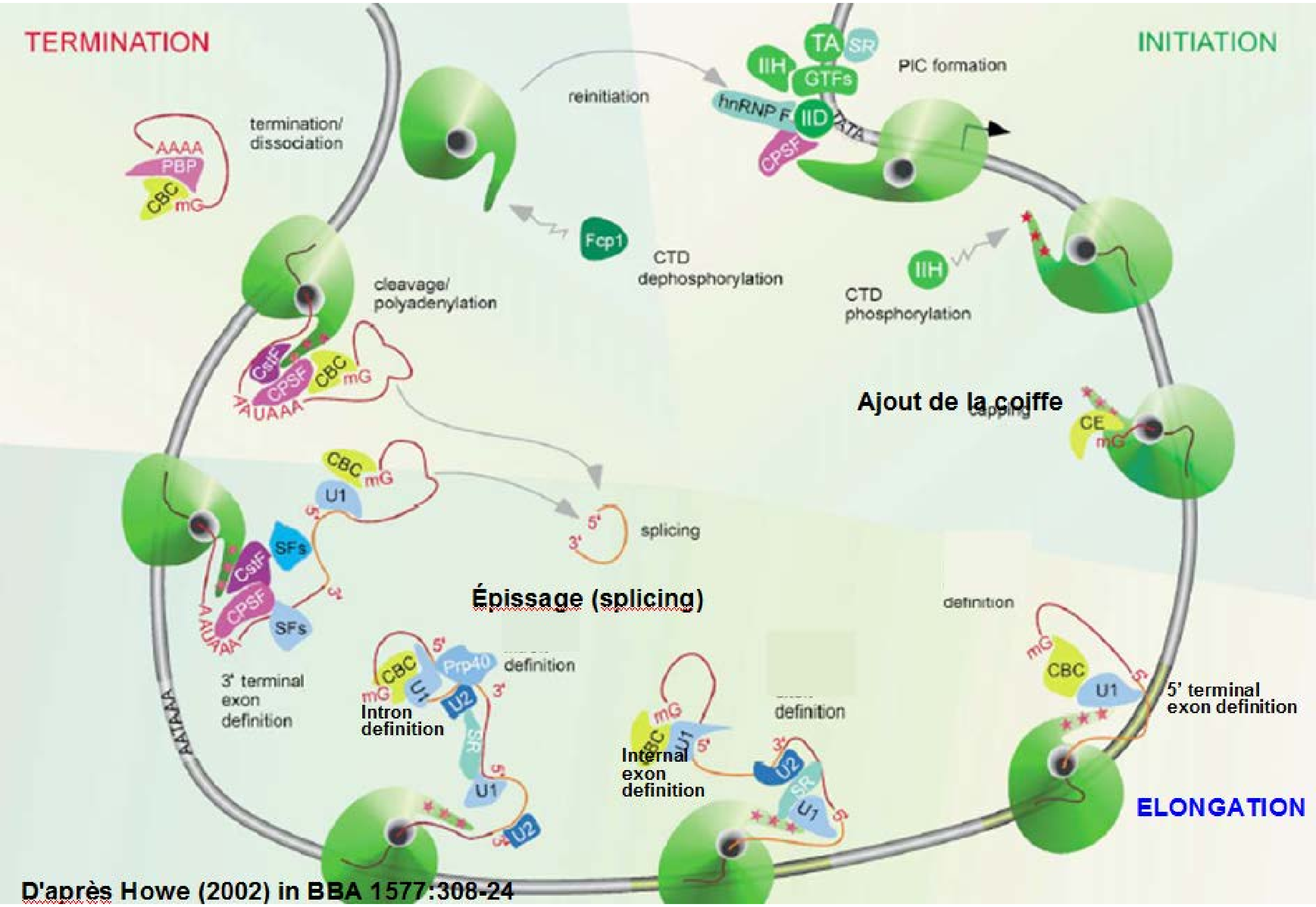
Splicing factors and the spliceosome interact with RNA polymerase II



The spliceosome is formed of 200 to 300 proteins and 5 snRNAs: U1, U2, U4, U5, U6.
The complexes assemble during the reactions

Nagai et al. 2019

Splicing of long gene RNAs occurs before RNA Pol2 reaches the end of the gene.



D'après Howe (2002) in BBA 1577:308-24

Transcription and splicing are coordinated

Examples of splicing factors that influence transcription and vice versa

Furthermore, in most cases, pre-mRNA maturation events have been described as being coupled with transcription, i.e. these processes influence each other.
For example, it has been shown that SFs must be present at the start of transcription to be effective in in vitro splicing, or are even required for transcriptional activation via pTEFb

En 2008

ELSEVIER
Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in
Genetics & Development

Splicing, transcription, and chromatin: a *ménage à trois*
Eric Allemand, Eric Batsché and Christian Muchardt

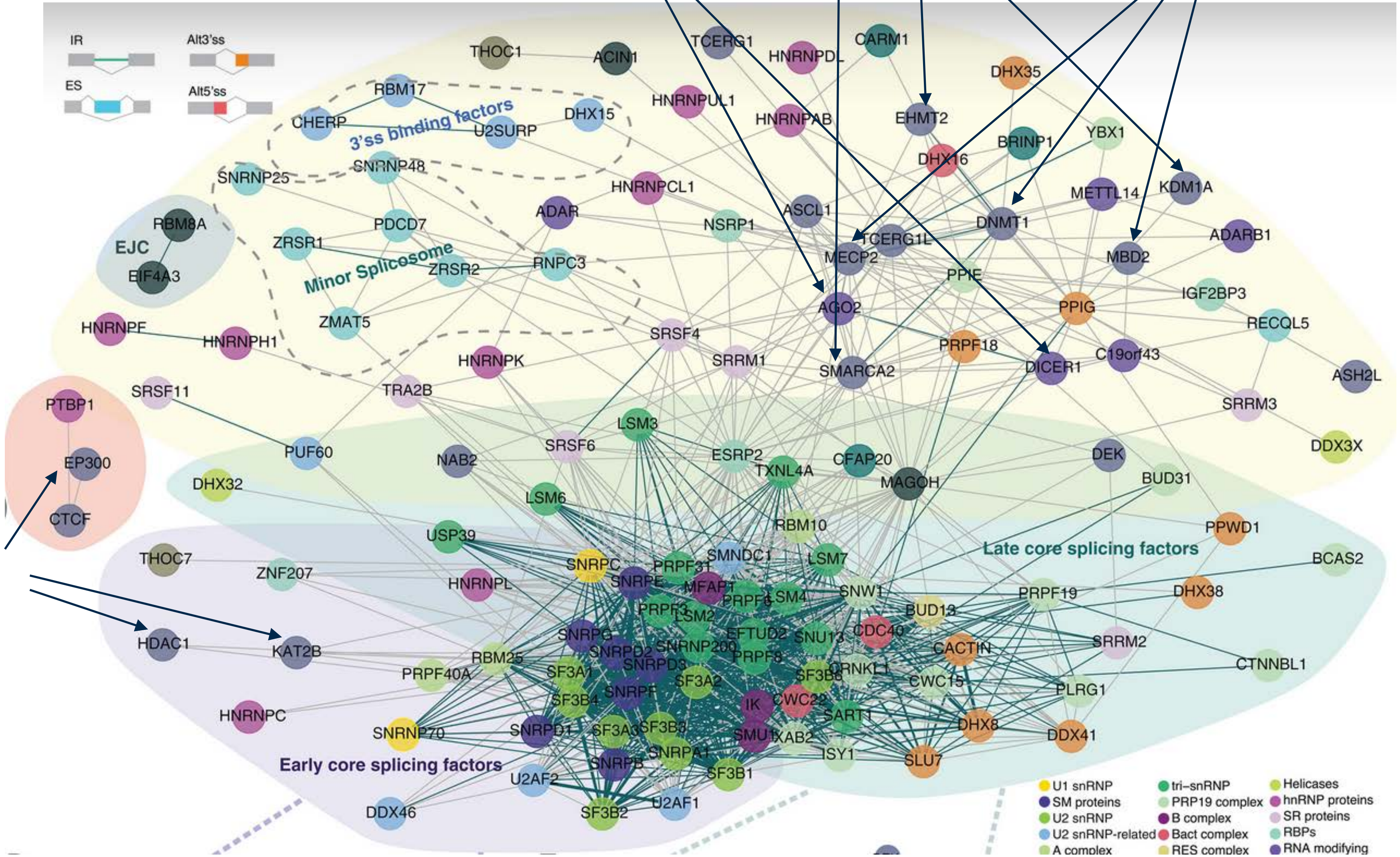
	Proteins	SPLICING		TRANSCRIPTION		References
		A/C	partners	+/-	partners	
Spliceosome	Prp6 / ANT-1	C	Bridge U4.U6/U5	+	AR(AF-1), GR, Brm	Batsché 06, Dellaire 02, Zhao 02, Fane 06
	SF3b130/SAP130	C	SU of U2 snRNP	?	SU of the STAGA complex	Brand 01, Martinez 01
	SF3a120	C	SU of U2 snRNP	+	ERα	Masuhiro 05
	SF1 (ZFM1)	C+A	SU of spliceosome	-	?	Zhang 98
	U2AF65	C	SU of spliceosome	?	uP-CTD	Bres 05, Robert 02, Ujvari 04
	hnRNP K	A	9G8, SRp20, Sam68	+/-	poly(C)-binding TF, Sp1, PcG	Expert-Bezancon 02 rev in Bomsztyk 04
SR proteins	SF2/ASF	C+A	SU of spliceosome	+	P-CTD	Cramer 99, Das 07, de la Mata 03, de la Mata 06
	SRp20	C+A	SU of spliceosome	+/-	Mediator complex, CHD1	Cramer 99, de laMata 06, Sato 04
	CAPER	A	SRp54	+	ER(AF-2), AP-1/ASC-2	Dowhan 05, Jung 02
Elongation factors	CA150	A	SF1, U2AF, U2 snRNP	-	P-CTD	Carty 00, Cheng 07, Goldstrohm 01, Neubauer 98
	SPT5	Mutation leads to splicing default in 43 yeast genes		+	SU of the DSIF complex, P/uP-CTD	Bourgeois 02, Ivanov 00, Kim 01, Lindstrom 03, Xiao 05
	COBRA1	A	?	-	SU of the NELF complex, CTD	Sun 07, Yamaguchi 99
	SPT6	A+C	REF/ALy	+	P-CTD	Yoh 07
	Tat-SF1	A	U snRNPs	+	RAP30 (TFIIF), SPT5, P-TEFb	Fong 01, Kim 99
RNA helicase	P68/Ddx5	A	U1 snRNP, mRNPs	+	ERα (AF-1)	Endoh 99, Liu 02, Merz 07 Lee 02, Shin 07, Watanabe 01 Rev in Fuller-Pace 06
	P72/Ddx17/RH70	A	SRp86, U1 snRNPs	+	ERα (AF-1)	
	RHA/Dhx9	?	Smn	+/-	TFs and coRegs	
Transcriptional regulators	SAF-B	A	SRp86	-	ER(DBD)	Nayler 98, Oesterreich 00
	TRAP150/ Med23	?	mRNPs	+	Mediator complex, CTD	Ito 99, Merz 07
	TFII H	?	U1 snRNA	+	CTD	O'Gorman 05
	PGC-1α/β (PRC/PERC)	A	U1 snRNP	+	PPARγ, ER, HNF4α, NRF1 RNAPII	Andersson 01, Kressler 02, Lin 02, Monsalve 00
	SKIP (NCoA2)	A	SU of spliceosome	+	TFs and NRs PTEFb	Baudino 98, Makarova 04, Neubauer 98, rev in Bres 05
	TLS (FUS)	A	SF1, SC35, TASR	+	NRs, TFIID, NF-KB	Hallier 98, Lerga 01, Powers 98, Yang 98
	CoAA	A	?	+	NRs, AP-1, NFkB	Auboeuf 04, Iwasaki 01
	P52	?	SF2/ASF	+	Sp1	Ge 98
	PSF p54nrb	C	U1 and U2 snRNPs	-	NRs, Sin3A, P-CTD	Emili 02, Kameoka 04, Mathur 01, Patton 93
	hnRNP U (GRIP120)	A	hnRNP K	-	GR(AF-2), ERα	Eggert 97, Masuhiro 05, Shnyreva 00
	FIR/PUF60	C+A	SU of spliceosome	-	TFIIH, FUSE	Hastings 07, Liu 06
	WT1	A	U2AF65, RBM4	-	Kruppel like Zn finger TF	Davies 98, Markus 06
	XAB2	?	spliceosome	-	RARa, HDAC3	Ohnuma-Ishikawa 07, Zhou 02
	EWS.Fli&.NOR1	A	U1C, TASR	+	TAFII55 & 100, P-CTD	Knoop 01, Ohkura 02, Yang 00
Chromatin regulators	Brm (SMARCA2)	A	PRP6, Sam68, U1 and U5 snRNAs	+/-	SU of the SWI/SNF complex, TFs and NRs	Batsché 06
	MeCP2	A	YB-1	-	SU of the Sin3A complex, SWI/SNF	Young 05, Rev in Moretti 06
	PRMT5	?	Sm B /B'proteins	+/-	NRs, YY1, p53, NFkB, TAT, HMG, CBP/p300, histones SPT5, CA150	Cheng 07 rev in McBride 05
	PRMT4 / CARM1	A	U1C, SmB, SAP49, CA150			Cheng 07, Ohkura 05 Rev in Bedford 05
	PRMT1	?	hnRNP K and Sam68			Chiou 07, Cote 03

Transcriptome-wide splicing network reveals specialized regulatory functions of the core spliceosome

MALGORZATA E. ROGALSKA, ESTEFANIA MANCINI, SOPHIE BONNAL, ANDRÉ GOHR, BRYAN M. DUNYAK, NICCOLÒ ARECCO, PETER G. SMITH, FRÉDÉRIC H. VAILLANCOURT, AND JUAN VALCÁRCEL Authors Info & Affiliations

SCIENCE · 31 Oct 2024 · Vol 386, Issue 6721 · pp. 551-560 · DOI: 10.1126/sc

…in 2024



SWI/SNF
Brm

AGO2
RNAi

H3K9me2/3

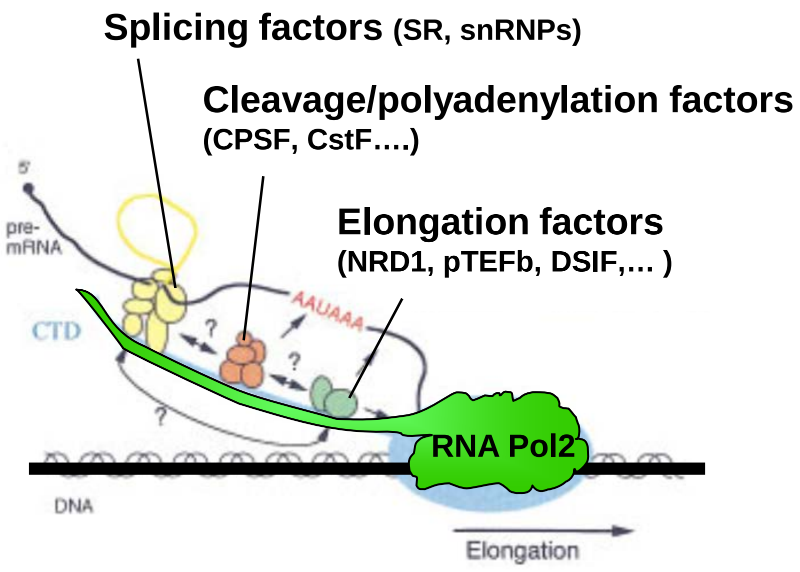
DNA methylation

Acetylation

Alternative splicing is regulated by transcription

Two mechanims for functional coupling which are not antagonistic

Pol2 recruits splicing factors

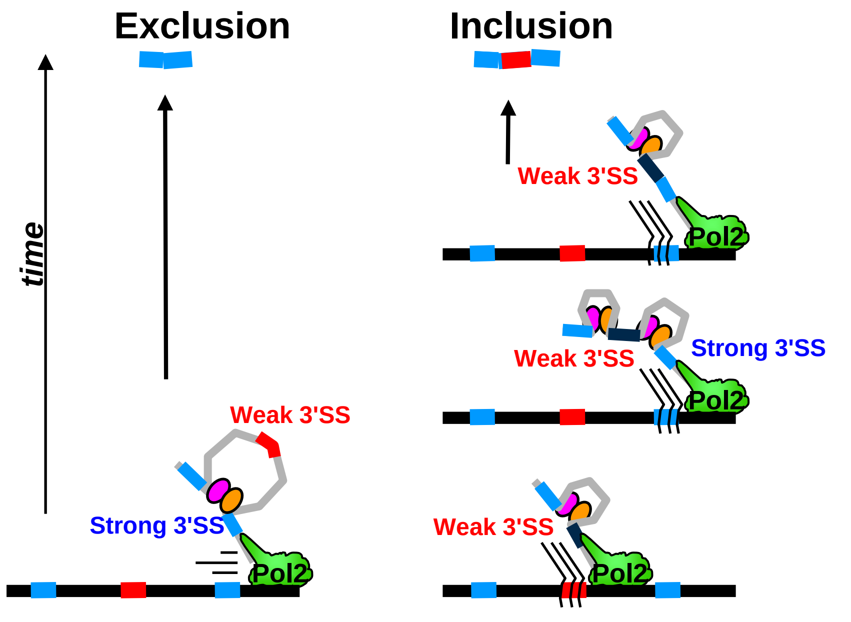

Splicing factors (SR, snRNPs)

Cleavage/polyadenylation factors
(CPSF, CstF….)

Elongation factors
(NRD1, pTEFb, DSIF,…)

The interaction of splicing factors with the nascent template and the transcriptional machinery (Bourquin et al. 1997; Kim et al. 1997; Tanner et al. 1997; Hirose et al. 1999; Robert et al. 2002; de la Mata and Kornblihtt 2006).

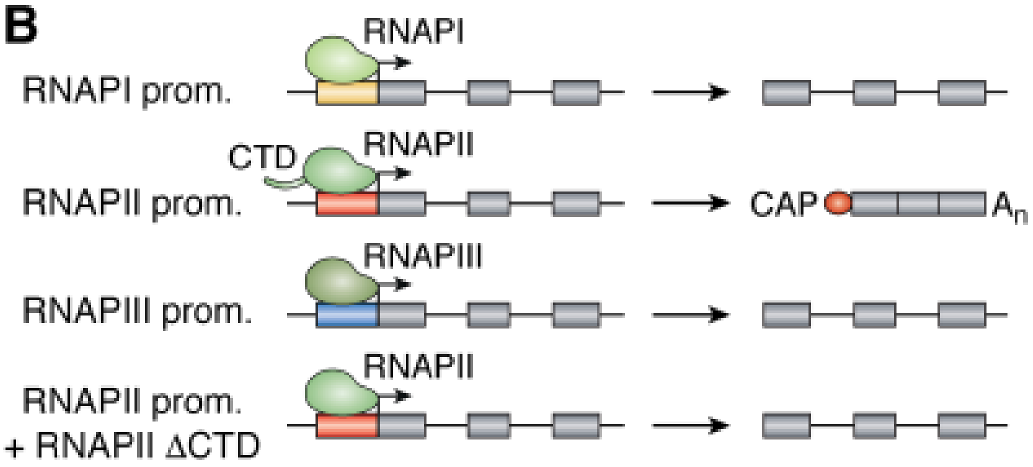
Transcription kinetics affect the choice of splicing sites: ‘first come, first served’ model

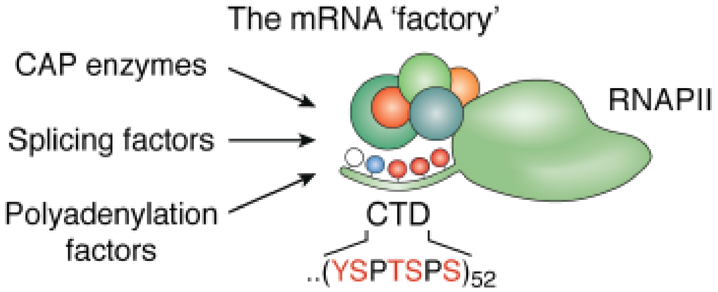


Fast elongation / no pause Slow elongation / pause

kinetic coupling, i.e., a “race” between the time it takes a transcript to be transcribed and cleaved from RNAPII by the end formation machinery versus the assembly of the spliceosome and the splicing reactions (de la Mata et al. 2003, 2010; Lacadie et al. 2006; Tardiff et al. 2006; Carrillo Oesterreich et al. 2010; Khodor et al. 2011).

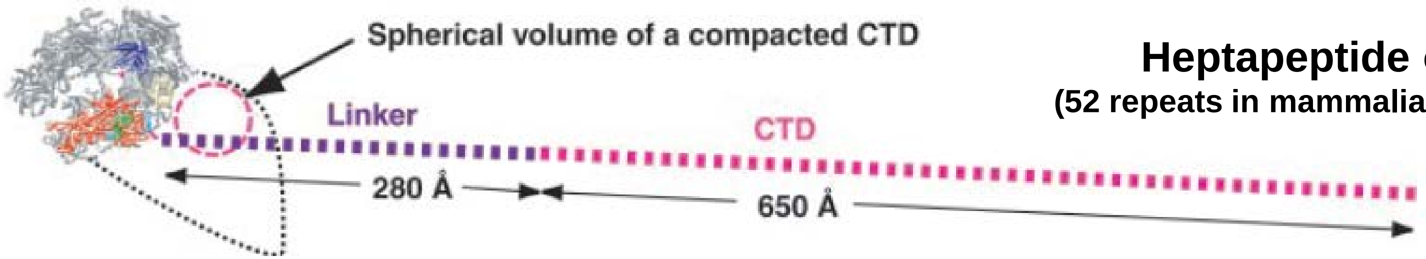
Splicing co-transcriptionality lies on the C-terminal Domain of the RNAPII





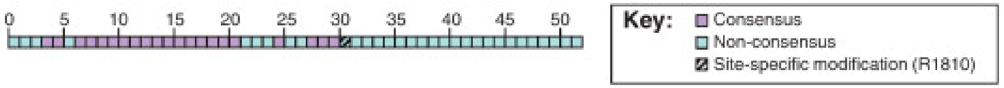
Reviewed in
Giono & Kornblihtt 2020 JB

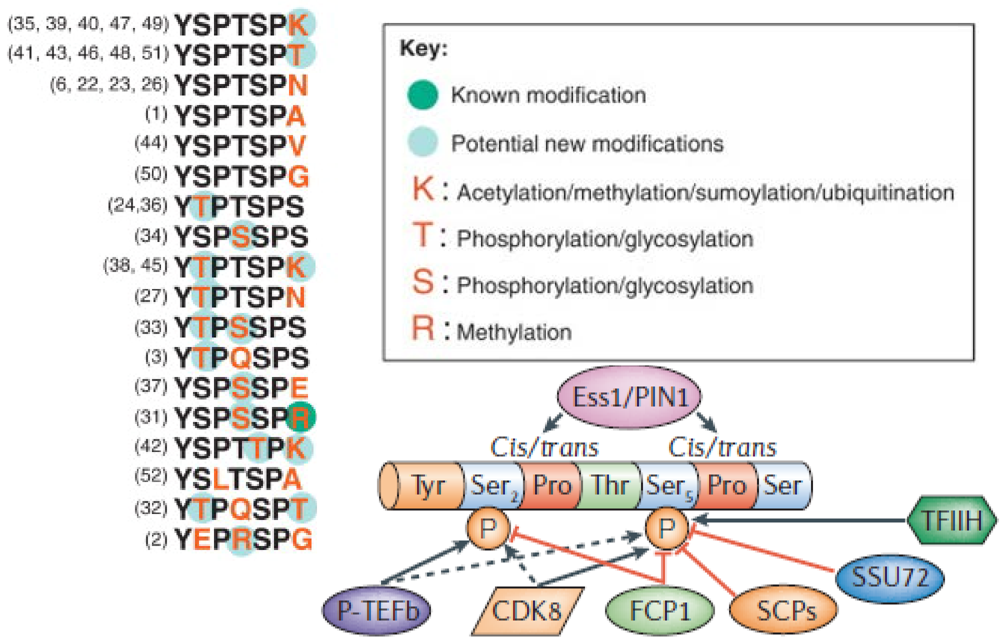
the carboxy-terminal domain (CTD) of RNA polymerase II



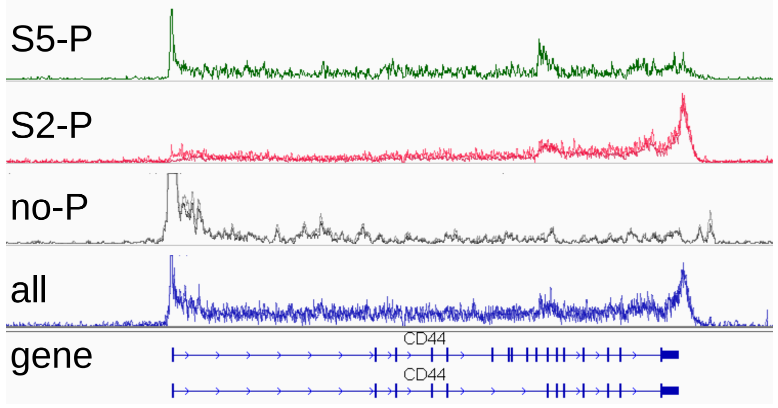
Heptapeptide of the CTD
(52 repeats in mammalian Rpb1, 27 in yeast)

Motifs répétés de 7 acides amines :





Transcribed gene by the RNA pol II (ChIP-seq)



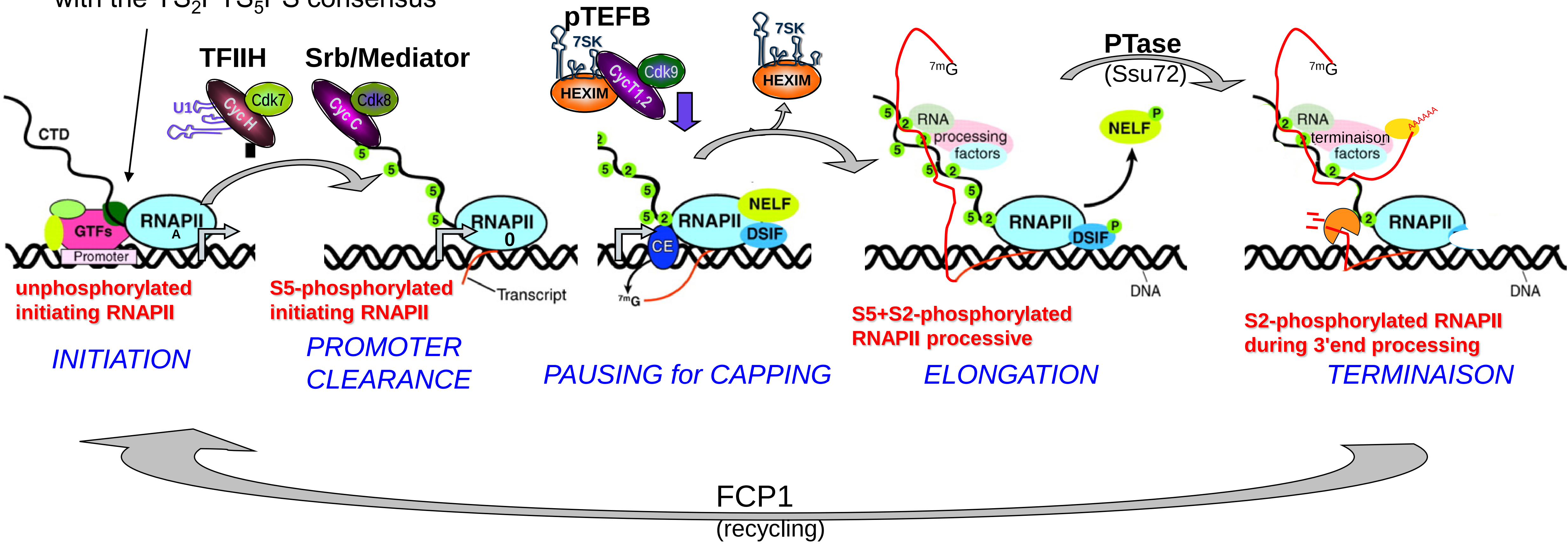
Rôles du CTD:

- unique to RNA pol II
- the CTD is not necessary for in vitro transcription
- the CTD is essential for cotranscriptional splicing, (and for life)
- The CTD is the site of numerous post-translational modifications.
- The CTD is predominantly phosphorylated at Ser5 at the promoter and predominantly phosphorylated at Ser2 at the termination site. (It can be simultaneously phosphorylated on both Ser2 and Ser5 of the same motif.)
- The nascent RNA from spliced exons is bound to CTD S5-P.

Egloff et al. 2012 TIG
Cramer et al. 2018 Science

CTD phosphorylation marks the stages of the RNAPII activity

The CTD contains 52 repeats
with the YS_2PTS_5PS consensus



unphosphorylated initiating RNAPII

INITIATION

S5-phosphorylated initiating RNAPII

PROMOTER CLEARANCE

PAUSING for CAPPING

S5+S2-phosphorylated RNAPII processive

ELONGATION

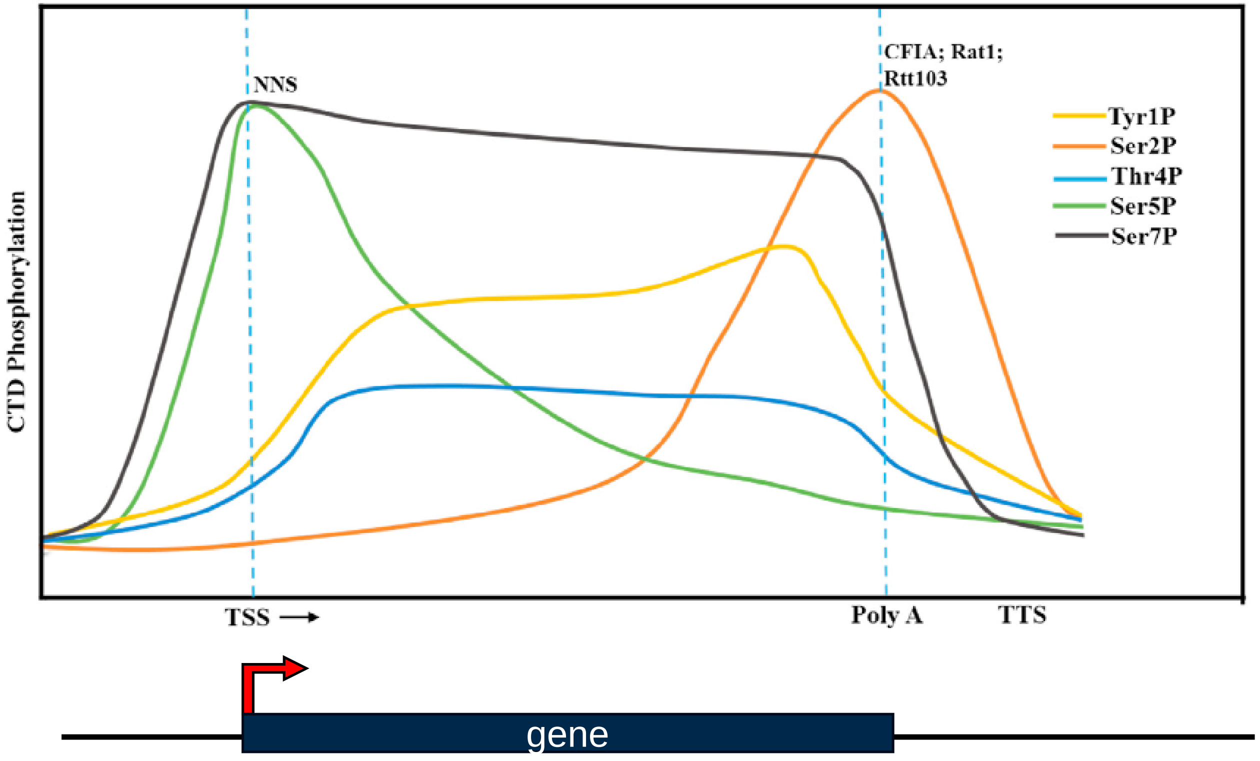
S2-phosphorylated RNAPII during 3'end processing

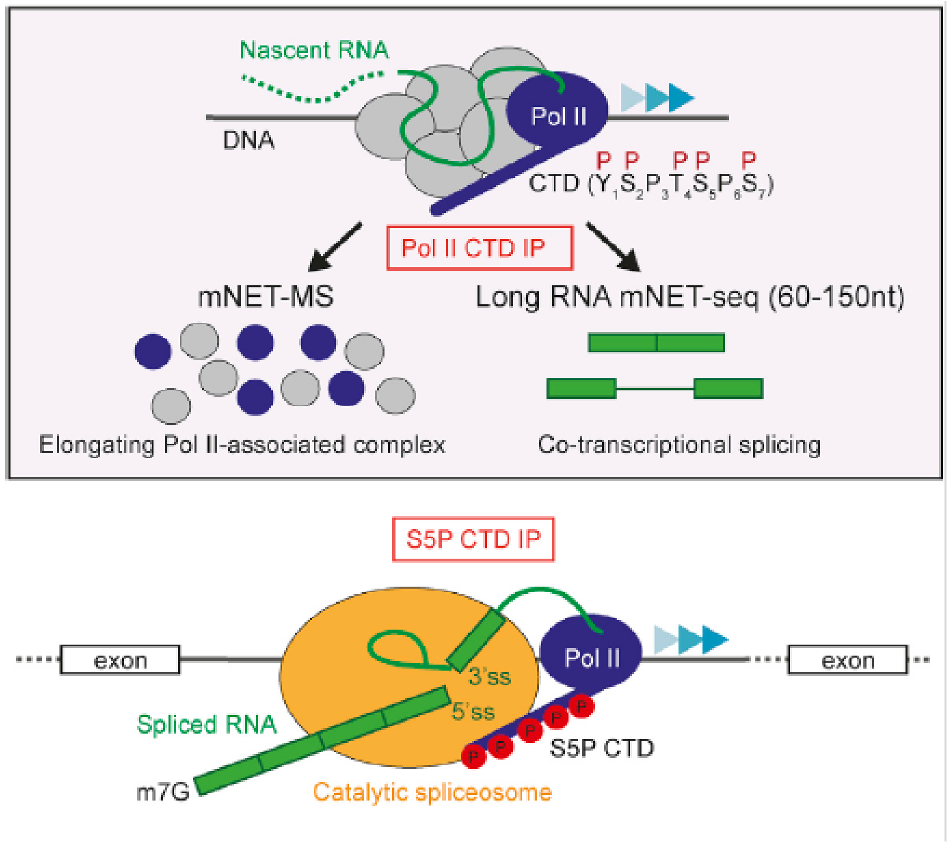
TERMINAISON

FCP1
(recycling)

Splicing co-transcriptionality lies on the C-terminal Domain of the RNAPII and its phosphorylation status

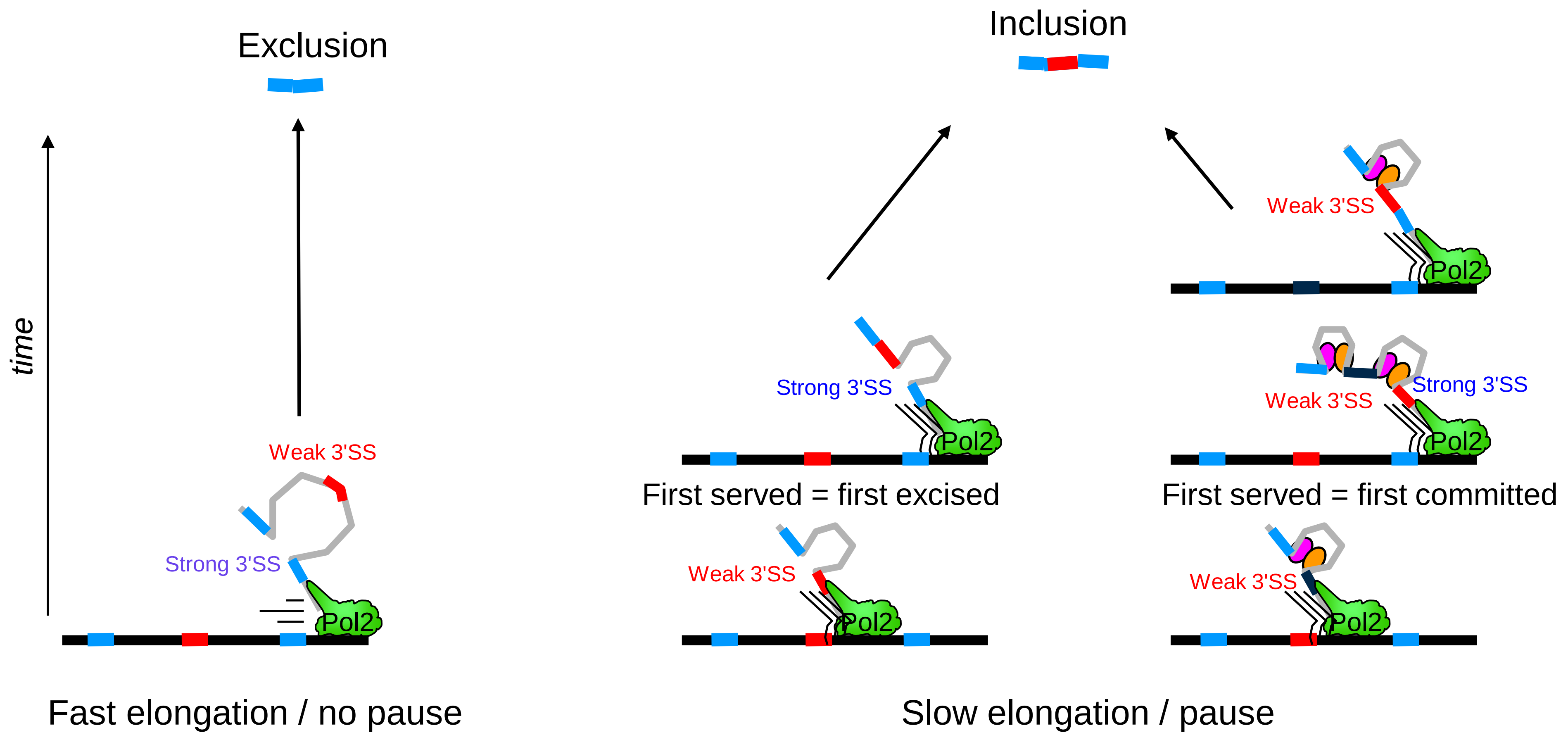
CTD phosphorylation status during the gene transcription





Nojima et al., 2018 Mol. Cell.

First come first served (revisited)



De la Mata et al. 2003 Cell
De la Mata et al. 2010 RNA
Giono & Kornblihtt 2020 JB

Alternative splicing is also regulated by transcription.

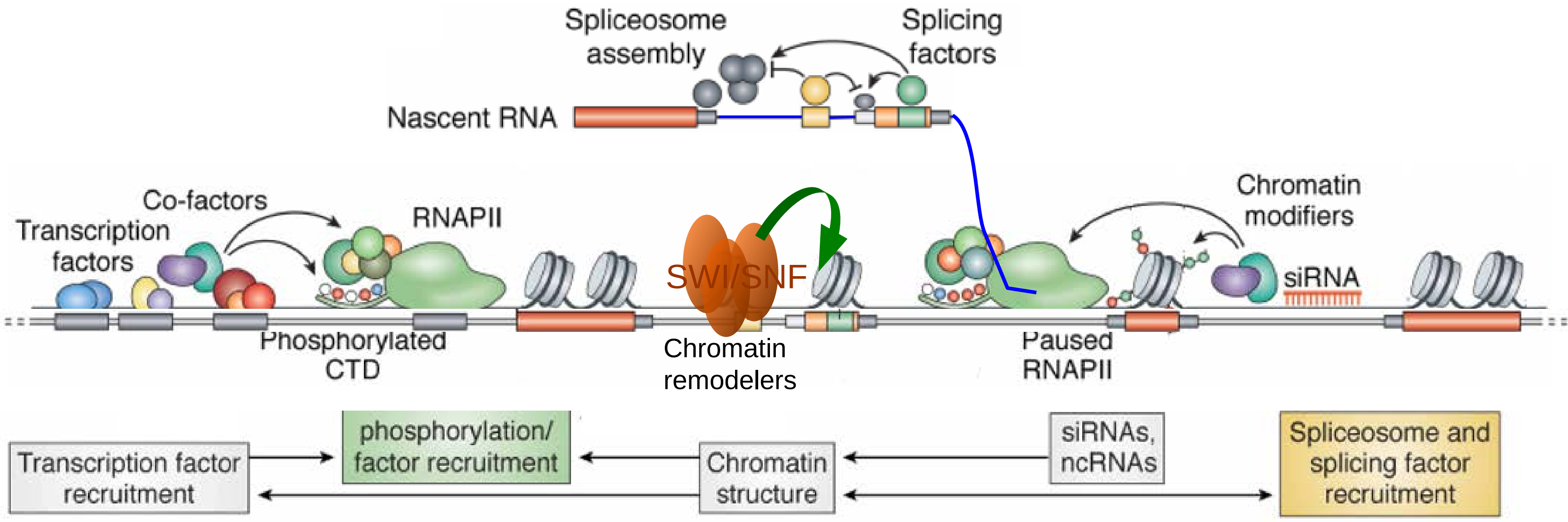
Overall, the inclusion of variant exons coincides with the slowing down of Pol2.

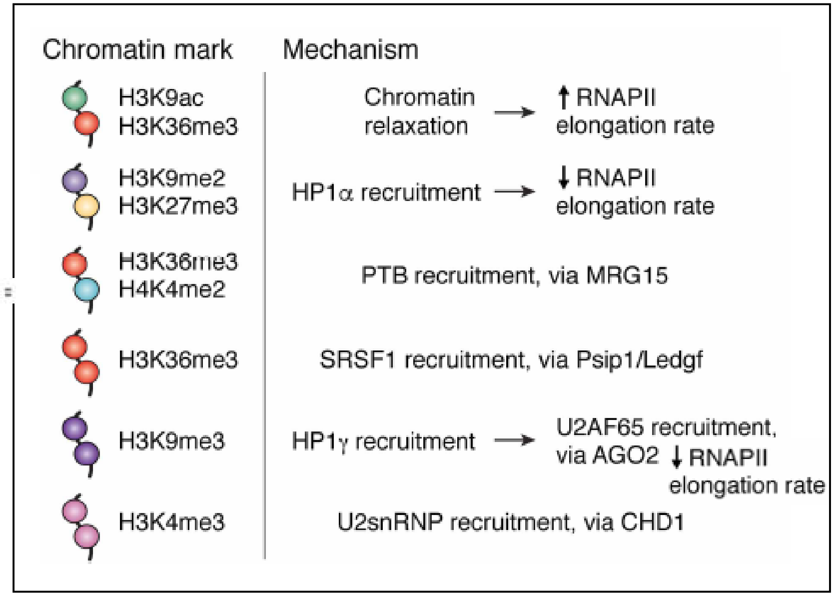
Slow RNA Pol2

Fast RNA Pol2

Variant exon can be included

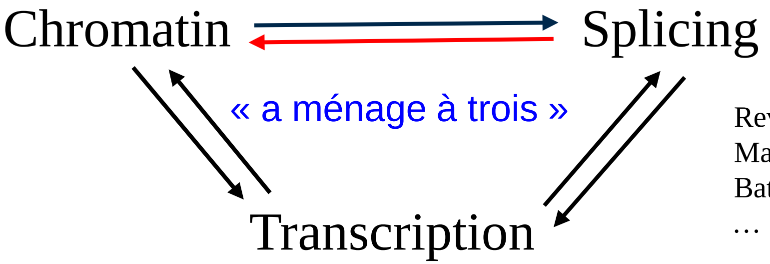
Alternative splicing decisions are linked to the chromatine





Chromatin mark	Mechanism
H3K9ac H3K36me3	Chromatin relaxation → ↑ RNAPII elongation rate
H3K9me2 H3K27me3	HP1α recruitment → ↓ RNAPII elongation rate
H3K36me3 H4K4me2	PTB recruitment, via MRG15
H3K36me3	SRSF1 recruitment, via Psip1/Ledgf
H3K9me3	HP1γ recruitment → U2AF65 recruitment, via AGO2 ↓ RNAPII elongation rate
H3K4me3	U2snRNP recruitment, via CHD1

Reviewed in Giono & Kornblihtt 2020

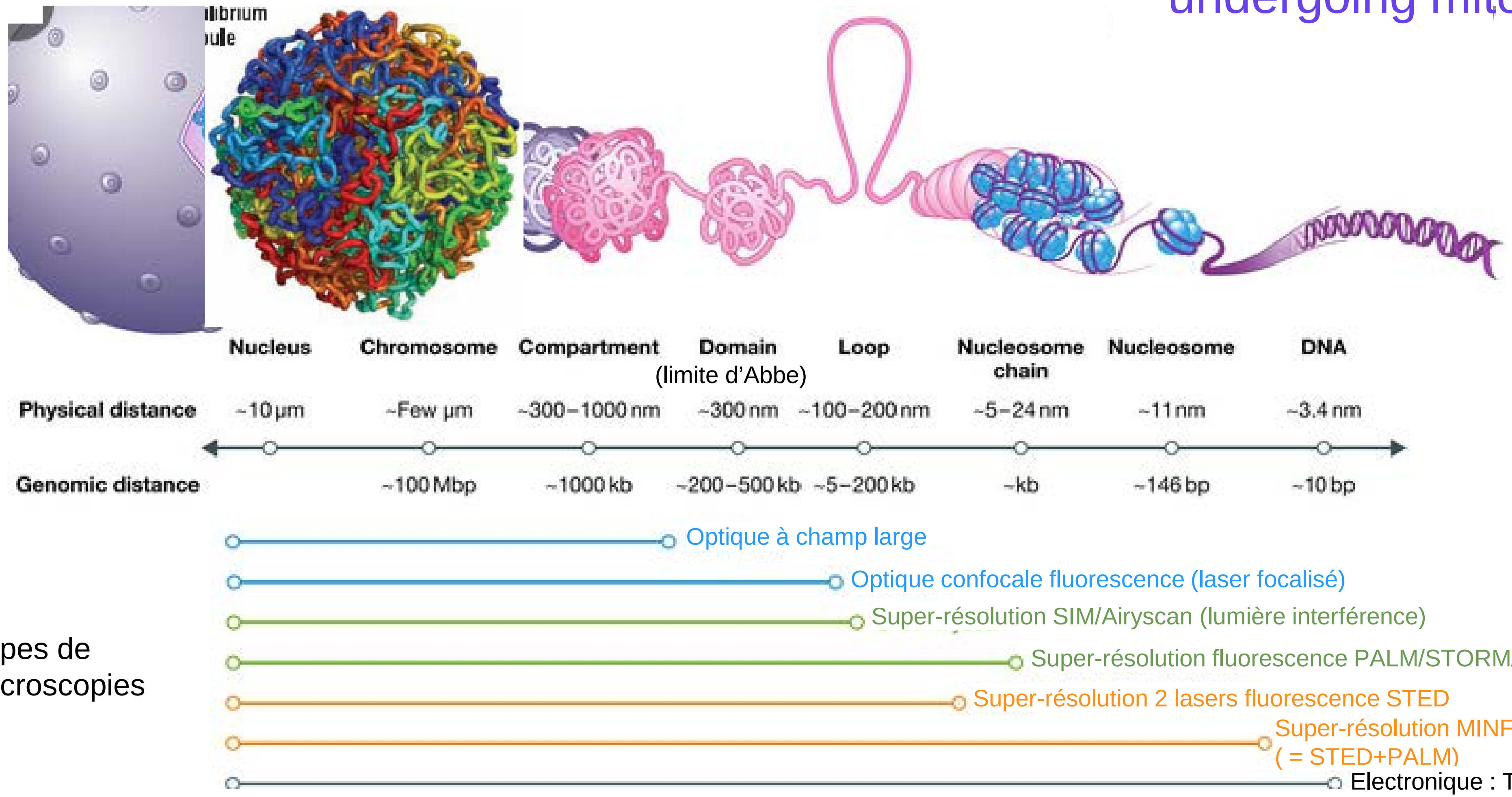


Reviewed in :
Mauger et al., 2015 *Nuc. Ac. Res.*
Batsché et al. 2015 *WIRE RNA*
…

chromatin and splicing

Chromatin: complex carrying genetic material

DNA is not free in the nucleus. Chromosomes are unpacked in cells that are not undergoing mitosis (cell division)



(limite d'Abbe)

Types de microscopies

Optique à champ large

Optique confocale fluorescence (laser focalisé)

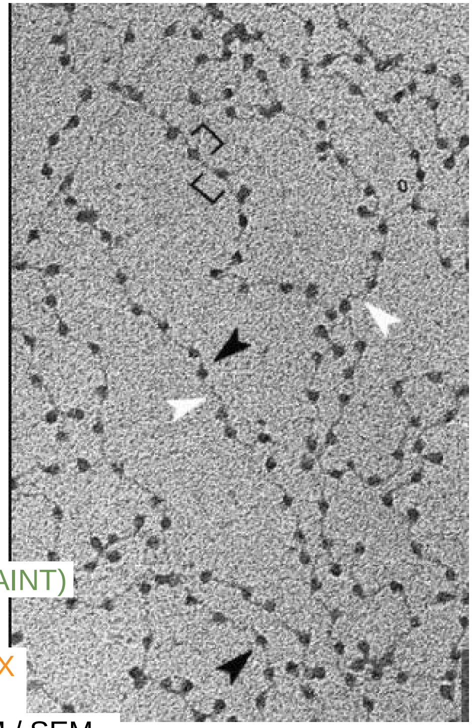
Super-résolution SIM/Airyscan (lumière interférence)

Super-résolution fluorescence PALM/STORM/ PAINT)

Super-résolution 2 lasers fluorescence STED

Super-résolution MINFLUX
(= STED+PALM)

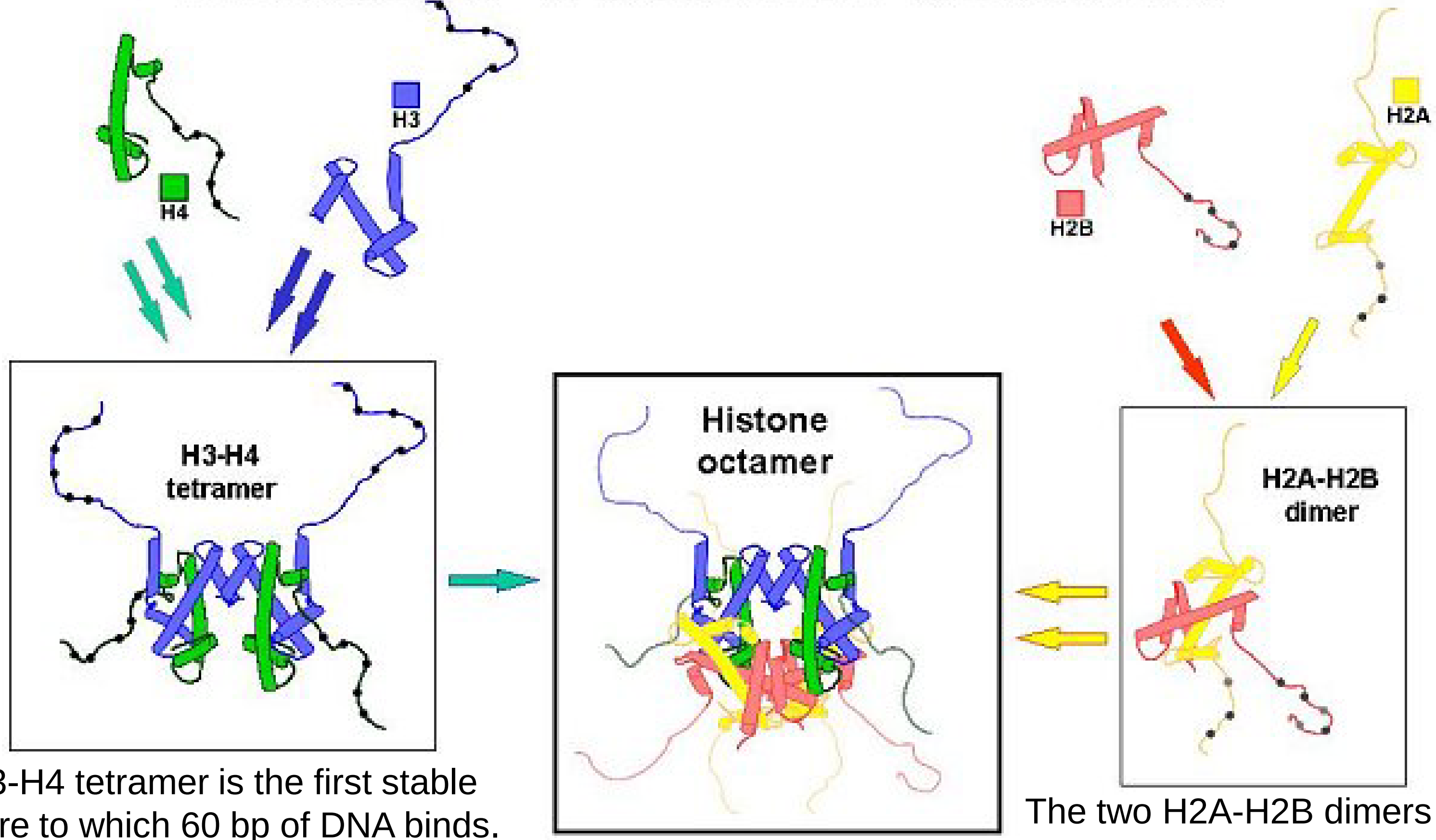
Electronique : TEM / SEM



Nucleosomes: repeating units of chromatin

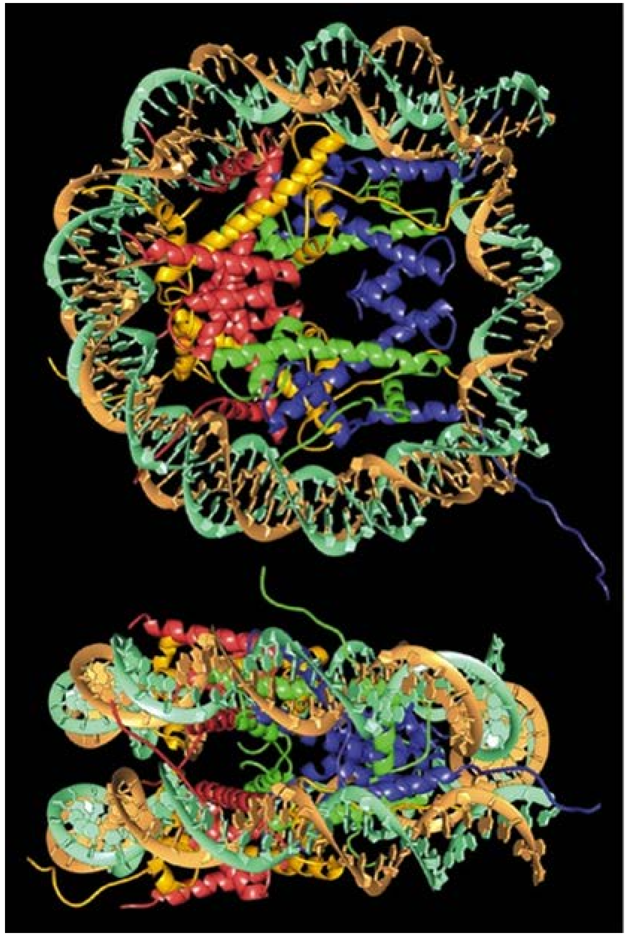
Nucleosomes = histone octamer
+ 146-7 pairs of nucleotides (1.65 turns)

Kornberg, R.D. (1974). *Science*



The H3-H4 tetramer is the first stable structure to which 60 bp of DNA binds.

The two H2A-H2B dimers then bind to the H3-H4-DNA complex.

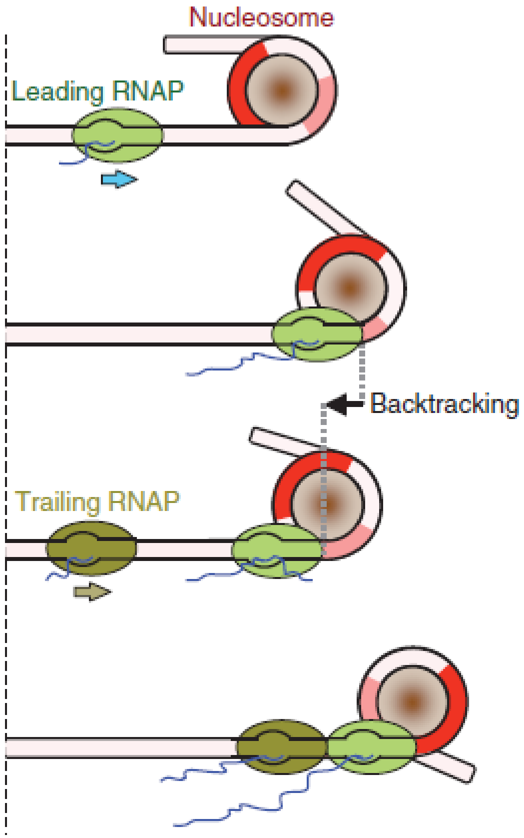


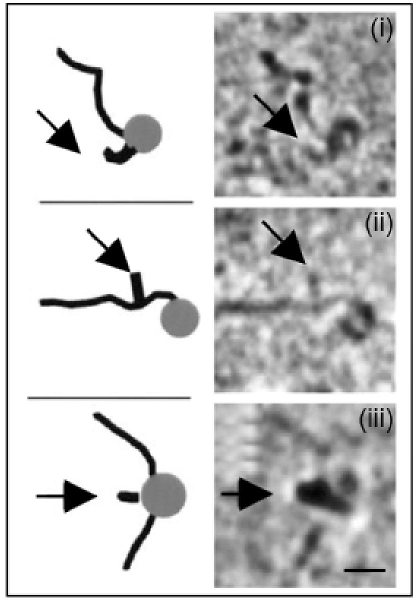
Luger et al. (1997). "Crystal structure of the nucleosome core particle at 2.8 Å resolution" — *Nature* 389(6648):251–260.

The nucleosome is complete when the DNA wraps around the octamer.

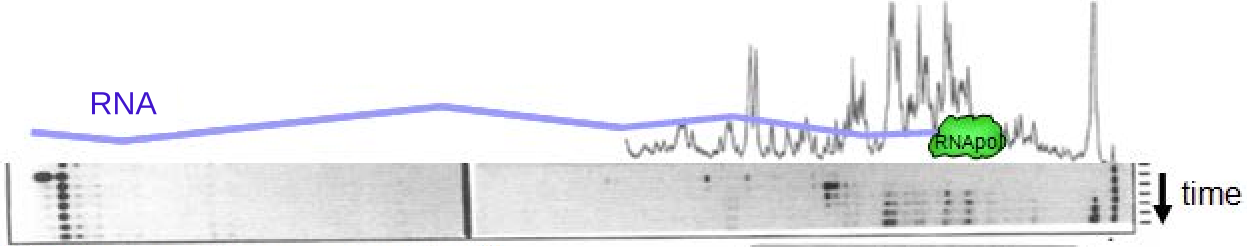
RNA polymerases II are blocked by the nucleosome.

Chromatin is a barrier to transcription in vitro

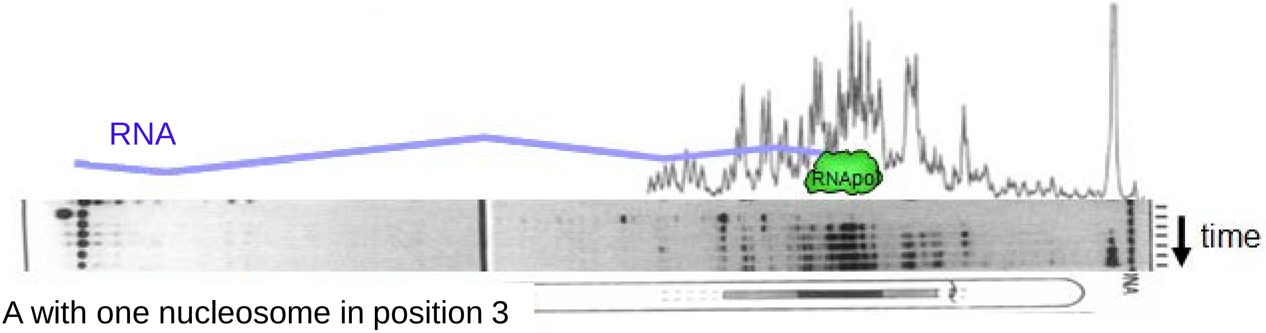




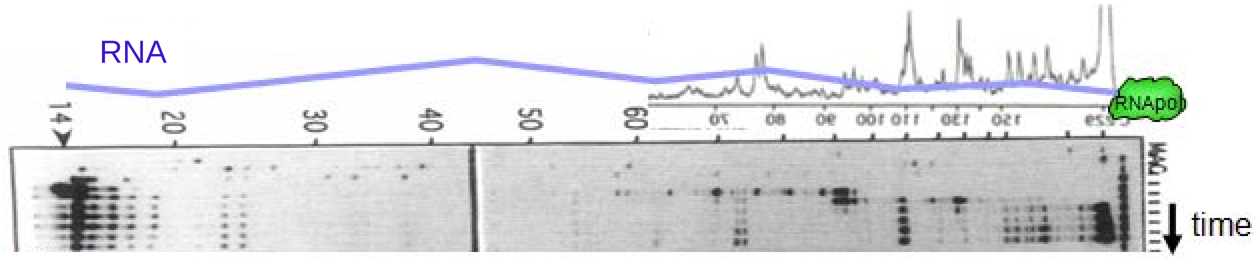
Jin et al. 2010 in NSMB



DNA with one nucleosome in position 2

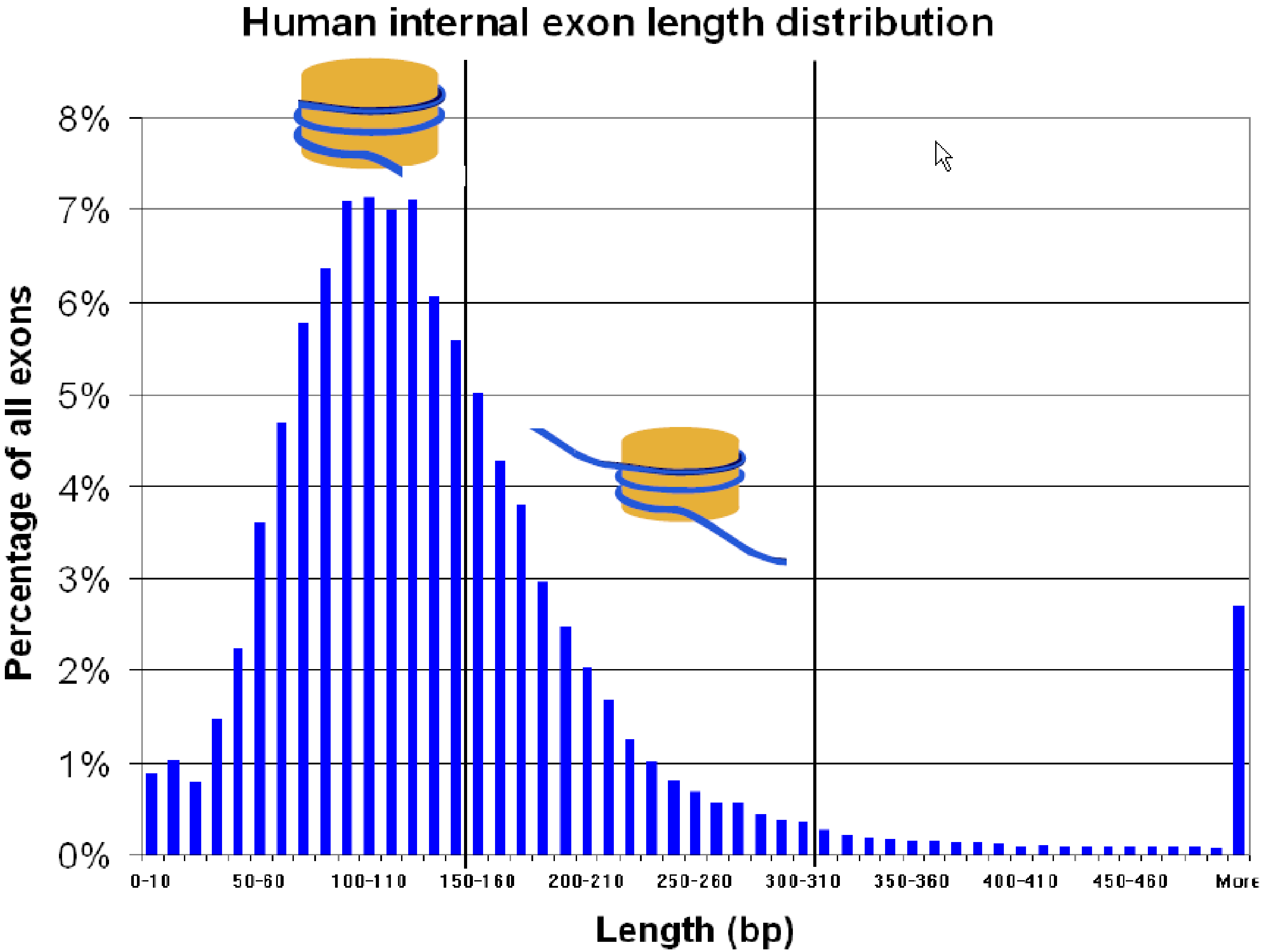


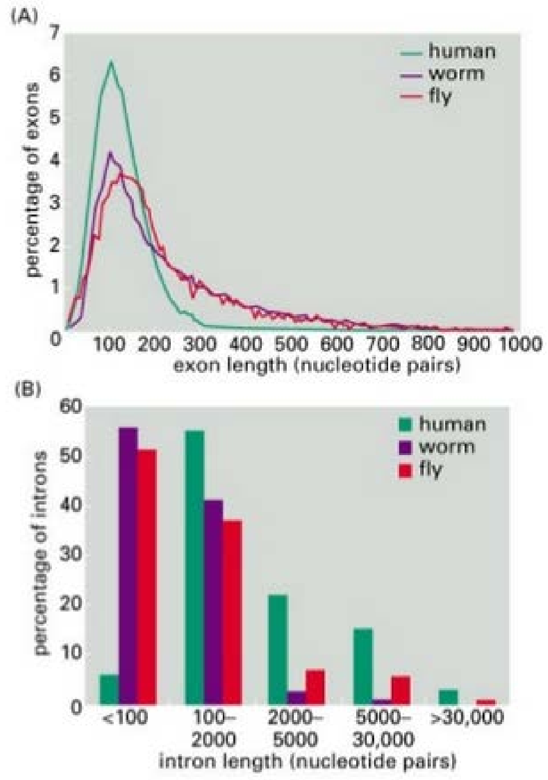
DNA with one nucleosome in position 3



Naked DNA

Studisky et al. 1995 Cell





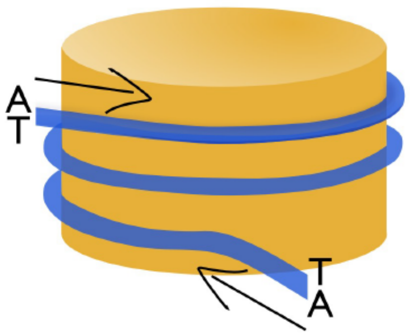
Exons are similar in size

Introns are highly variable in size

Bioinformatic reassessment

Genome-wide nucleosome positioning maps in CD4+ T cells

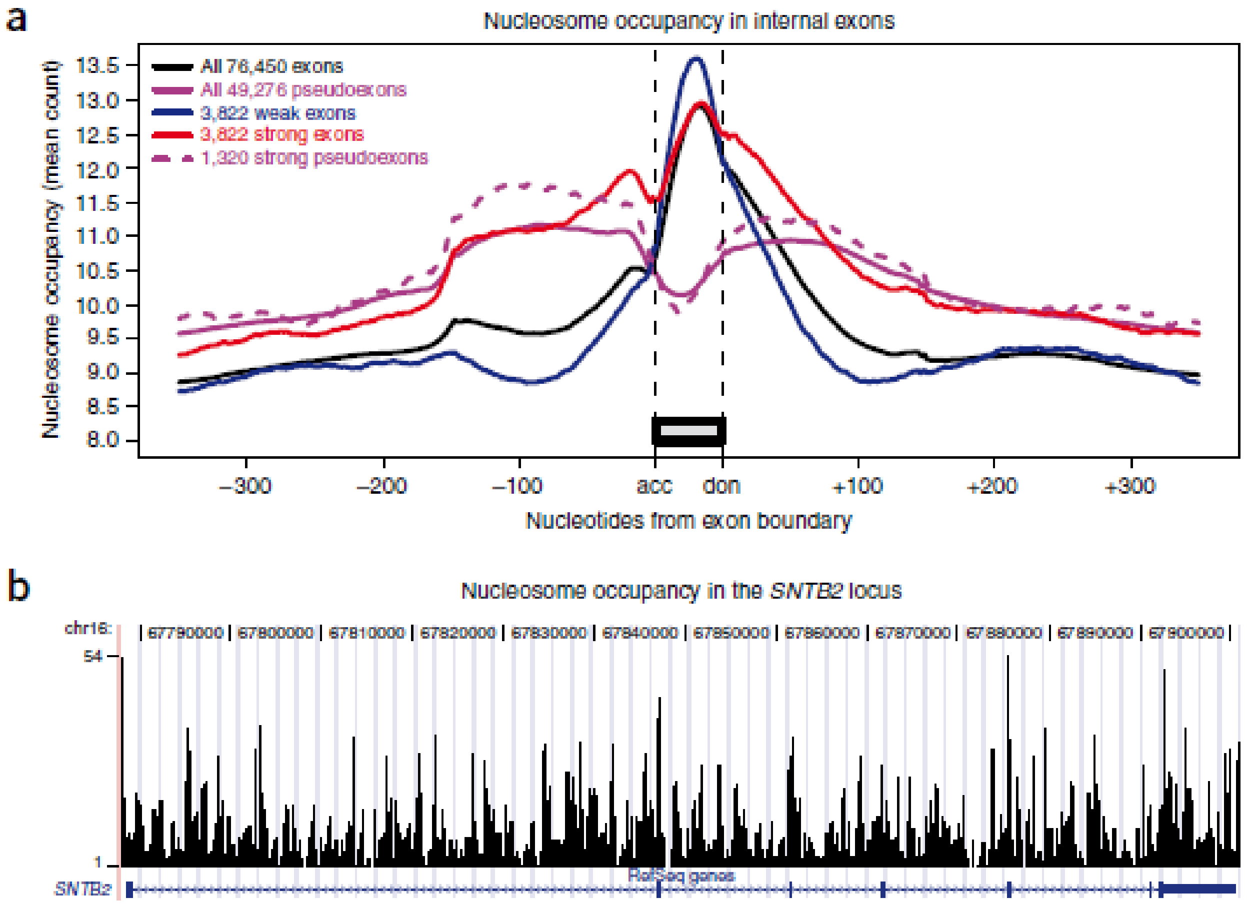
- Transcription start site studies by Barski, Schones *et al*.
- Linker DNAs are cut and consumed with MNase treatment
- Up to 36 bp from the 5’ ends of the chromatin associated DNA strands is deep sequenced by Solexa and aligned with the reference genome
- Barski: ChIP-Seq
- Schones: just Seq



(Raw data from
Barski *et al.*, 2007,
Schones *et al.*, 2008)

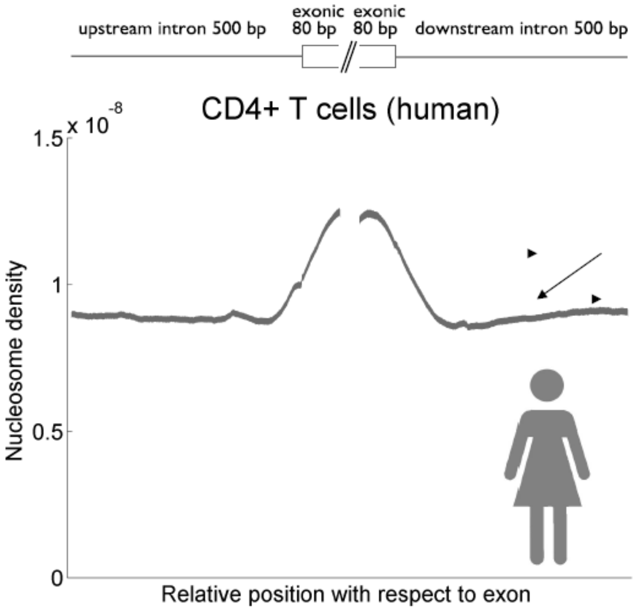
Nucleosome positioning as a determinant of exon recognition

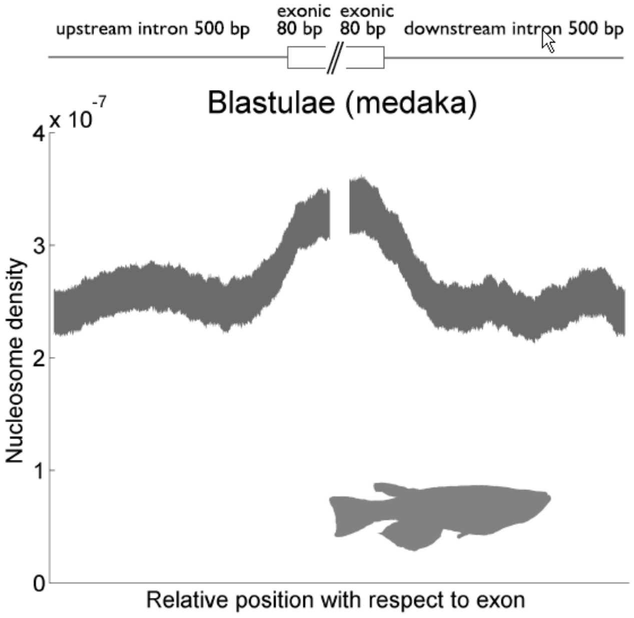
Hagen Tilgner[1,3], Christoforos Nikolaou[1,3], Sonja Althammer[1], Michael Sammeth[1], Miguel Beato[1],
Juan Valcárcel[1,2] & Roderic Guigó[1]



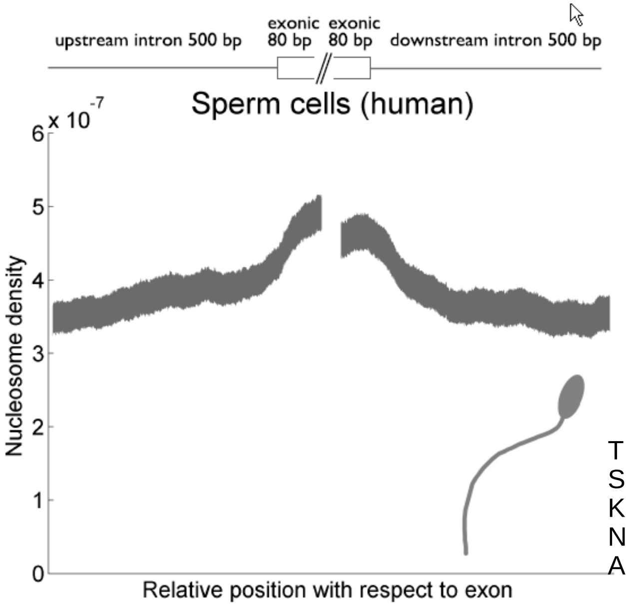
Nucleosomes appear to be enriched on internal exons and depleted in introns.

The location of internal exons is encoded in the chromatin structure and can be inherited across generations.





(Raw data from Sasaki *et al*., 2009)



(Raw data from Hammoud *et al*., 2009)

Tilgner et al. 2009 NSMB
Schwartz et al. 2009 NSMB
Kolasinska-Zwierz et al. 2009 Nat. Gen.
Nahkuri et al. 2009 Cell cycle
Andersson et al. 2009 Gen. Res.

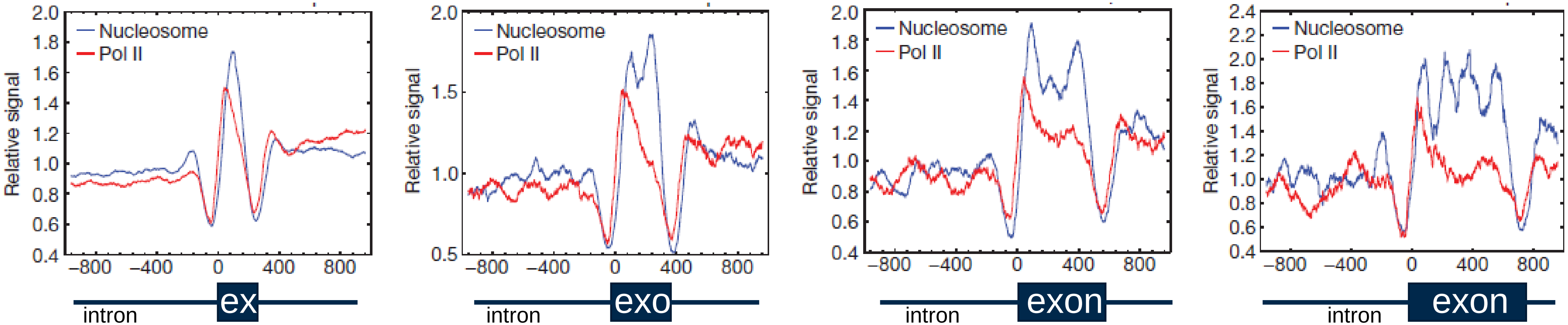
The positioning of nucleosomes on exons coincides with the distribution of RNA polymerase II.

In vivo, when nucleosomes are less mobile at a specific location in the chromatin (exons), RNA-Pol2 is more frequently found at the first nucleosome.

ChIP-Seq signal average

Accumulation of nucleosomes on exons

→ Direction of Pol2 RNA movement



Chodavarapu et al. (2010) in *Nature*

Taille des exons: 170-240 bp 315-350 bp 480-500 bp 645-715 bp

Hard way = slowing-down

Chromatin (real life)

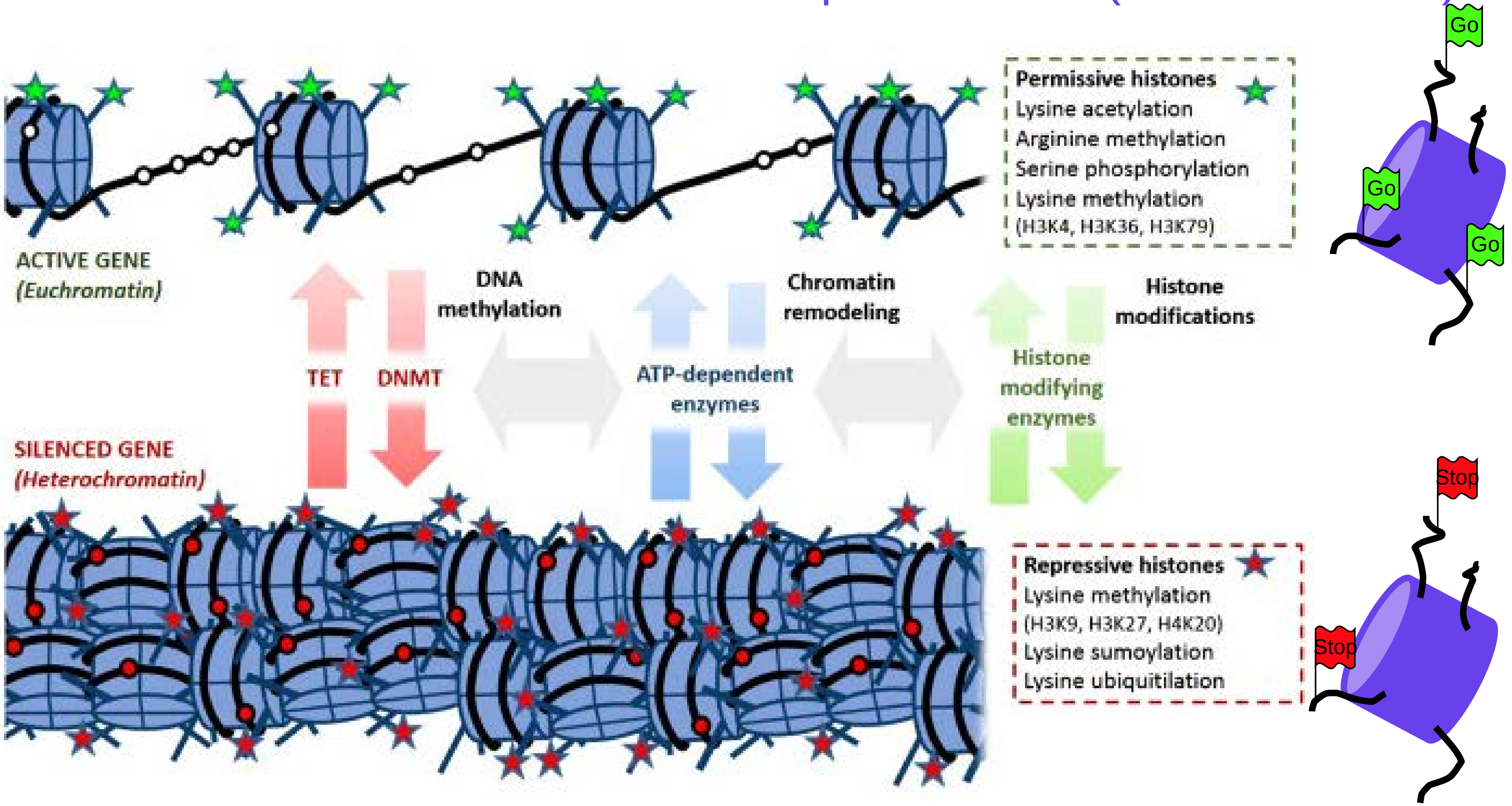
Slow RNAPII

Naked DNA

Fast RNAPII

Euchromatine (ON) or heterochromatine (OFF) ?

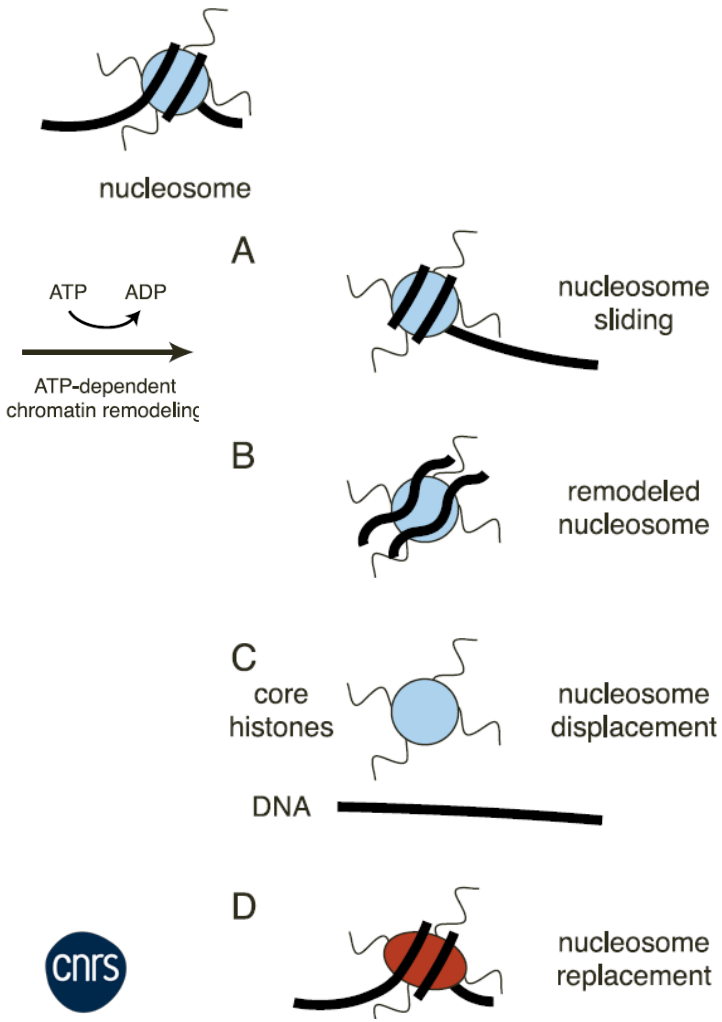
The state of chromatin controls the action of transcription factors (and vice versa).

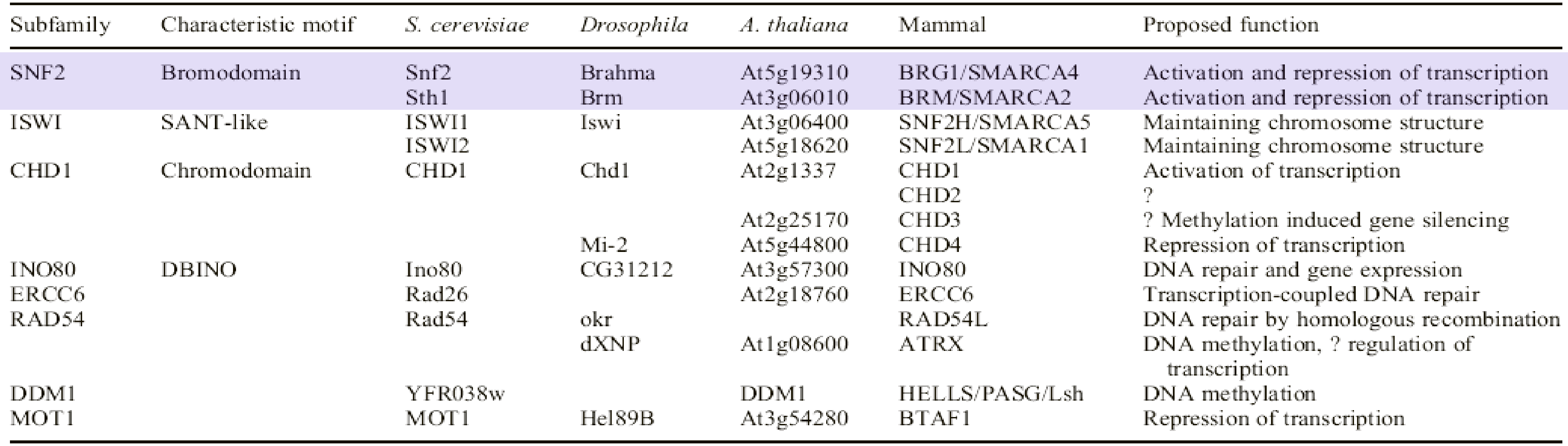


CAUTION: The definition of the role of genes depends on their location within gene regions. Most of these roles are defined according to their role at the promoter level**.**

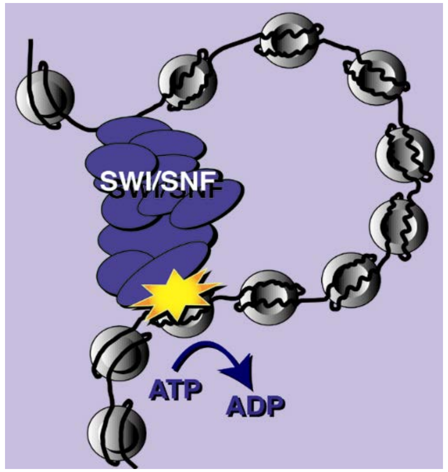
How does chromatin become competent for transcription?

Chromatin remodeling





Subfamily	Characteristic motif	*S. cerevisiae*	*Drosophila*	*A. thaliana*	Mammal	Proposed function
SNF2	Bromodomain	Snf2	Brahma	At5g19310	BRG1/SMARCA4	Activation and repression of transcription
		Sth1	Brm	At3g06010	BRM/SMARCA2	Activation and repression of transcription
ISWI	SANT-like	ISWI1	Iswi	At3g06400	SNF2H/SMARCA5	Maintaining chromosome structure
		ISWI2		At5g18620	SNF2L/SMARCA1	Maintaining chromosome structure
CHD1	Chromodomain	CHD1	Chd1	At2g1337	CHD1	Activation of transcription
					CHD2	?
				At2g25170	CHD3	? Methylation induced gene silencing
			Mi-2	At5g44800	CHD4	Repression of transcription
INO80	DBINO	Ino80	CG31212	At3g57300	INO80	DNA repair and gene expression
ERCC6		Rad26		At2g18760	ERCC6	Transcription-coupled DNA repair
RAD54		Rad54	okr		RAD54L	DNA repair by homologous recombination
			dXNP	At1g08600	ATRX	DNA methylation, ? regulation of transcription
DDM1		YFR038w		DDM1	HELLS/PASG/Lsh	DNA methylation
MOT1		MOT1	Hel89B	At3g54280	BTAF1	Repression of transcription

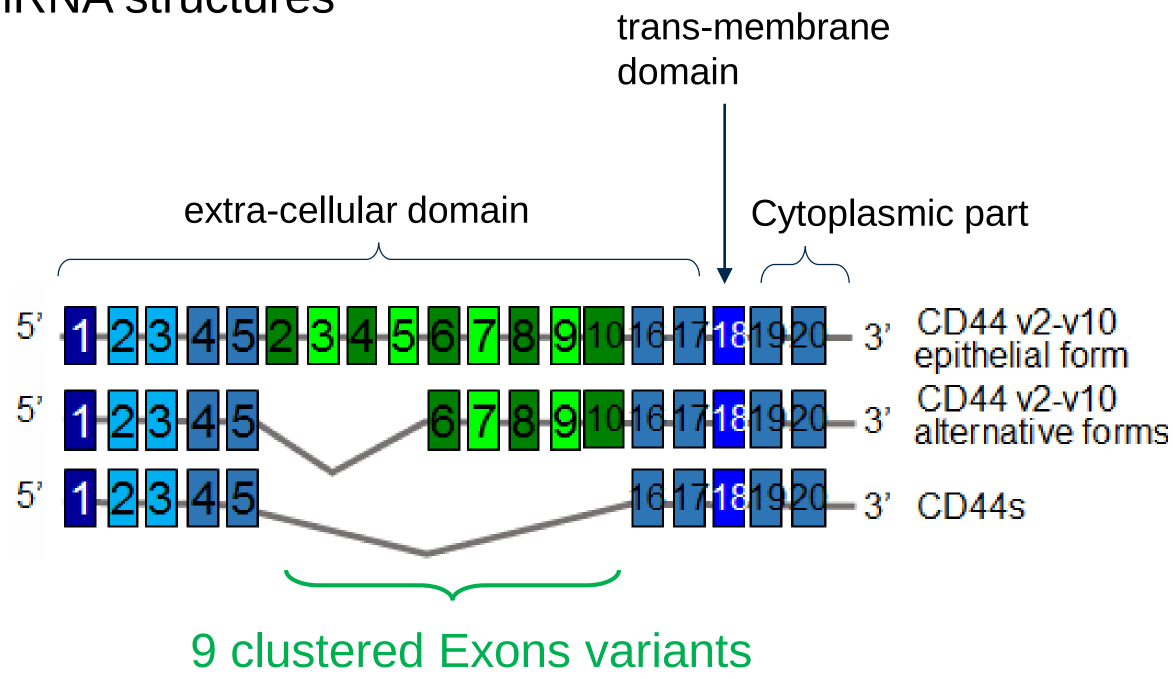


The SWI/SNF chromatin remodelling complex promotes the inclusion of variant exons of

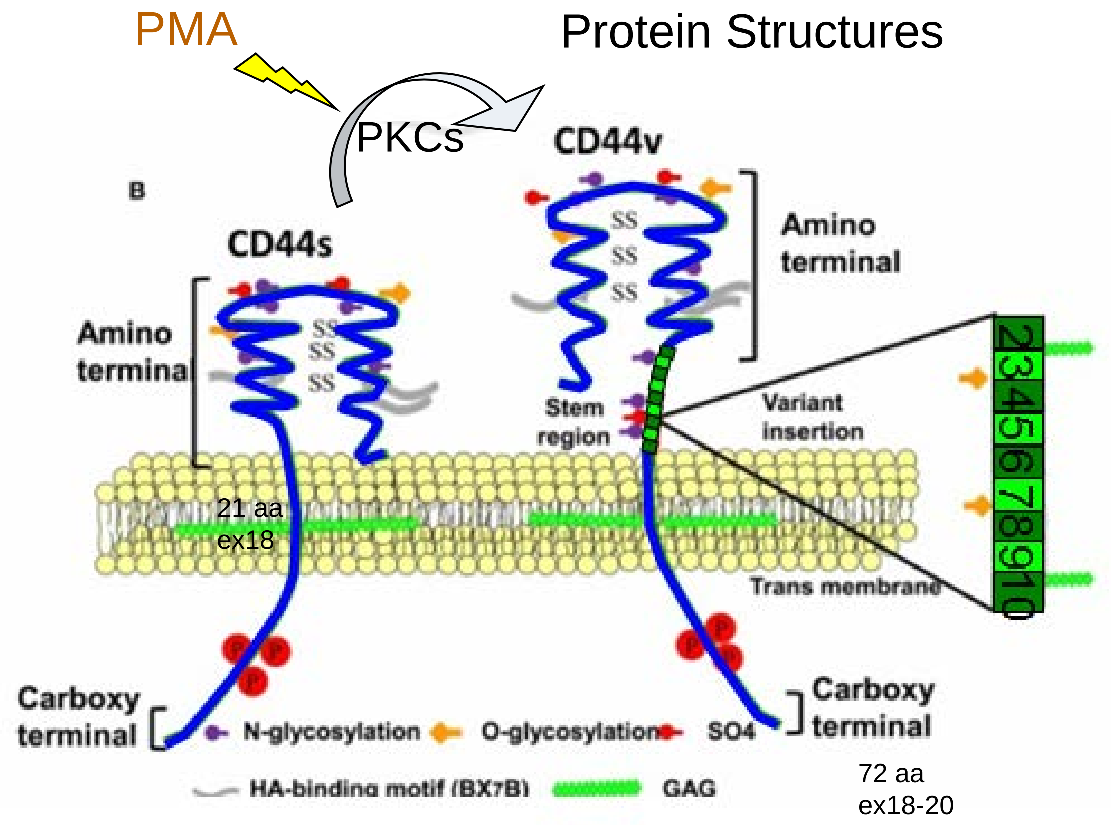
The CD44 gene as a model for alternative splicing

The CD44 gene contains nine variable exons in the centre of two sets of constant exons.

mRNA structures

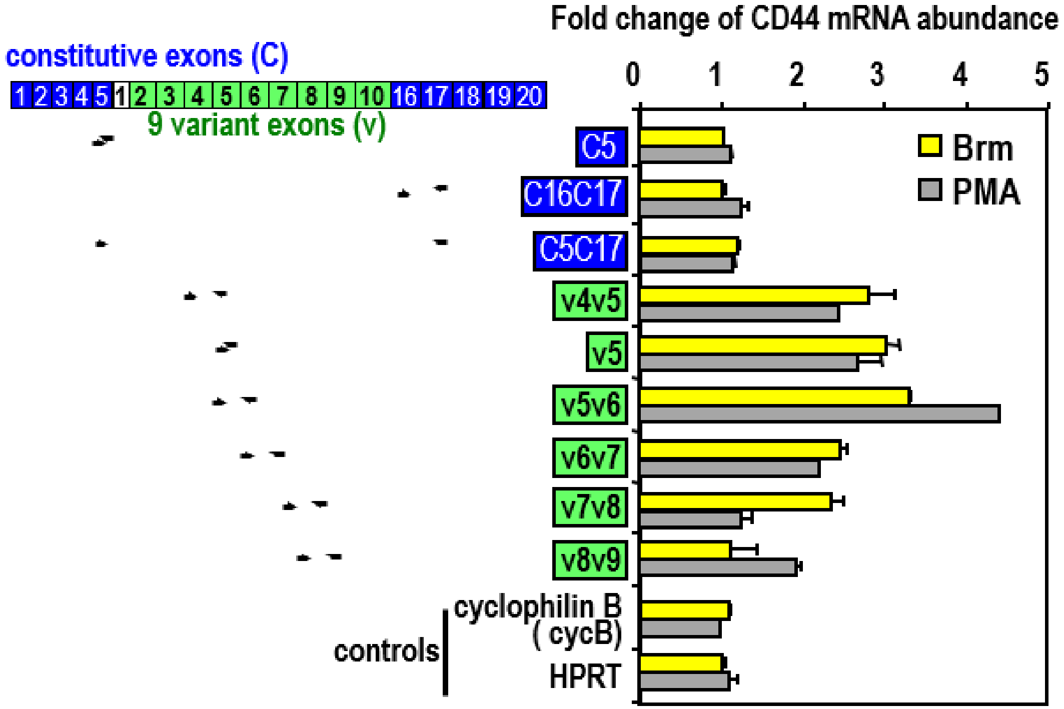


Protein Structures

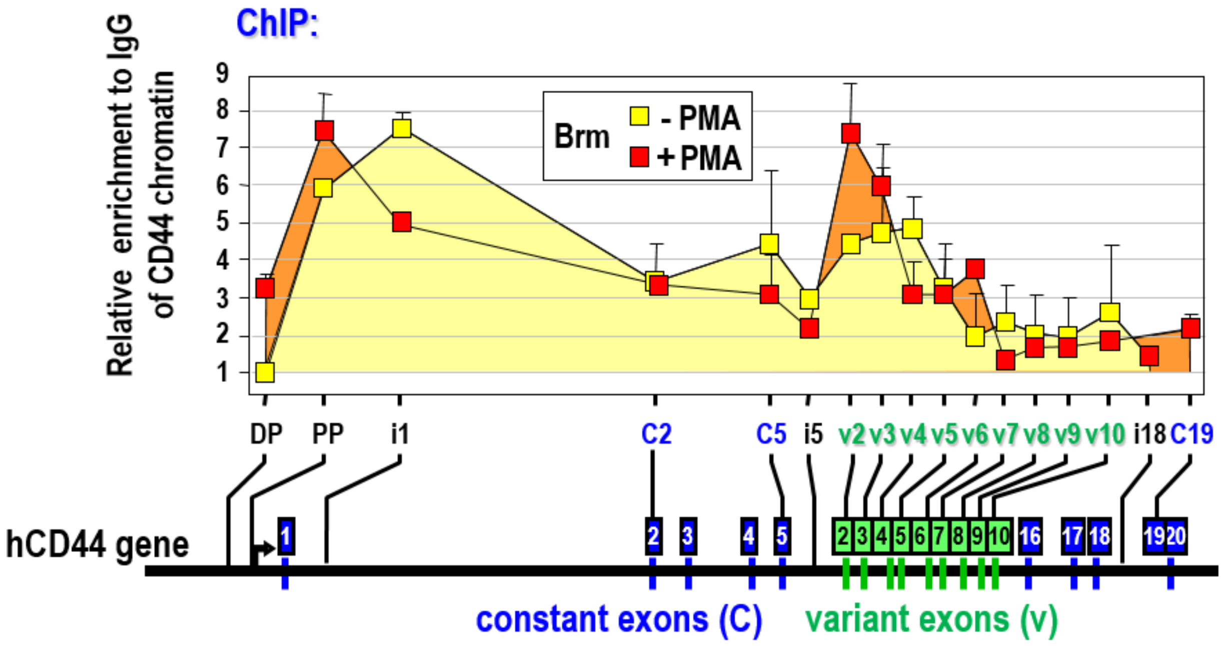


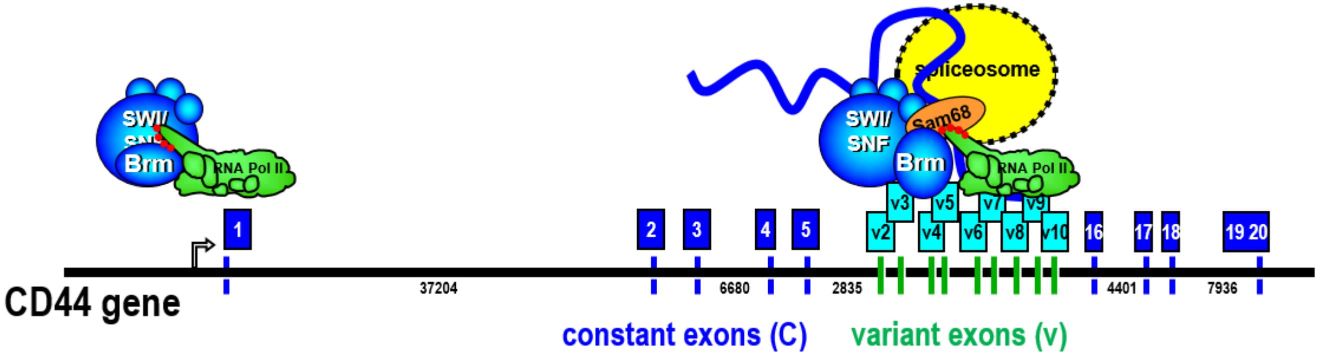
The CD44 gene is a transcriptional target of SWI/SNF and its alternative splicing is regulated by Brm

Real time RT-PCR

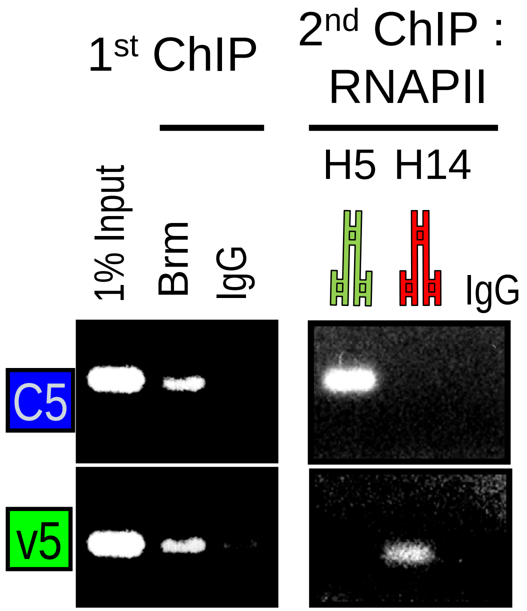


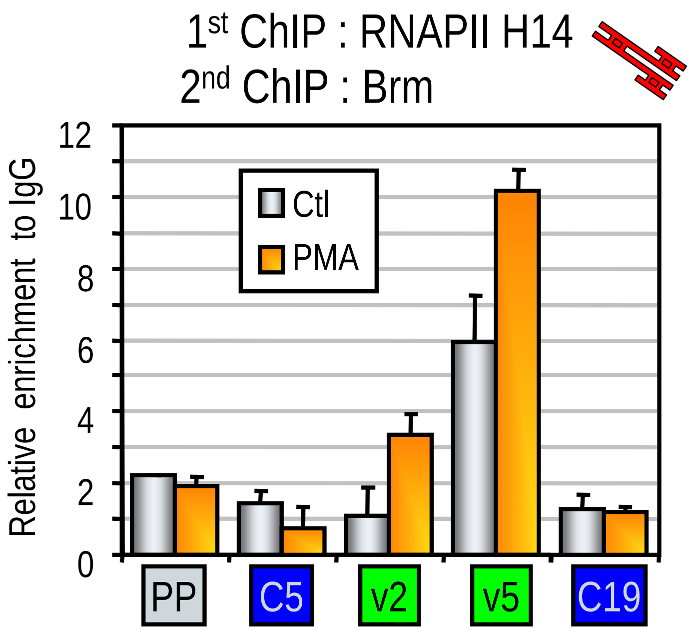
Batsché et al., 2006 NSMB

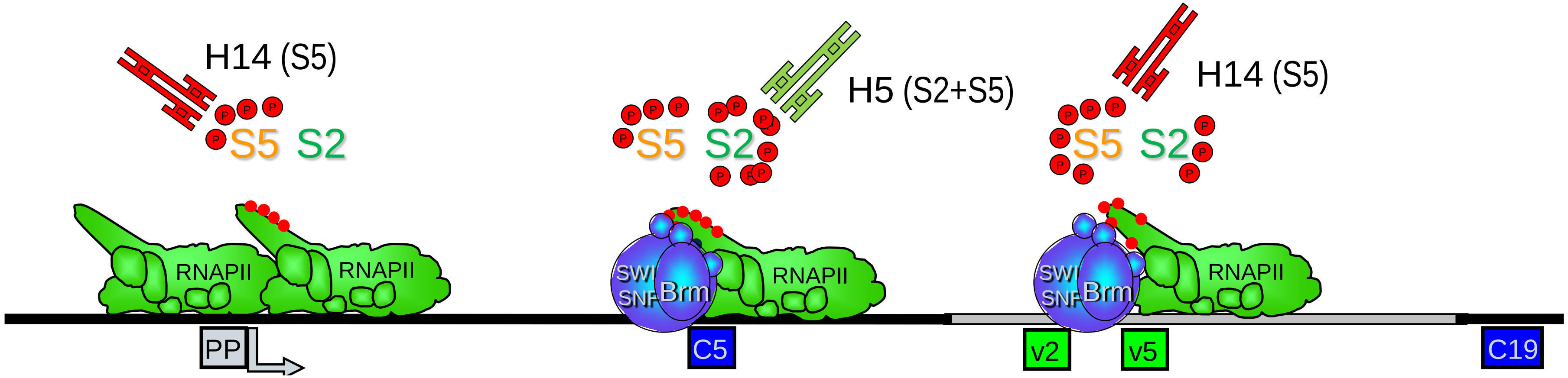




Brm is associated to the S2 or S5 phosphorylated RNAPII depending of its localization in the CD44 gene







"paused RNAPII "
promoter

Tous droits

"fast RNAPII "
constant region

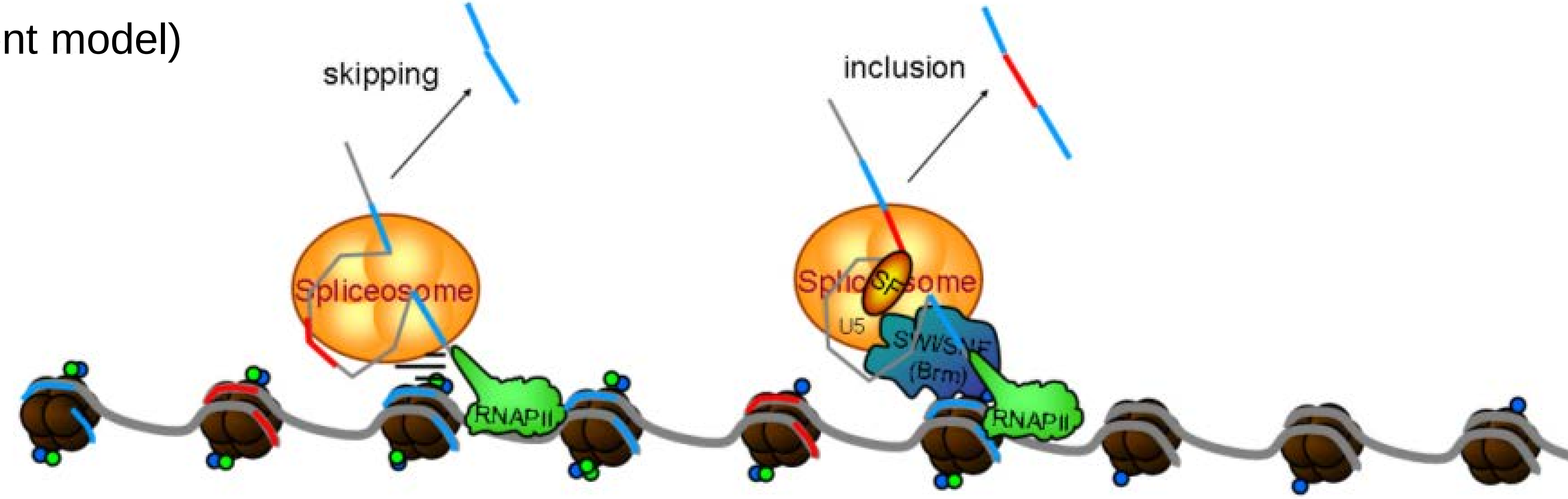
"slow RNAPII"
variable region

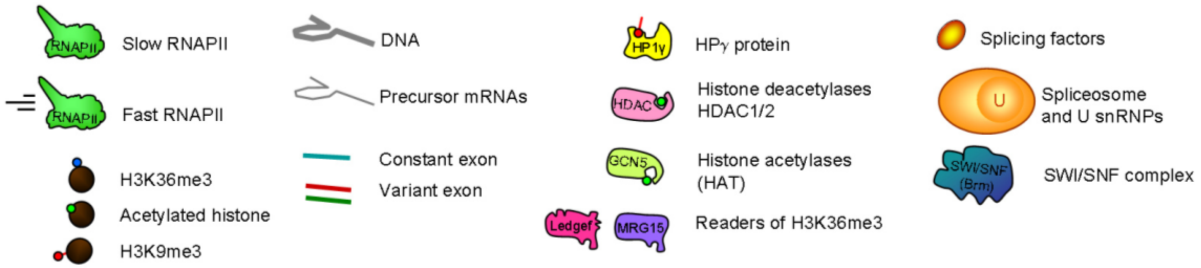
Batsché et al., 2006

Chromatin modifiers affect alternative splicing decisions

SWI/SNF complex favors a slowing-down of elongative RNAPII and interacts with spliceosome components. This promotes inclusion of CD44 alternative exons.

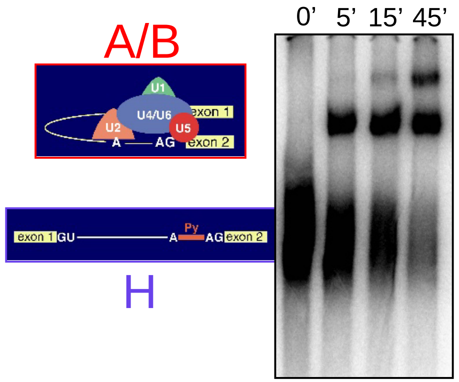
(kinetic and recrutment model)





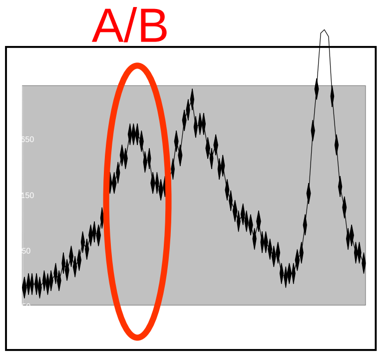
Batsché et al. 2006 *NSMB*
Ito et al., 2008 *Biochem J*
Waldholm et al., 2011 *BMC Mol. Bio*
Zraly and Dingwall, 2012 *NAR*

Spliceosome purified with U2 snRNP



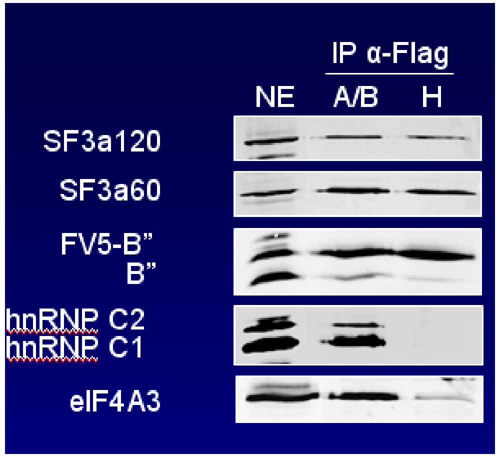
Spliceosomal complexes

chromatography →



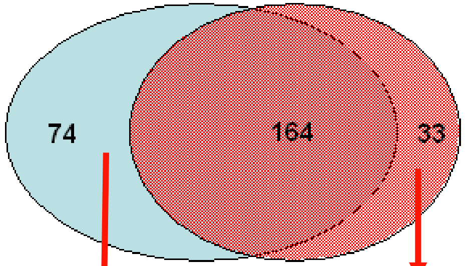
Gel filtration

IP of Flag-B''



Western blot analysis

Mass spectrometry analysis



New factors associated to Spliceosome

Factors previously described

But also among others :

- AGO1
- AGO2
- DICER1
- HP1g
- HP1BP3
- EHMT1/2
- MBD3
- ZBTB33 (Kaiso)
- RB1
- TAFII250
- NELFB
- …

- BAF200
- Brm / Brg1
- BAF170
- BAF155
- Snf5/Ini1
- BAF60a/b/c
- BAF57
- mSIN3A
- HDAC1
- HDAC2
- RbAP48

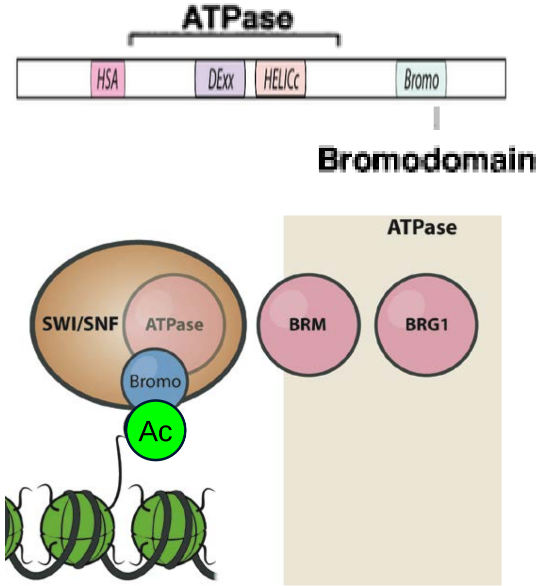
SWI/SNF factors Associated to Spliceosome

Allemand et al., 2016 PloS Genet.

Chromatin remodellers recognise modified nucleosomes

4 main families of complexes:

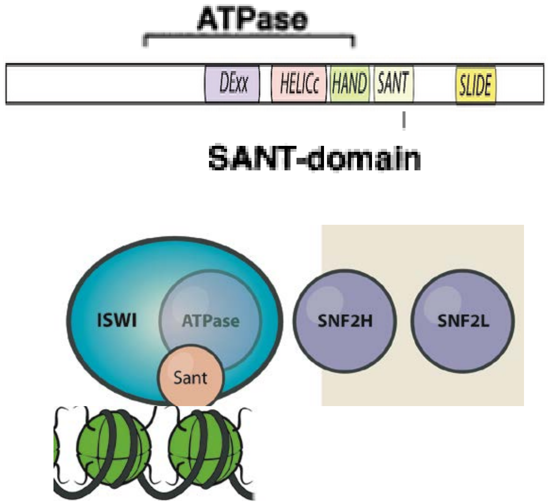
SWI/SNF (BAF, PBAF)



Activation and/or repression

Nucleosome sliding

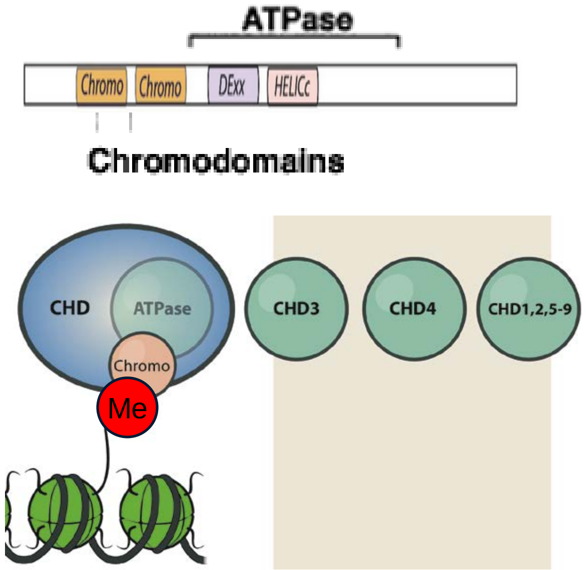
ISWI (NURF, BPTF, ACF1, CHRAC, RSF…)



Transcriptional Activation

nucleosome assembly
nucleosome sliding

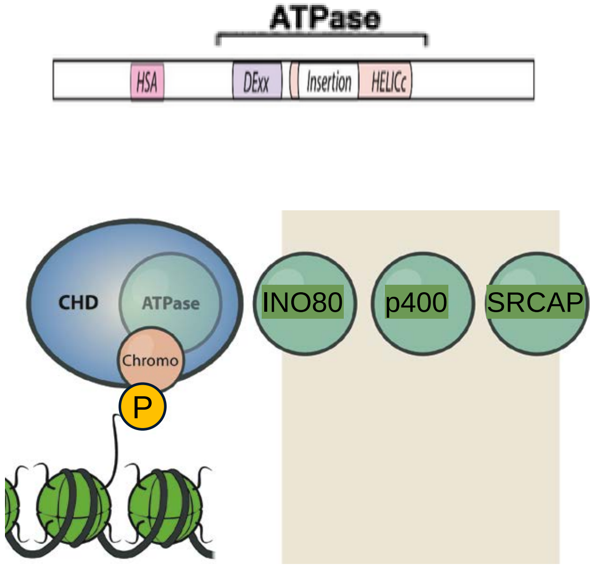
Mi-2 (CHDs, NURD)



Transcriptional Repression

nucleosome assembly
nucleosome sliding

INO80 (p400, SRCAP)



Activation and/or repression

Nucleosome editing
replacement of histones

Epigenetic regulation of alternative splicing

Molecular supports for epigenetics

m
hm
ADN
ca
f

histones
me
ac
p
cit
ub

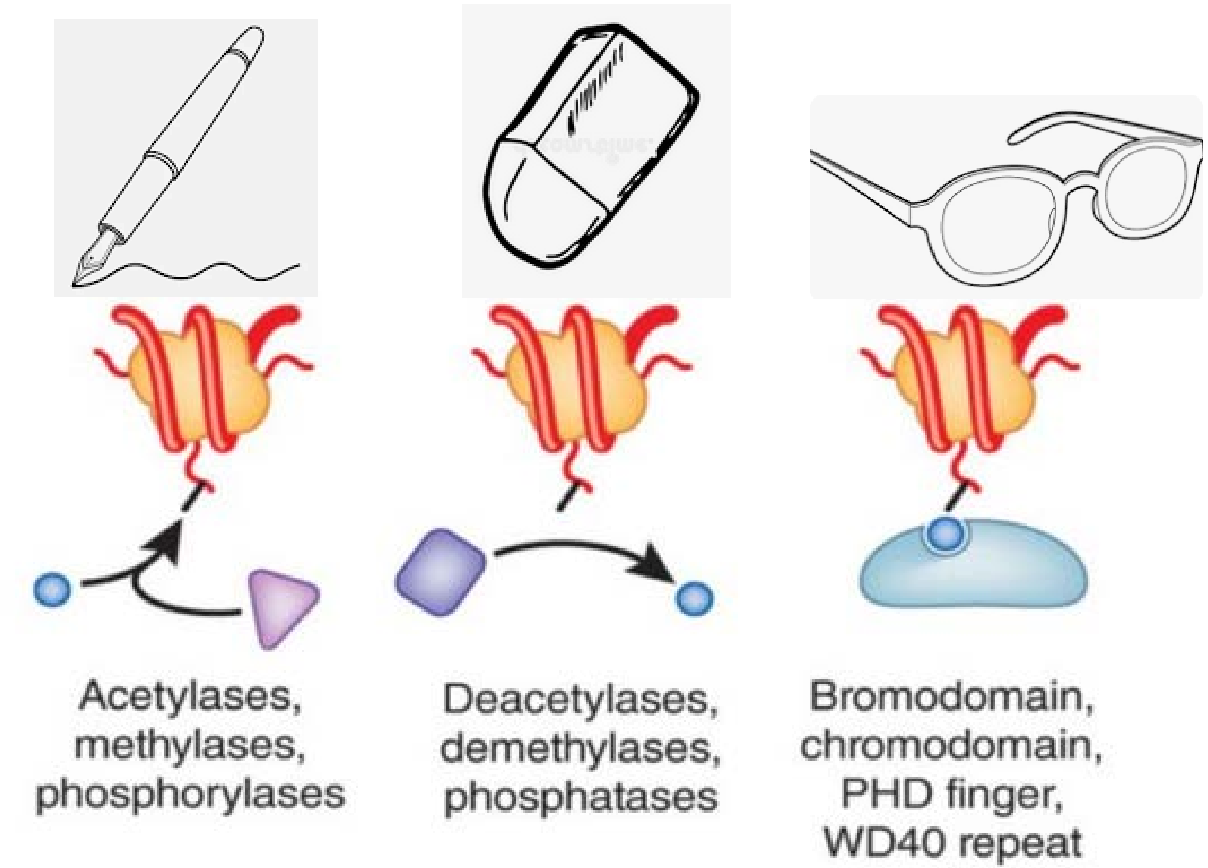
ARN
mi
nc
lnc

1948 (Hotchkiss)
Méthylation de l’ADN (5mC)

1967 (Allfrey et al.) acétylation
1970-80 Méthylation
1997 « code des histone »
(T. Jenuwein & D. Allis)

1980 lncRNA XIST
1993 / 2006 miRNA /siRNA / piRNA
2000s lncRNAs : HOTAIR, MALAT…

The players in epigenetic regulation



The histone code

Post-translational modifications of histones

Nomenclature

amino acid residue

type of modification

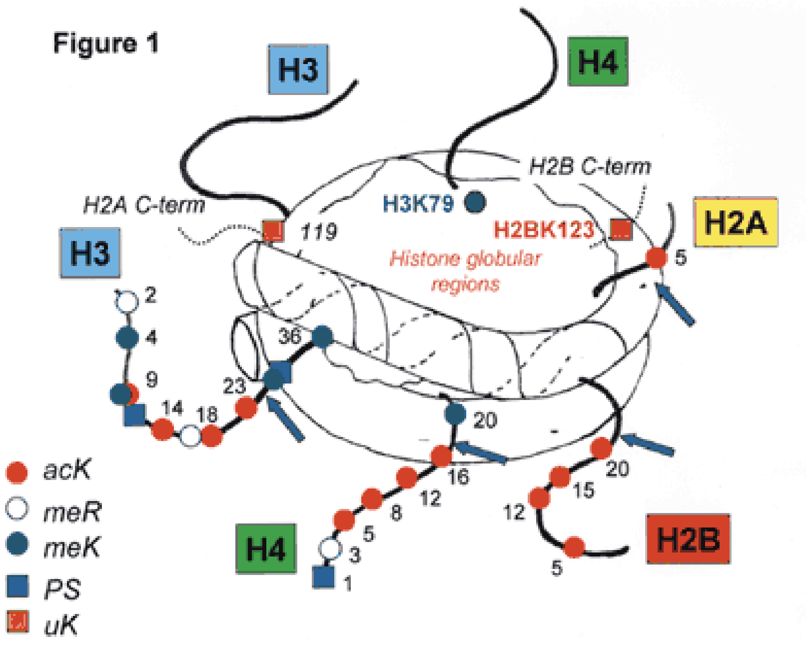
H3K27me3

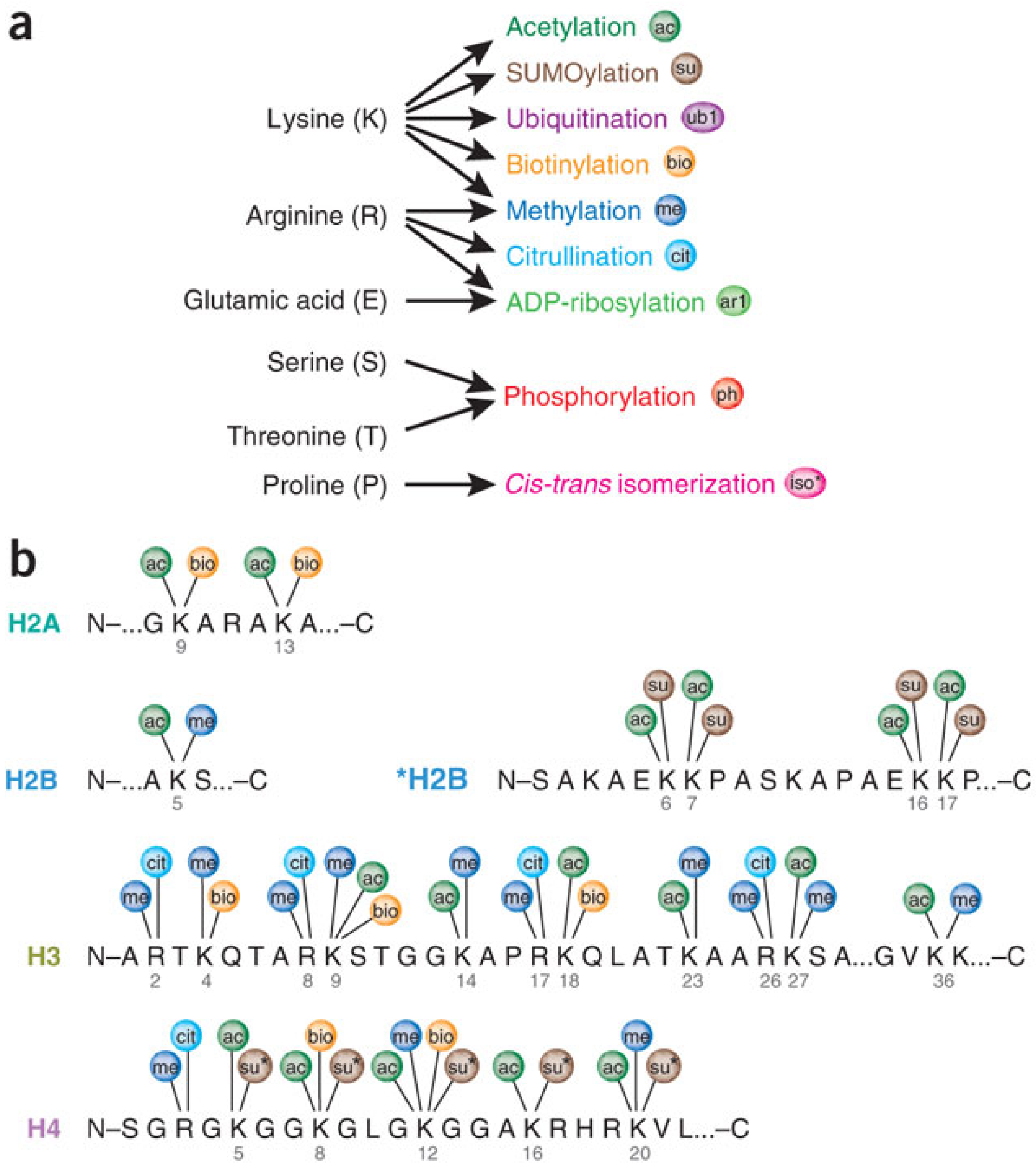
histone 3

position of the amino acid from the N terminal

Tri-méthylation
de la Lysine 27
de l'histone H3

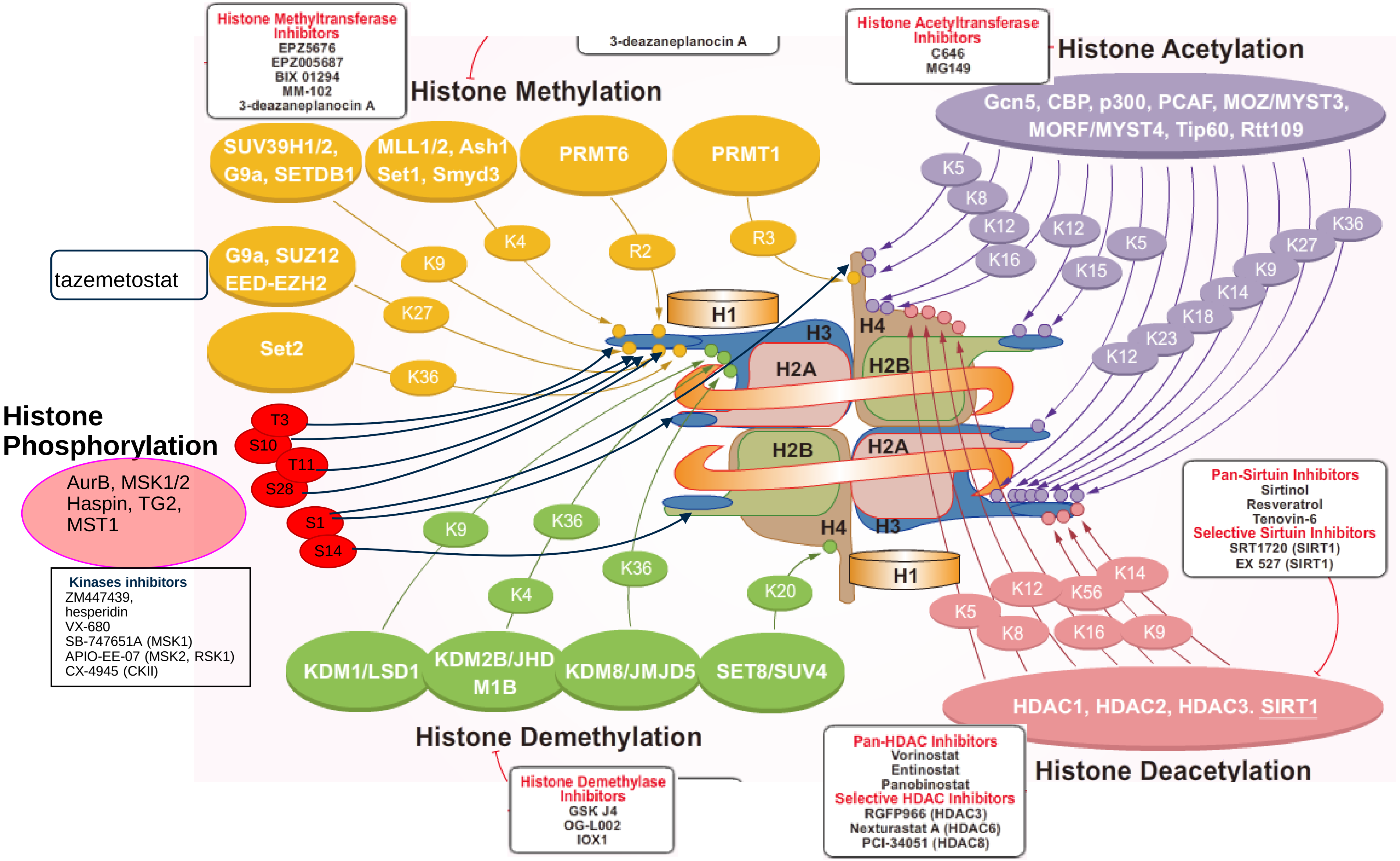
Figure 1





Katie Ris-Vicari

Some histone modifiers



Histone Methyltransferase Inhibitors
EPZ5676
EPZ005687
BIX 01294
MM-102
3-deazaneplanocin A

3-deazaneplanocin A

Histone Methylation

tazemetostat

Histone Acetyltransferase Inhibitors
C646
MG149

Histone Acetylation

Histone Phosphorylation

AurB, MSK1/2
Haspin, TG2,
MST1

Kinases inhibitors
ZM447439,
hesperidin
VX-680
SB-747651A (MSK1)
APIO-EE-07 (MSK2, RSK1)
CX-4945 (CKII)

Pan-Sirtuin Inhibitors
Sirtinol
Resveratrol
Tenovin-6
Selective Sirtuin Inhibitors
SRT1720 (SIRT1)
EX 527 (SIRT1)

Histone Demethylation

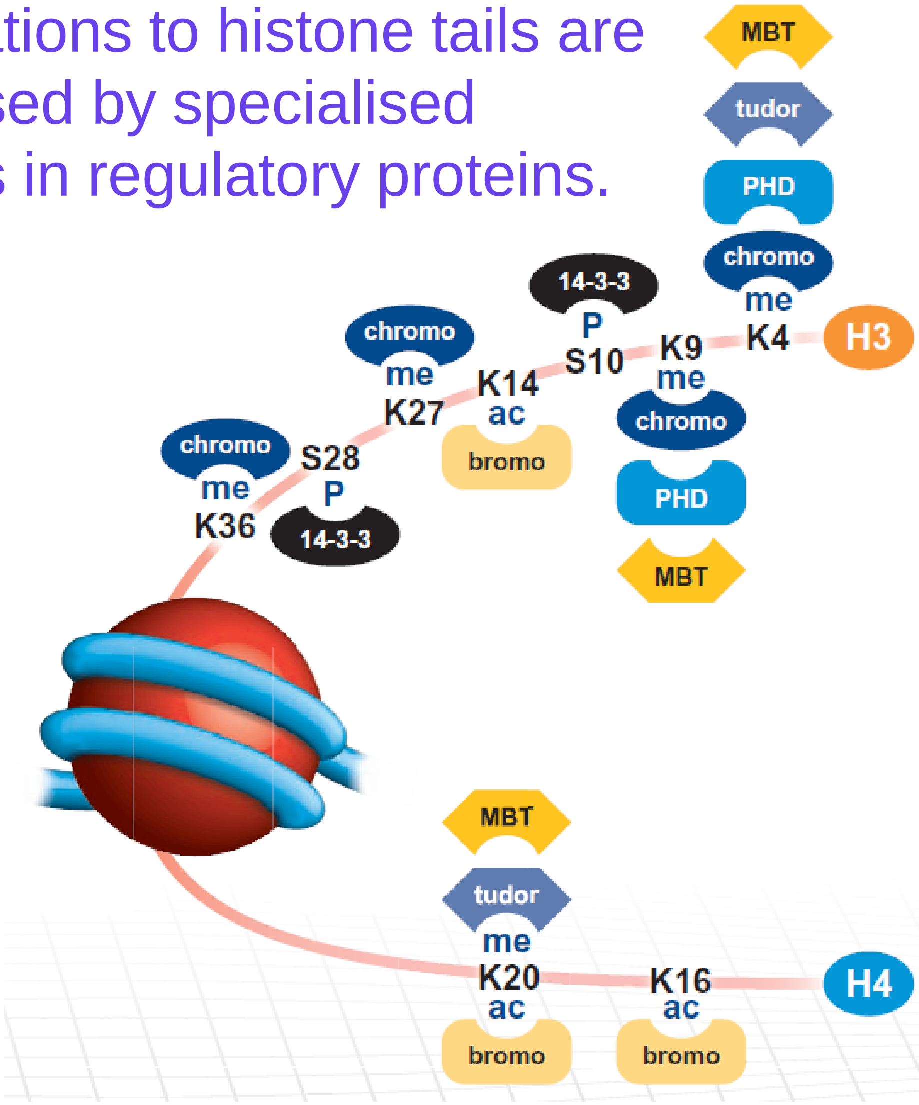
Histone Demethylase Inhibitors
GSK J4
OG-L002
IOX1

Pan-HDAC Inhibitors
Vorinostat
Entinostat
Panobinostat
Selective HDAC Inhibitors
RGFP966 (HDAC3)
Nexturastat A (HDAC6)
PCI-34051 (HDAC8)

Histone Deacetylation

Histone code readers

Modifications to histone tails are recognised by specialised domains in regulatory proteins.



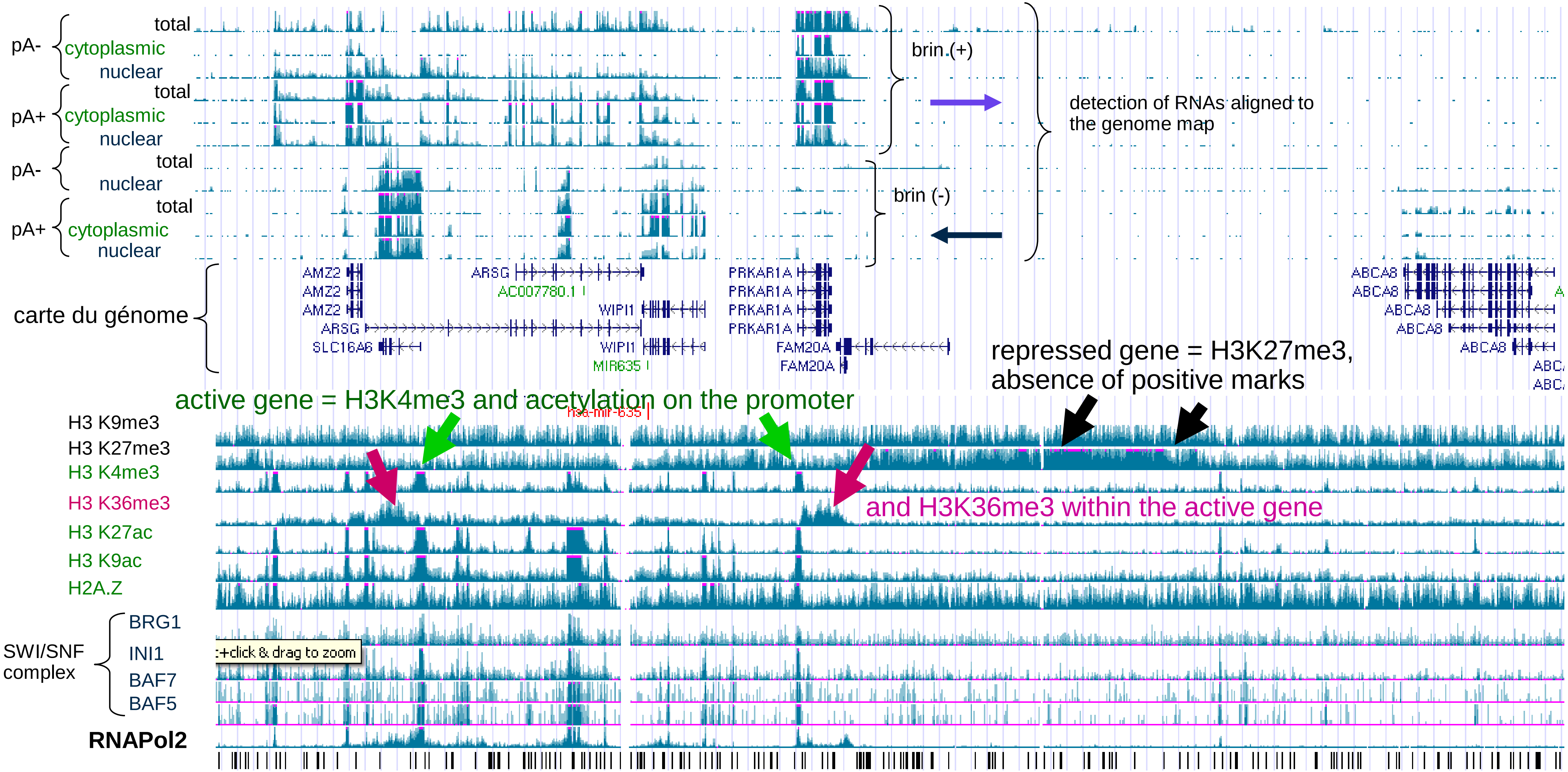
Key

Chromodomain Proteins	chromo	CHD1	H3K4me2/3
		HP1/spSwi6	H3K9me2/3
		spChp1	H3K9me2/3?
		CDY1	H3K9me2/3
		PC1/PC2/PC/LHP1	H3K27me3, H3K9me3
		MSL3	H3K36me3
		hMRG15	H3K36me, H3K4me
		scEaf3	H3K36me, H3K4me
MBT Proteins	MBT	PHF20L1	H3K4me1, H4K20me1
		SFMBT	H3K9me1/2, H4K20me1/2
		L(3)MBTL1	H4K20me1/2, H1bK26me1/2
PHD Proteins	PHD	scYng1	H3K4me2/3
		ING1,2,3,4,5	H3K4me2/3
		BPTF/dmNURF301	H3K4me2/3
		scSpp1	H3K4me2/3
		scSet3	H3K4me2/3
		scJhd1	H3K4me3
		RAG2	H3K4me3
		TAF3	H3K4me3
		ICBP90 (Np95)	H3K9me2/3
		JARID1C	H3K9me3
14-3-3 Proteins	14-3-3	14-3-3	H3S10ph, H3S28p
Tudor Proteins	tudor	JMJD2A	H3K4me3, H4K20me3
		53BP1	H4K20me1/2
		spCrb2	H4K20me2
		PHF20	H4K20me2
Bromodomain Proteins	bromo	Polybromo/BAF180	H3ac
		scSnf2	H3ac, H4ac
		Brd2,3,4,7	H3ac, H4ac
		TAF1	H3ac, H4ac
		P/CAF	H3ac, H4ac, H4K16ac
		CBP/p300	H3ac, H4ac
		scBdf1	H4ac
		hBRG1	H3K14ac
		scRsc1,2,4	H3K14ac (Rsc4)
		scGcn5	H4ac, H4K16ac

The histone code correlates with gene activity

The RNAs produced in HeLa cells (RNA-seq)

Epigenetic marks in HeLa cells (ChIP-seq)



detection of RNAs aligned to the genome map

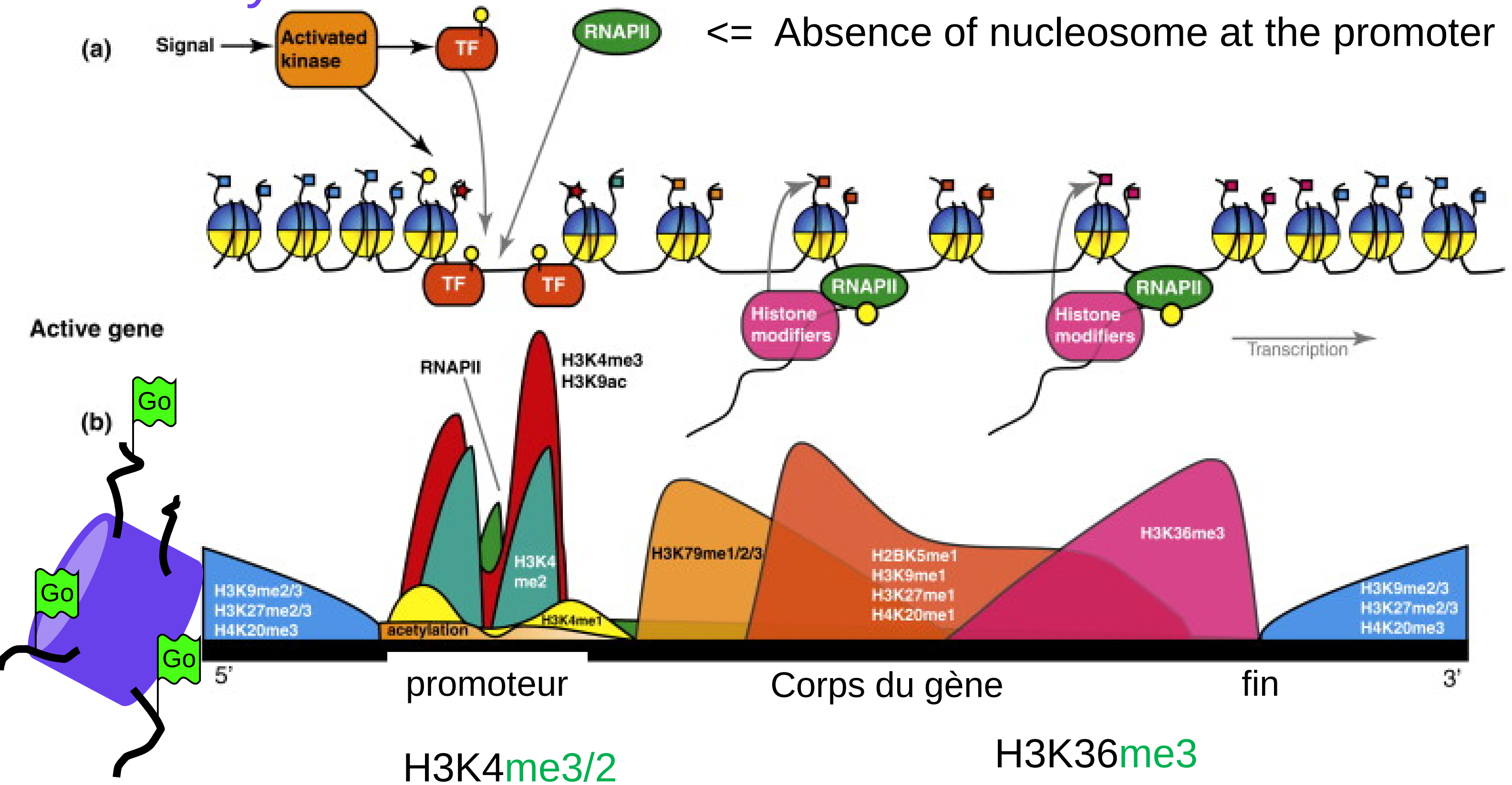
active gene = H3K4me3 and acetylation on the promoter

and H3K36me3 within the active gene

repressed gene = H3K27me3, absence of positive marks

The histone marks

Their distribution defines the regions of the gene according to their transcriptional activity.

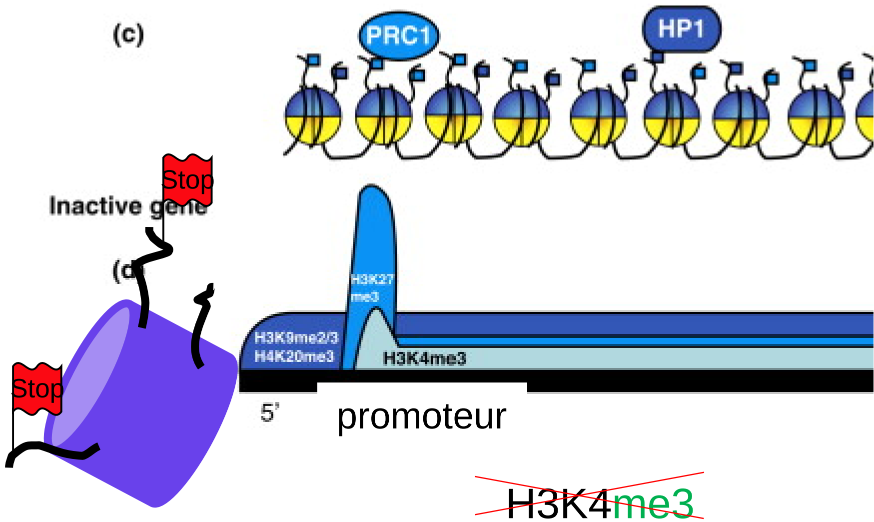


<= Absence of nucleosome at the promoter

H3K4me3/2
H3K9ac
H3K27ac

H3K79me1/2/3

H3K36me3



~~H3K4me3~~
H3K9me2/3
H3K27me3

Is there a specific mark for alternative exons ?
pattern of the H3K36me3

Differential chromatin marking of introns and expressed exons by H3K36me3

VOLUME 41 | NUMBER 3 | MARCH 2009 **NATURE GENETICS**

Paulina Kolasinska-Zwierz[1], Thomas Down[1], Isabel Latorre[1], Tao Liu[2], X Shirley Liu[2,3] & Julie Ahringer[1]

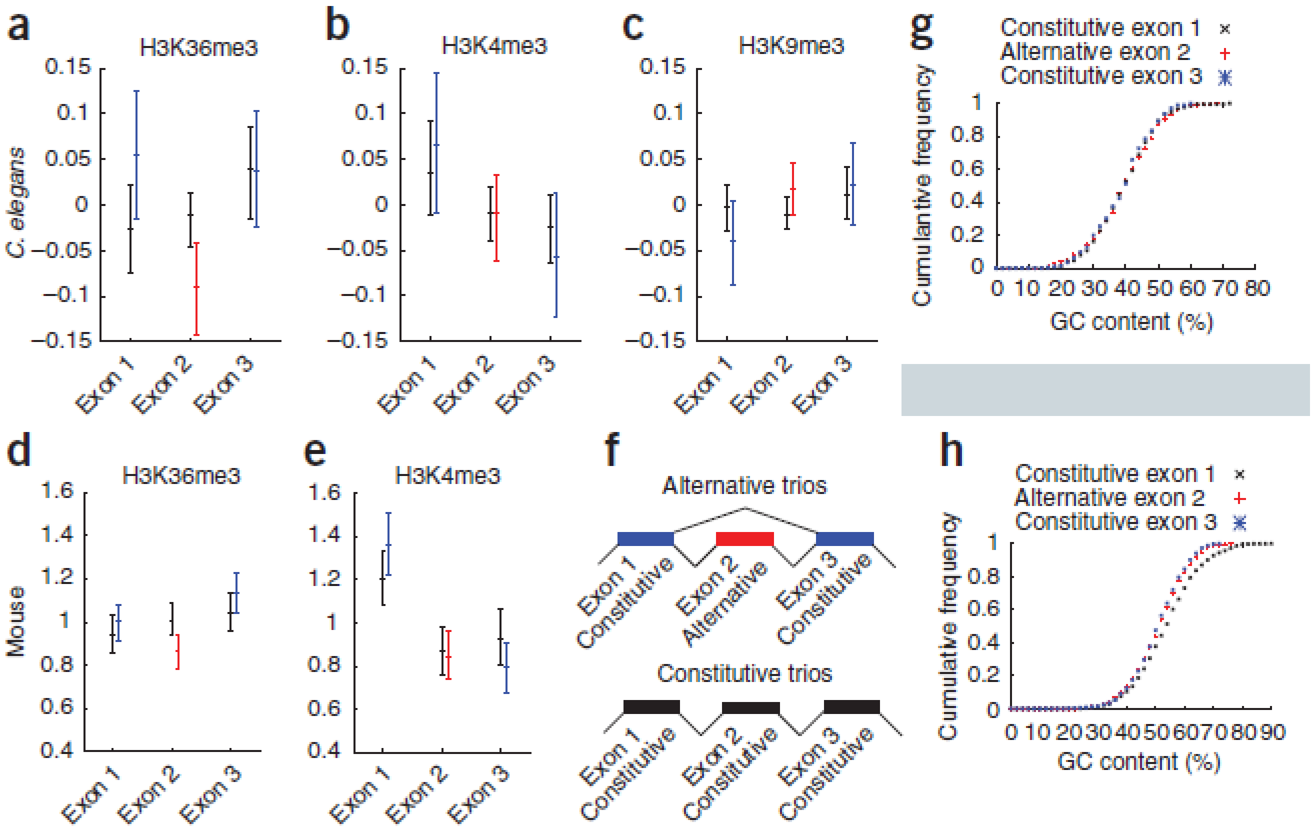


Figure 3 Alternative exons have lower H3K36me3 signal than constitutive exons. (**a**–**e**) Mean $\log_2$ ChIP signal of each *C. elegans* trio exon (**a**–**c**) or the average tag count for each mouse trio exon (**d**,**e**) is

Coupling between chromatin, transcription and splicing

Transcriptional Activators Differ in Their Abilities to Control Alternative Splicing*

Guadalupe Nogués, Sebastián Kadener, Paula Cramer, David Bentley, and Alberto R. Kornblihtt

jbc 2002

CHD1 associates with NCoR and histone deacetylase as well as with RNA splicing proteins

Helen H. Tai,[a,b,*] Margit Geisterfer,[a] John C. Bell,[a,c] Mariko Moniwa,[d] James R. Davie,[d] Lorrie Boucher,[a] and Michael W. McBurney[a,c]

BBRC 2003

Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2

Juan I. Young, Eugene P. Hong, John C. Castle, Juan Crespo-Barreto, Aaron B. Bowman, Matthew F. Rose, Dongcheul Kang, Ron Richman, Jason M. Johnson, Susan Berget, and Huda Y. Zoghbi

PNAS 2005

The human SWI/SNF subunit Brm is a regulator of alternative splicing

Eric Batsché, Moshe Yaniv & Christian Muchardt

nature structural & molecular biology 2006

Recognition of Trimethylated Histone H3 Lysine 4 Facilitates the Recruitment of Transcription Postinitiation Factors and Pre-mRNA Splicing

Robert J. Sims III,[1] Scott Millhouse,[3] Chi-Fu Chen,[4] Brian A. Lewis,[1] Hediye Erdjument-Bromage,[5] Paul Tempst,[5] James L. Manley,[3] and Danny Reinberg[1,2,*]

Molecular Cell 2007

Control of alternative splicing through siRNA-mediated transcriptional gene silencing

Mariano Alló[1], Valeria Buggiano[1], Juan P Fededa[1], Ezequiel Petrillo[1], Ignacio Schor[1], Manuel de la Mata[1], Eneritz Agirre[2], Mireya Plass[2], Eduardo Eyras[2], Sherif Abou Elela[3], Roscoe Klinck[3], Benoit Chabot[3] & Alberto R Kornblihtt[1]

nature structural & molecular biology 2009

Chromatin Binding of SRp20 and ASF/SF2 and Dissociation from Mitotic Chromosomes Is Modulated by Histone H3 Serine 10 Phosphorylation

Rebecca J. Loomis,[1,6] Yoshinori Naoe,[1,4,6] J. Brandon Parker,[1,2] Velibor Savic,[2] Matthew R. Bozovsky,[1] Todd Macfarlan,[2,5] James L. Manley,[3] and Debabrata Chakravarti[1,*]

Molecular Cell 2009

Differential chromatin marking of introns and expressed exons by H3K36me3

Paulina Kolasinska-Zwierz[1], Thomas Down[1], Isabel Latorre[1], Tao Liu[2], X Shirley Liu & Julie Ahringer[1]

nature genetics 2009

SWI/SNF Associates with Nascent Pre-mRNPs and Regulates Alternative Pre-mRNA Processing

Anu Tyagi[1], Jessica Ryme[2], David Brodin[3], Ann Kristin Östlund Farrants[2], Neus Visa[1*]

PLoS GENETICS 2009

Nucleosomes are well positioned in exons and carry characteristic histone modifications

Robin Andersson,[1,4] Stefan Enroth,[1,4] Alvaro Rada-Iglesias,[1,4] Claes Wadelius, and Jan Komorowski[1,3,5]

GENOME RESEARCH 2009

Chromatin organization marks exon-intron structure

Schraga Schwartz[1], Eran Meshorer[2] & Gil Ast[1]

nature structural & molecular biology 2009

Nucleosome positioning as a determinant of exon recognition

Hagen Tilgner[1,3], Christoforos Nikolaou[1,3], Sonja Althammer[1], Michael Sammeth[1], Miguel Beato[1], Juan Valcárcel[1,2] & Roderic Guigó[1]

nature structural & molecular biology 2009

Complex Exon-Intron Marking by Histone Modifications Is Not Determined Solely by Nucleosome Distribution

Pawandeep Dhami, Peter Saffrey, Alexander W. Bruce, Shane C. Dillon, Kelly Chi, Nicolas Bonhoure[2], Christoph M. Koch[1], Jackie Bye[1], Keith James[1], Nicola S. Foad[3], Peter Ellis[1], Nicholas A. Watkins[3], Willem H. Ouwehand[1,3], Cordelia Langford[1], Robert M. Andrews[1], Ian Dunham, David Vetrie

PLoS one 2010

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*]

Science 2010

Pre-mRNA splicing is a determinant of histone H3K36 methylation

Soojin Kim, Hyunmin Kim[1], Nova Fong[1], Benjamin Erickson, and David L. Bentley[2]

PNAS 2010

Histone H3 lysine 9 trimethylation and HP1γ favor inclusion of alternative exons

Violaine Saint-André[1–3], Eric Batsché[1–3], Christophe Rachez[1–3] & Christian Muchardt[1–3]

nature structural & molecular biology 2011

Hu proteins regulate alternative splicing by inducing localized histone hyperacetylation in an RNA-dependent manner

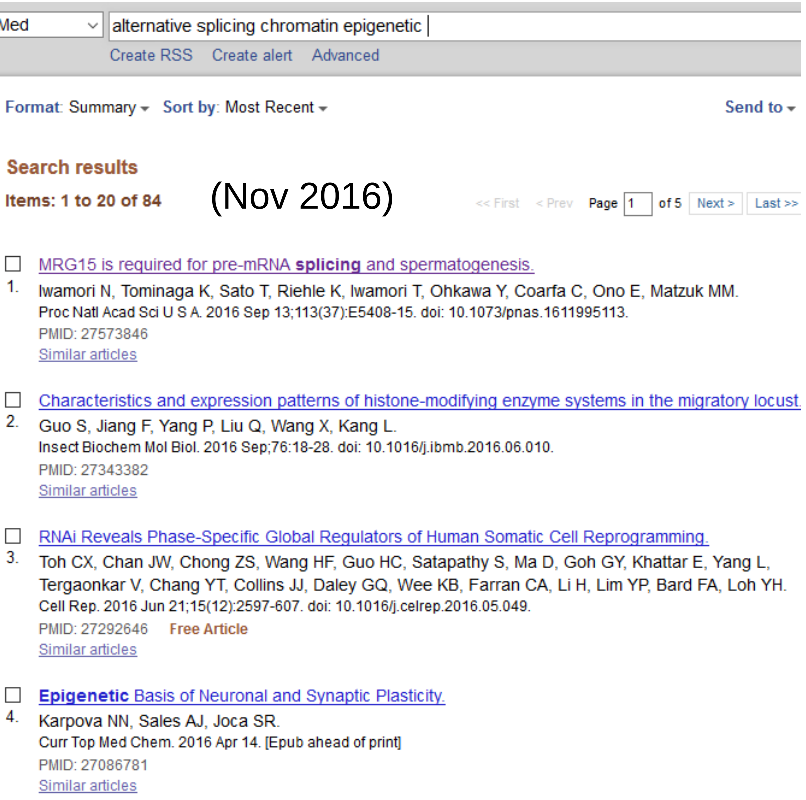
Hua-Lin Zhou, Melissa N. Hinman, Victoria A. Barron, Cuiyu Geng, Guangjin Zhou, Guangbin Luo, Ruth E. Siegel, and Hua Lou

PNAS 2011

Splicing enhances recruitment of methyltransferase HYPB/Setd2 and methylation of histone H3 Lys36

Sérgio Fernandes de Almeida[1], Ana Rita Grosso[1], Frederic Koch[2], Romain Fenouil[2], Sílvia Carvalho[1], Jorge Andrade[1], Helena Levezinho[1], Marta Gut[3], Dirk Eick[4], Ivo Gut[3], Jean-Christophe Andrau[2], Pierre Ferrier[2] & Maria Carmo-Fonseca[1]

nature structural & molecular biology 2011



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(Nov 2016)

☐ 1. MRG15 is required for pre-mRNA **splicing** and spermatogenesis.
Iwamori N, Tominaga K, Sato T, Riehle K, Iwamori T, Ohkawa Y, Coarfa C, Ono E, Matzuk MM.
Proc Natl Acad Sci U S A. 2016 Sep 13;113(37):E5408-15. doi: 10.1073/pnas.1611995113.
PMID: 27573846
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☐ 2. Characteristics and expression patterns of histone-modifying enzyme systems in the migratory locust.
Guo S, Jiang F, Yang P, Liu Q, Wang X, Kang L.
Insect Biochem Mol Biol. 2016 Sep;76:18-28. doi: 10.1016/j.ibmb.2016.06.010.
PMID: 27343382
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☐ 3. RNAi Reveals Phase-Specific Global Regulators of Human Somatic Cell Reprogramming.
Toh CX, Chan JW, Chong ZS, Wang HF, Guo HC, Satapathy S, Ma D, Goh GY, Khattar E, Yang L, Tergaonkar V, Chang YT, Collins JJ, Daley GQ, Wee KB, Farran CA, Li H, Lim YP, Bard FA, Loh YH.
Cell Rep. 2016 Jun 21;15(12):2597-607. doi: 10.1016/j.celrep.2016.05.049.
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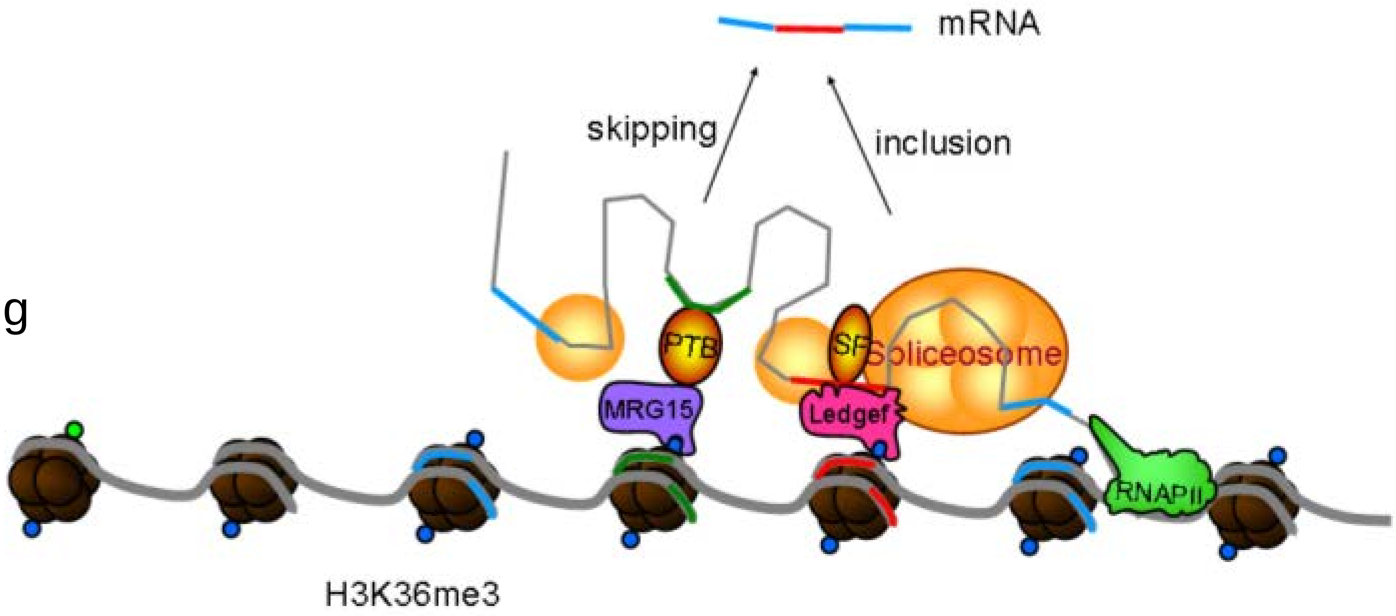
☐ 4. **Epigenetic** Basis of Neuronal and Synaptic Plasticity.
Karpova NN, Sales AJ, Joca SR.
Curr Top Med Chem. 2016 Apr 14. [Epub ahead of print]
PMID: 27086781
Similar articles

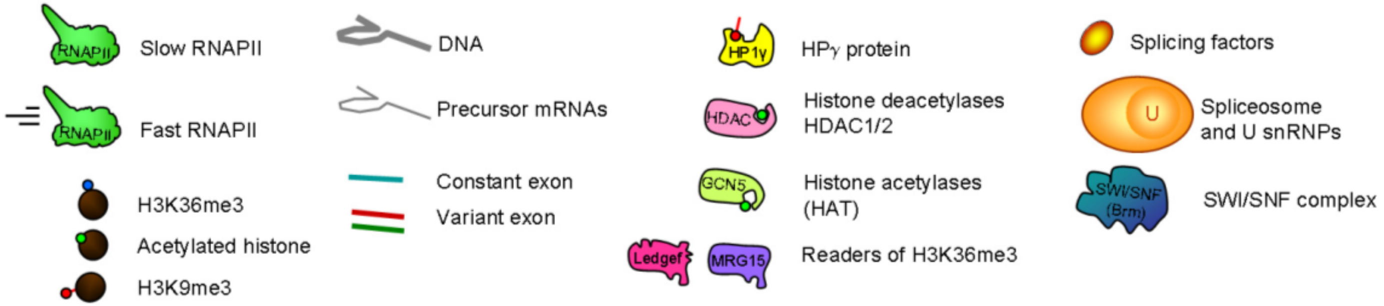
And this continues…

Chromatin readers and modifiers of H3K36me3 affect alternative splicing

H3K36me3 mark is recognized by adaptators that bind to splicing factors (Recrutment model)

Defects of SETD2 (the H3K36me3 writer) in Renal Carcinoma impact widely on splicing





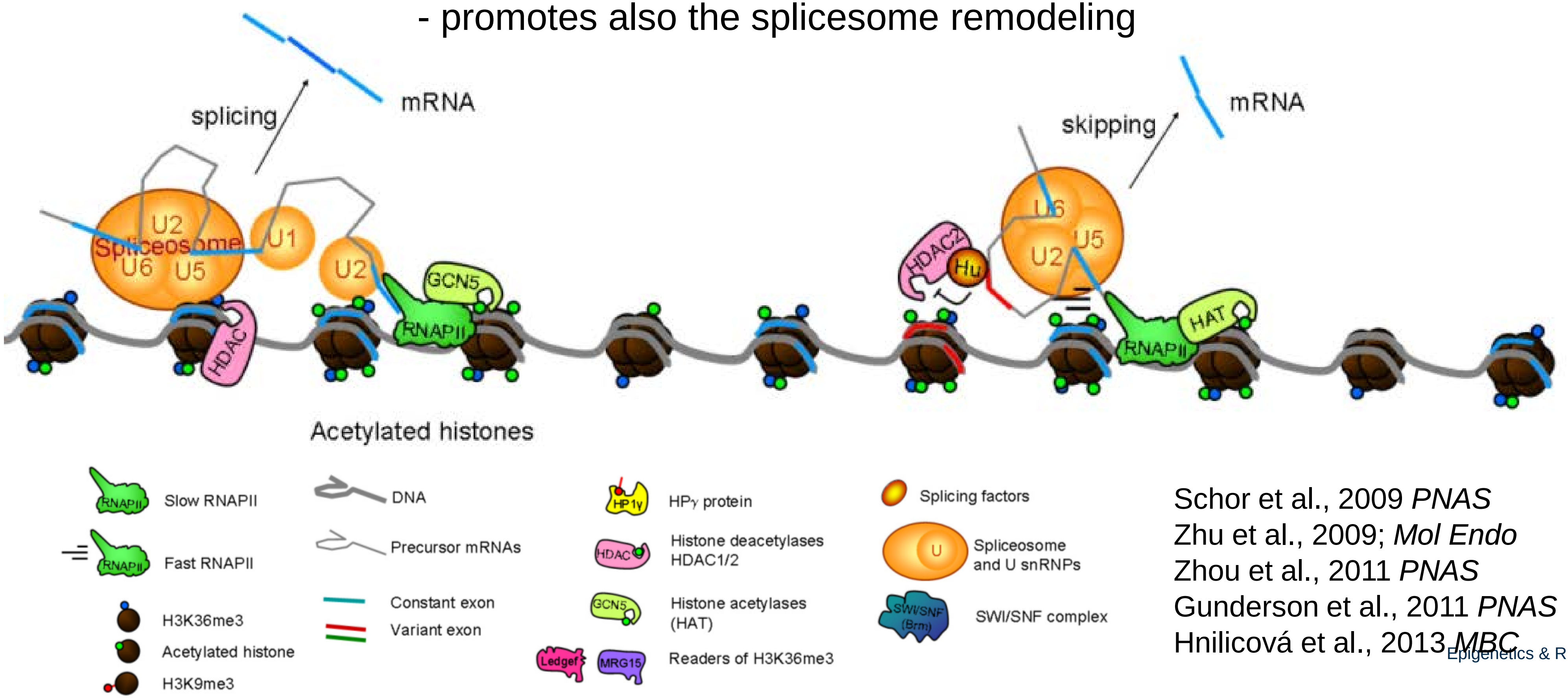
Luco et al. 2010 *Science*

Pradeepa et al., 2012 *Plos Genet*

Simon et al., 2013 *Gen. Res.*

Modifiers and readers of acetylated histones affect alternative splicing

Acetylation of histones :
- is inhibited by splicing regulator Hu
- increases the RNAPII elongation speed
- promotes also the splicesome remodeling



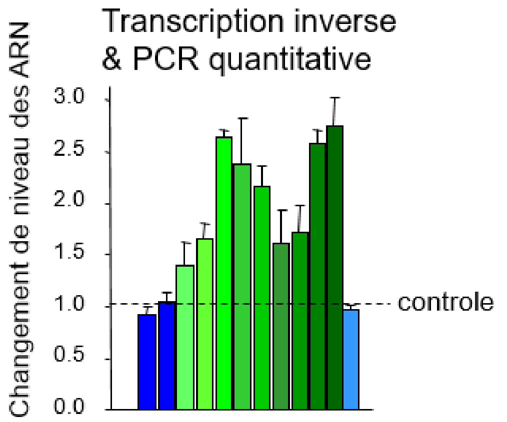
Schor et al., 2009 *PNAS*
Zhu et al., 2009; *Mol Endo*
Zhou et al., 2011 *PNAS*
Gunderson et al., 2011 *PNAS*
Hnilicová et al., 2013 *MBC*

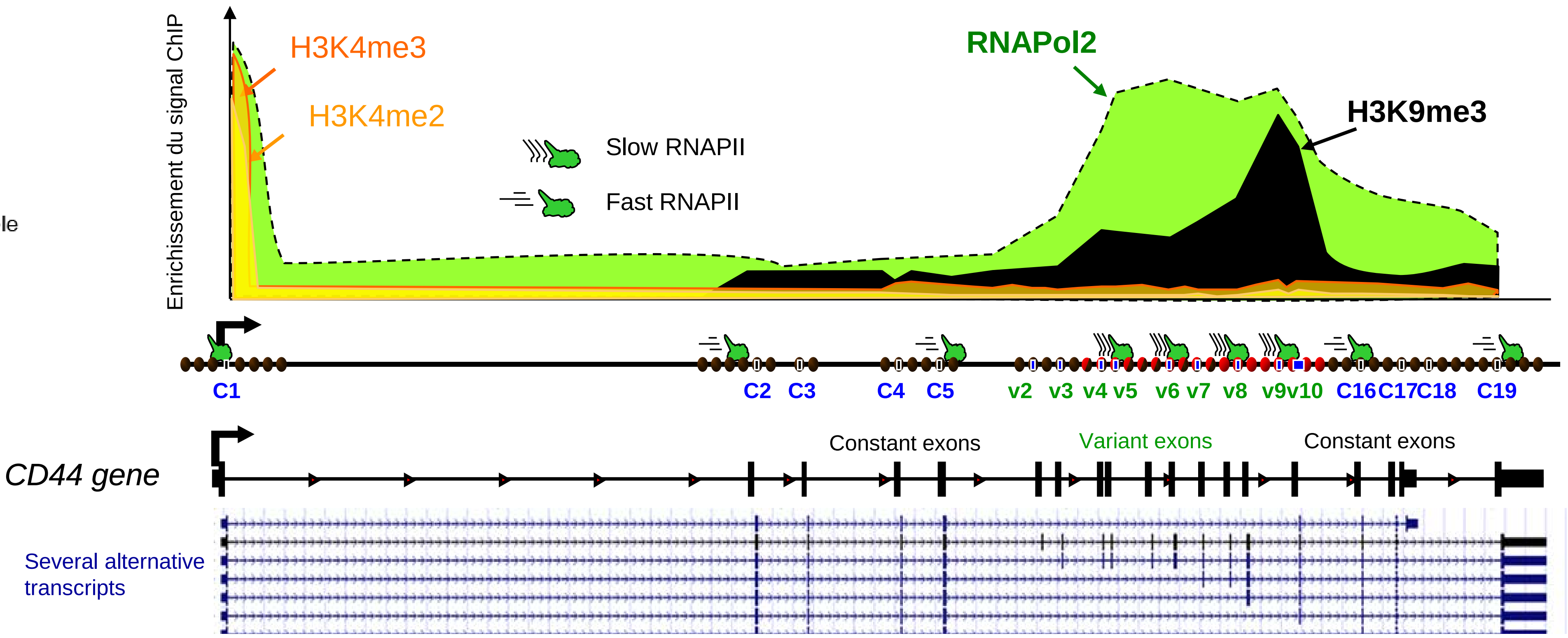
The exon composition of mRNAs is also regulated by epigenetics

H3K9me3 coincides with a slowdown in RNA Pol2

Repressive H3K9me3 mark in a transcriptionally active gene causes RNA Pol2 to slow down and variant exons to be included.

After stimulation of the cells by an activating agent (PMA)

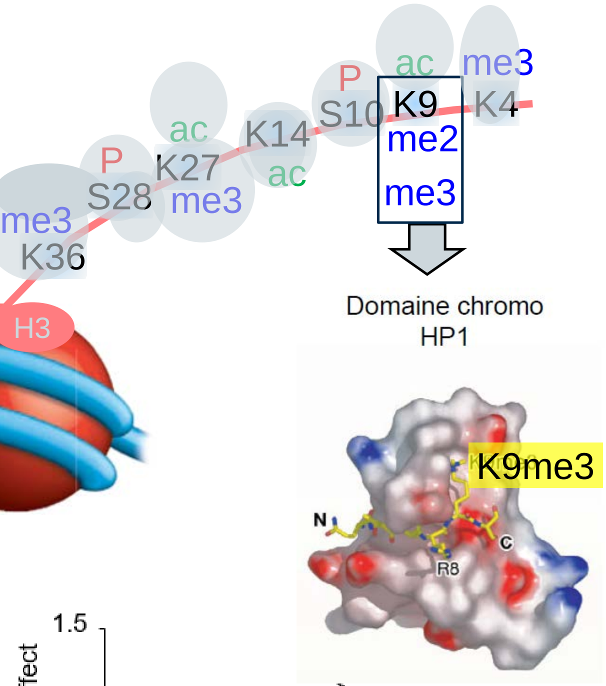


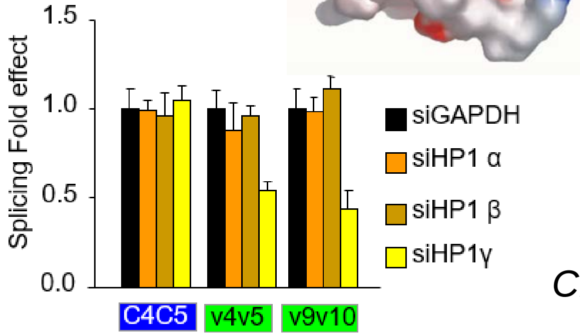


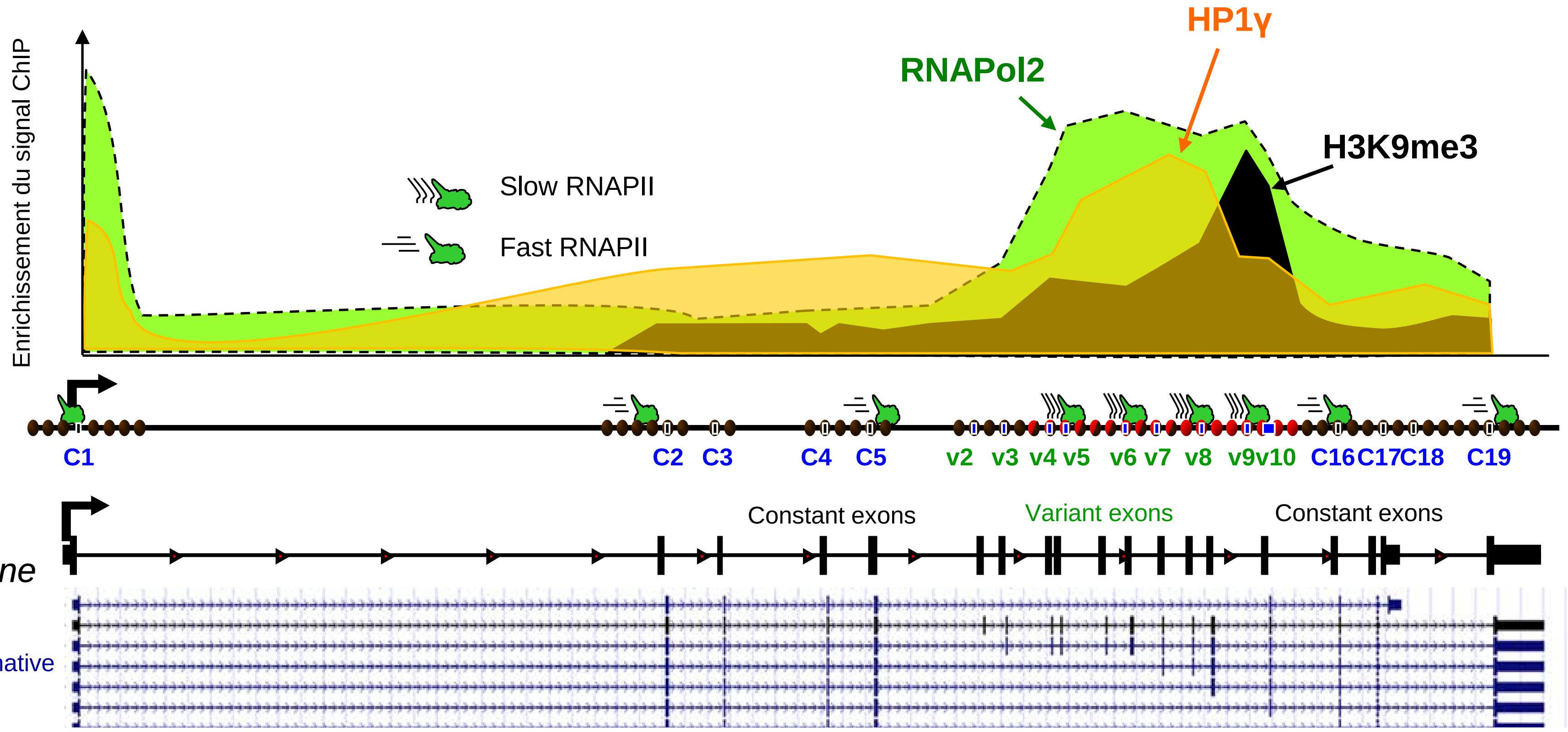
Saint-André et al. 2011 *NSMB*
Ameyar-Zazoua et al. 2012 *NSMB*

The slowing down of RNA Pol2 depends on HP1γ

Inhibition of HP1γ reduces the inclusion of variant exons and decreases the accumulation of Pol2 in the region of variant exons.



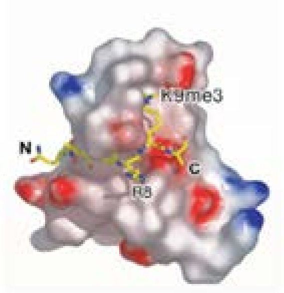


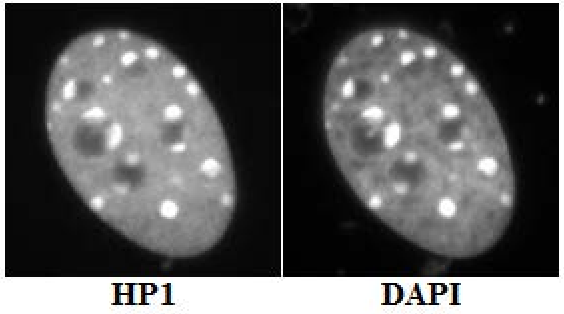


Saint-André et al. 2011 *NSMB*
Ameyar-Zazoua et al. 2012 *NSMB*

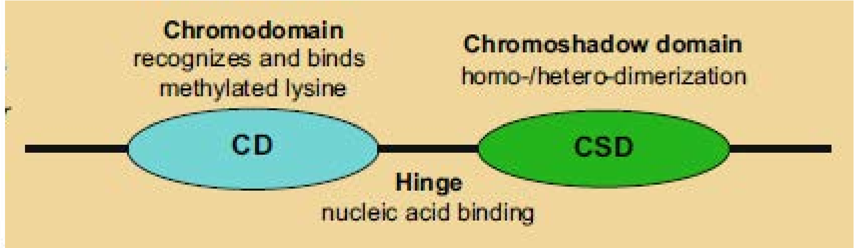
HP1γ (CBX3) is an H3K9me3 reader that binds to RNAs.

Domaine chromo HP1



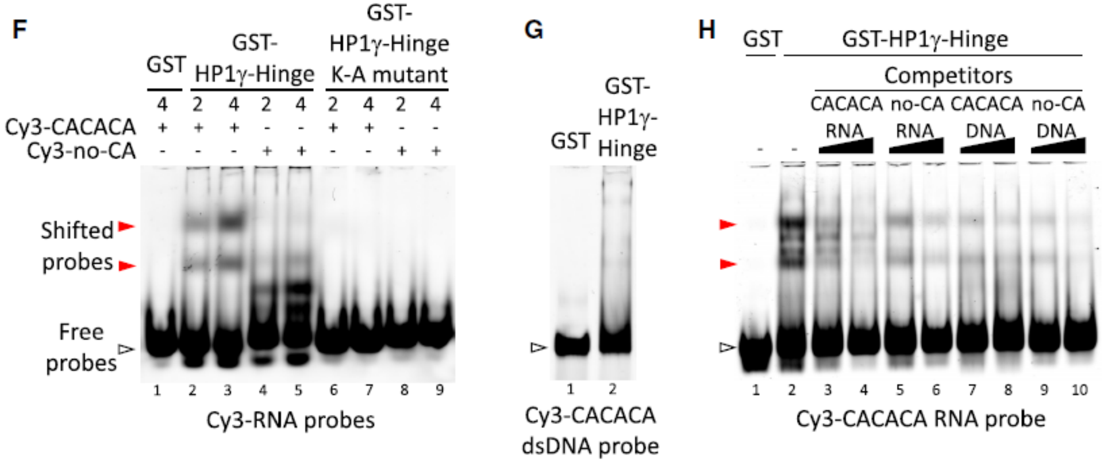


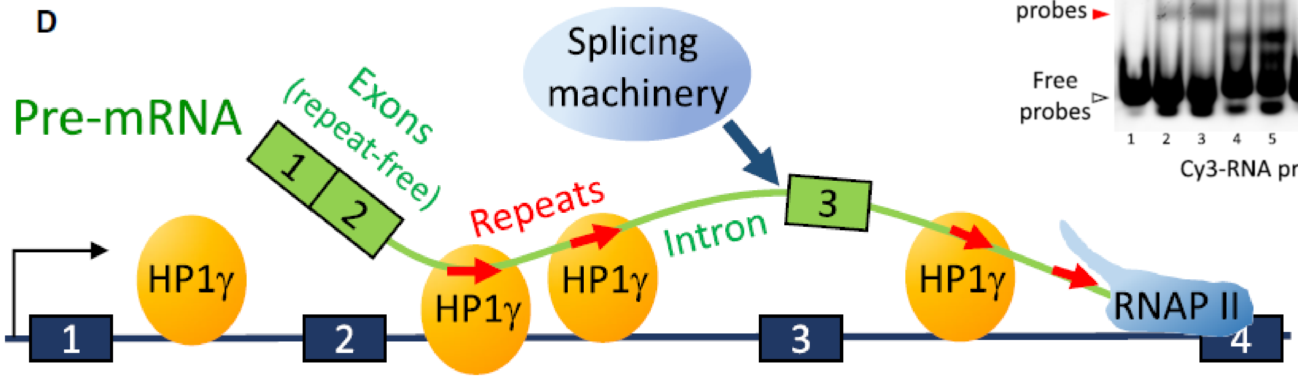
HP1 associates with chromatin


Chromodomain recognizes and binds methylated lysine

Chromoshadow domain homo-/hetero-dimerization

Hinge nucleic acid binding



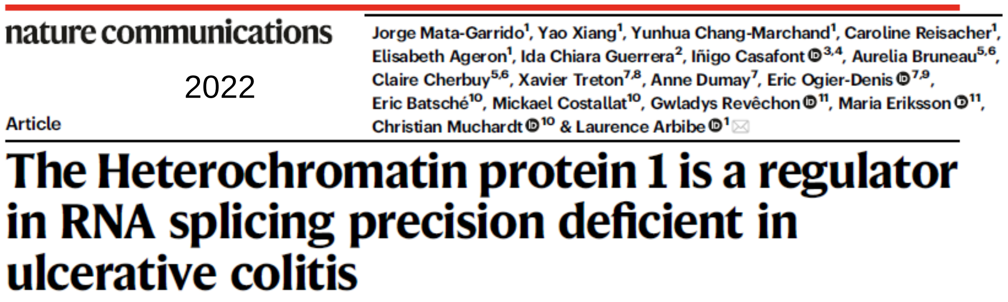


EMBO reports 2021

HP1γ binding pre-mRNA intronic repeats modulates RNA splicing decisions

Christophe Rachez[1,2,3,*], Rachel Legendre[4,5], Mickaël Costallat[1,2,3], Hugo Va
Etienne Kornobis[3,†] & Christian Muchardt[1,2,3,*]

Ulcerative colitis: a disease associated with the function of HP1γ (CBX3) in splicing regulation

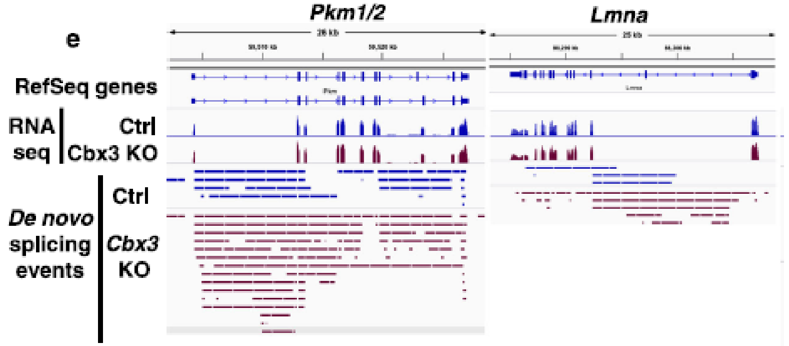

nature communications

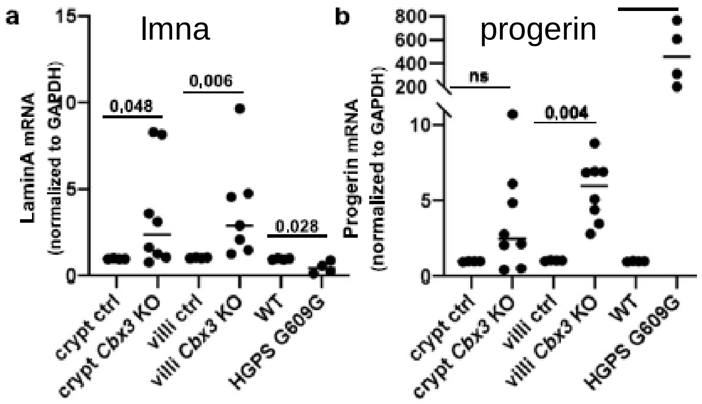
2022

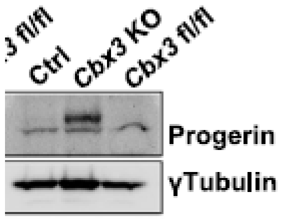
Article

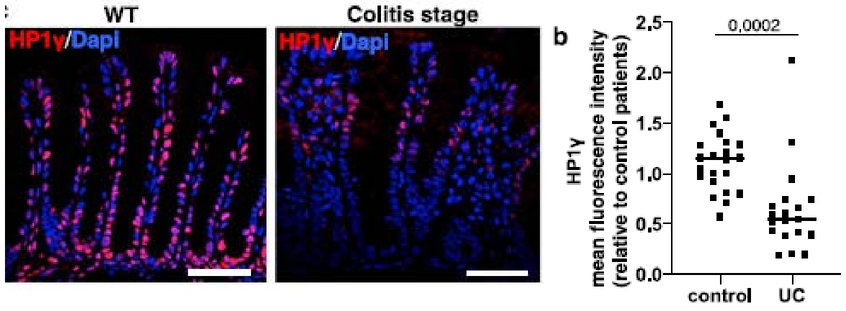
Jorge Mata-Garrido[1], Yao Xiang[1], Yunhua Chang-Marchand[1], Caroline Reisacher[1], Elisabeth Ageron[1], Ida Chiara Guerrera[2], Iñigo Casafont[3,4], Aurelia Bruneau[5,6], Claire Cherbuy[5,6], Xavier Treton[7,8], Anne Dumay[7], Eric Ogier-Denis[7,9], Eric Batsché[10], Mickael Costallat[10], Gwladys Revêchon[11], Maria Eriksson[11], Christian Muchardt[10] & Laurence Arbibe[1] ✉

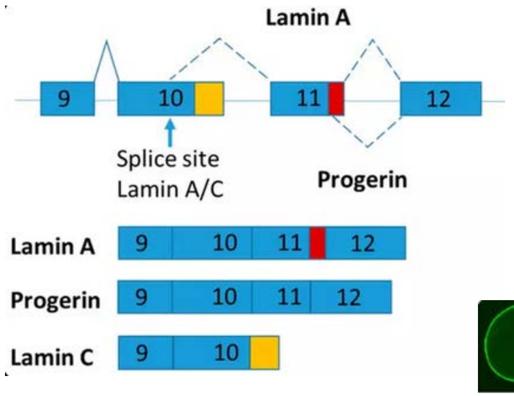
The Heterochromatin protein 1 is a regulator in RNA splicing precision deficient in ulcerative colitis

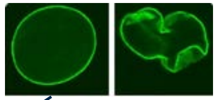


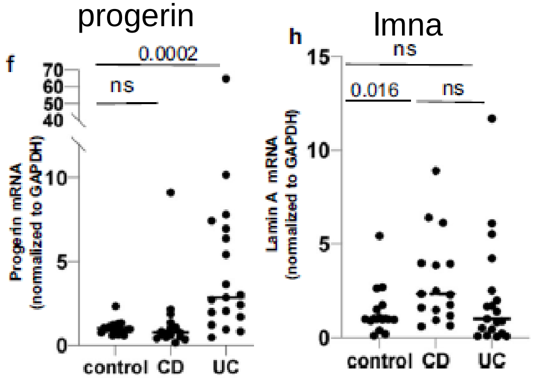




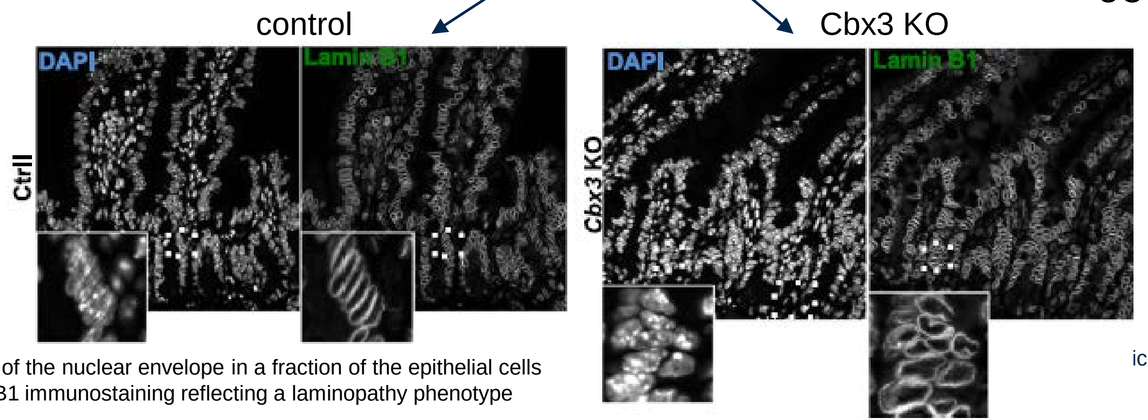








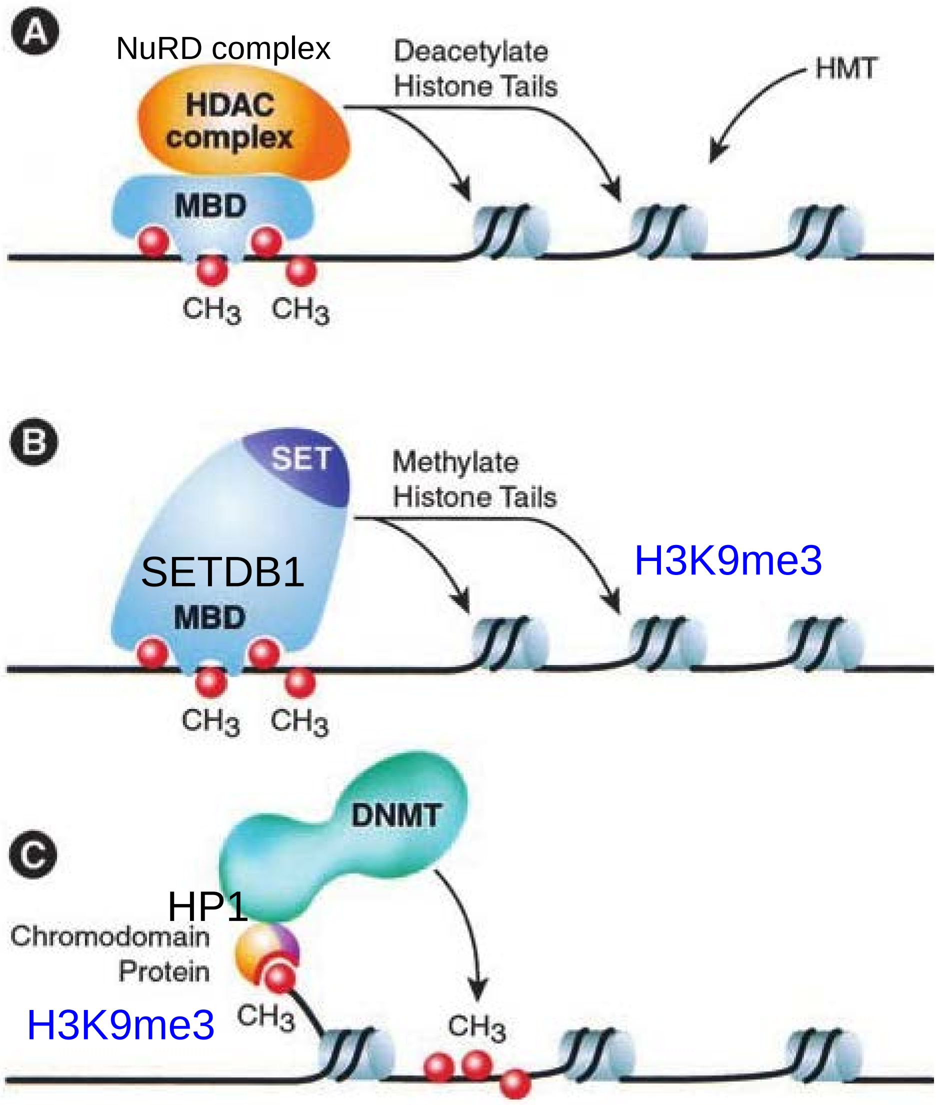
CD = Crohn's disease
UC = ulcerative colitis



misshaping of the nuclear envelope in a fraction of the epithelial cells upon laminB1 immunostaining reflecting a laminopathy phenotype

Epigenetic regulations

DNA methylation modifications are linked to histone tail modifications and *vice versa*.

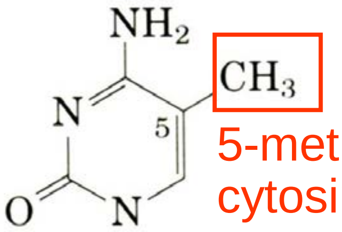


DNA methylation
indirectly and directly influences
alternative splicing decisions.

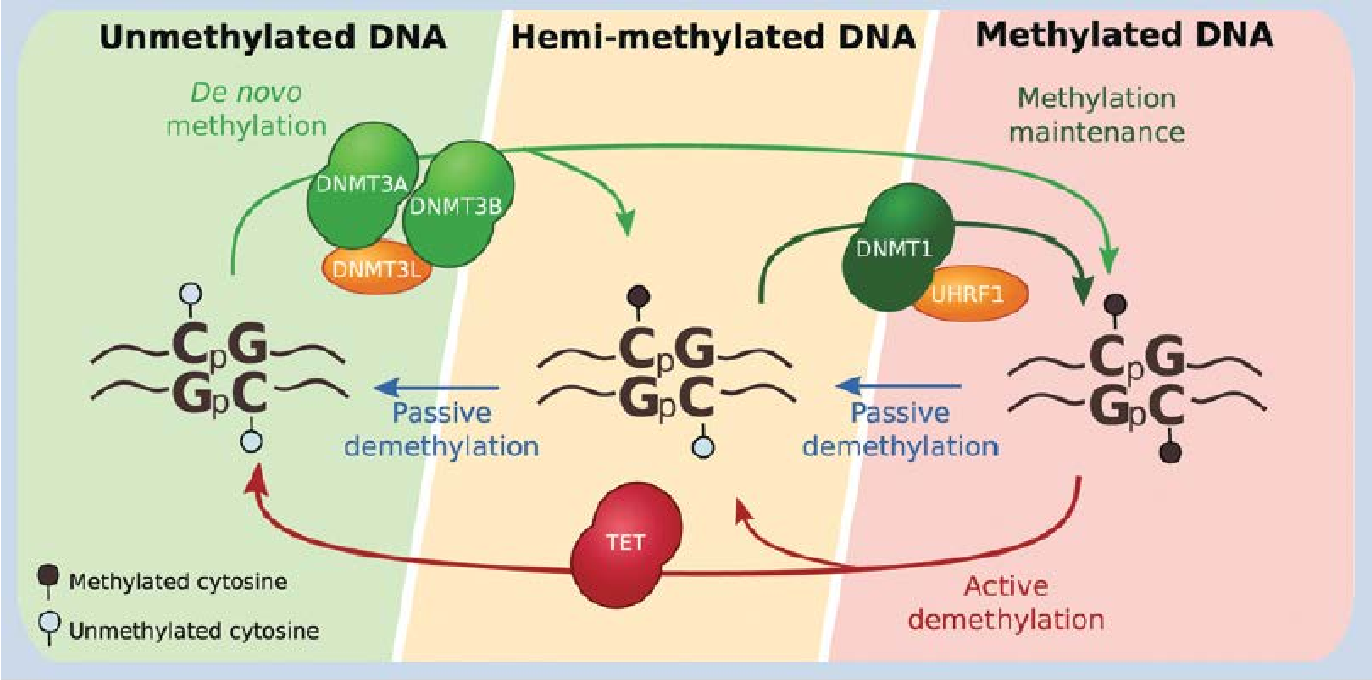
The DNA methylation

Identified in 1948, the ‘fifth base’ is the oldest canonical epigenetic mark.

- In mammalian cells, cytosine is methylated at carbon 5,
- mainly on CpG dinucleotides (underrepresented except in ‘CpG islands’).
- The majority of CpGs are methylated, except those in the islands of transcribed genes, which are the signature of active promoters.



5-methyl-cytosine



Roles of the DNA methylation

Repression of transcriptional initiation

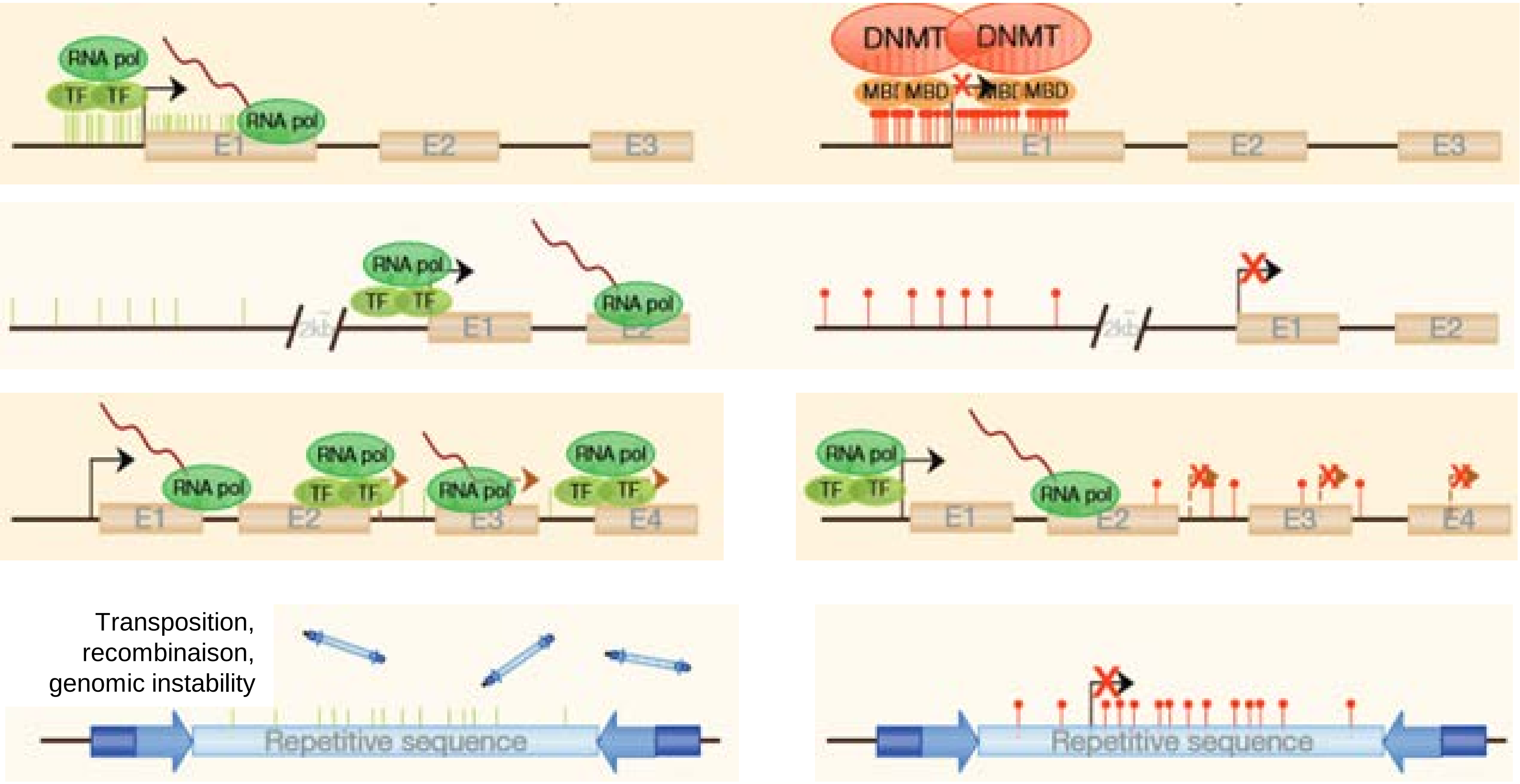
unmethylated **methylated**

CpG islands of promoters

The regions bordering CpG islands

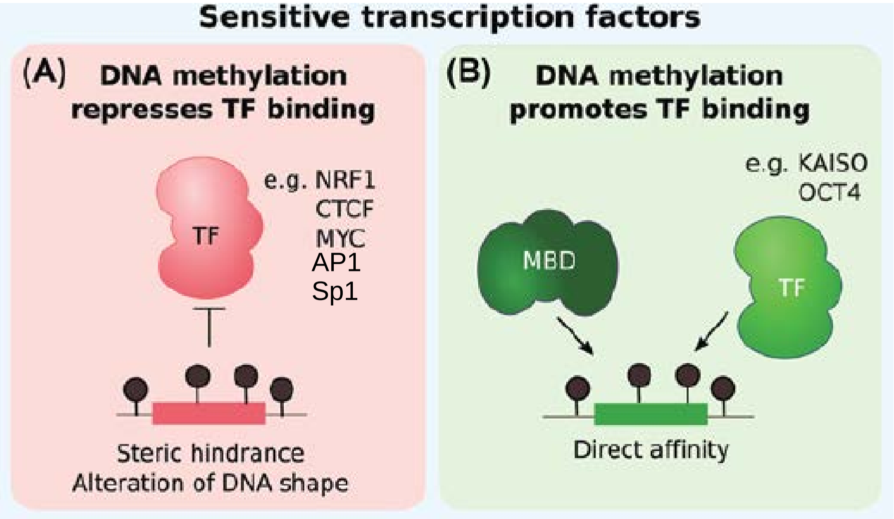
Gene bodies

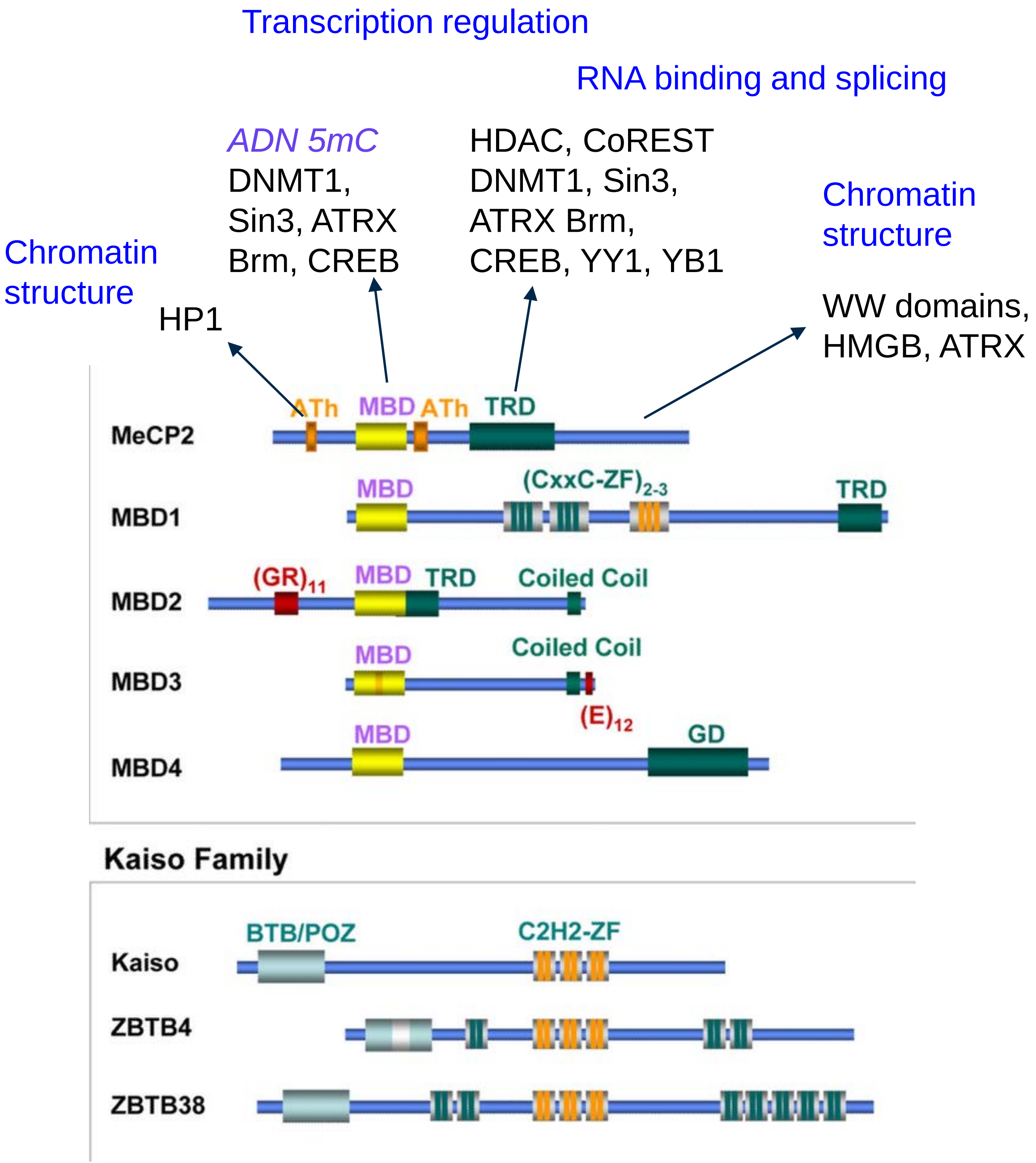
Repeated sequences in the genome


Transposition, recombinaison, genomic instability

Epigenetic regulations

DNA methylation readers enable the recruitment of other factors





Transcription regulation

RNA binding and splicing

Chromatin structure

HP1

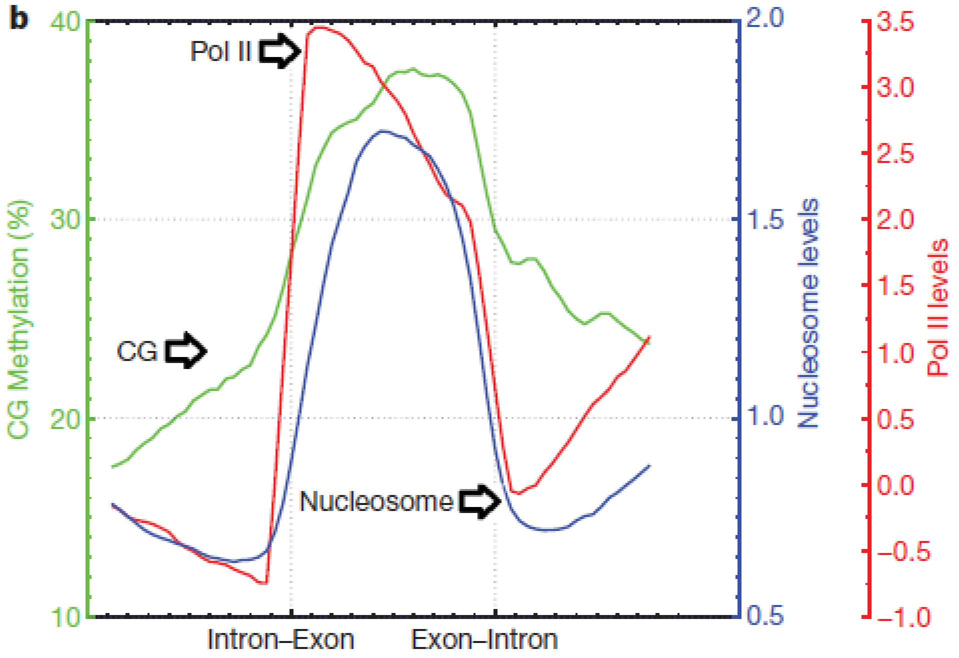
ADN 5mC
DNMT1,
Sin3, ATRX
Brm, CREB

HDAC, CoREST
DNMT1, Sin3,
ATRX Brm,
CREB, YY1, YB1

Chromatin structure

WW domains,
HMGB, ATRX

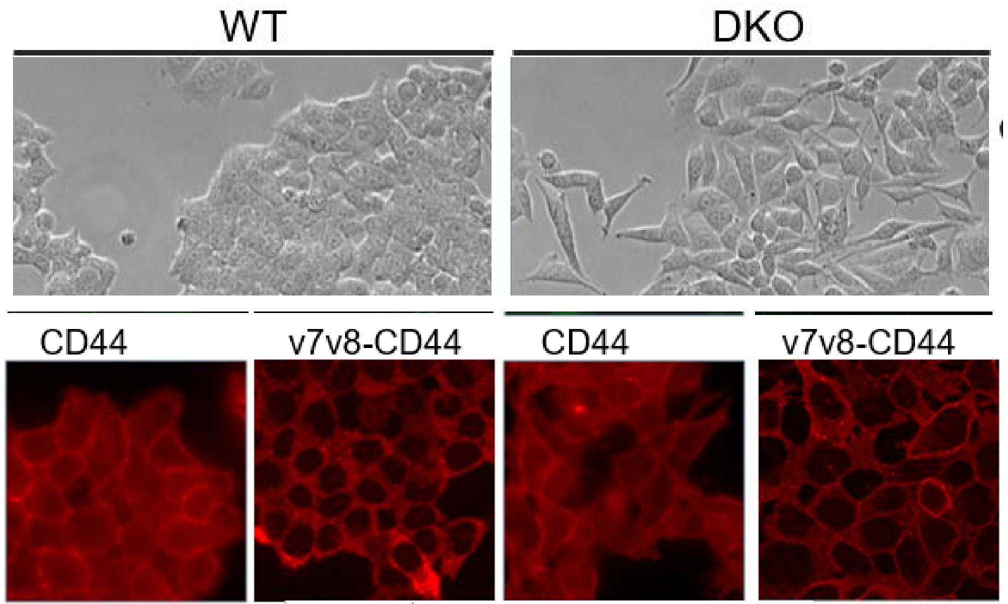
DNA methylation, nucleosome positioning, and Pol2 slowing down



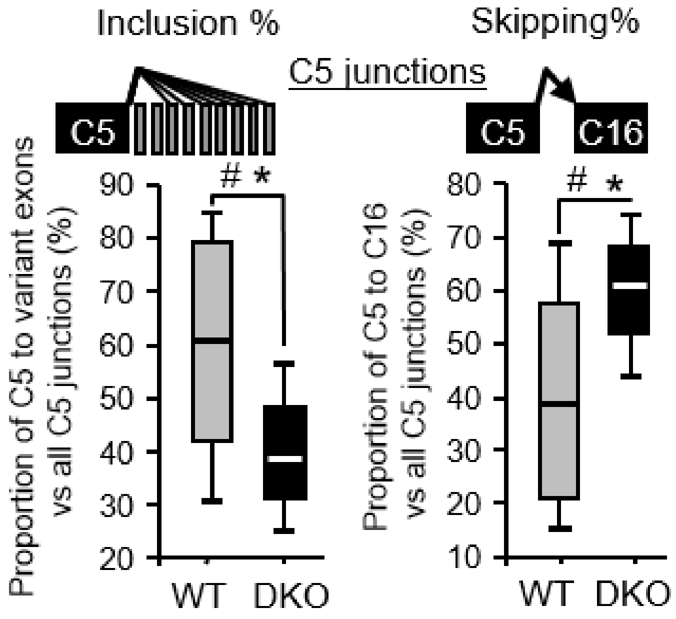
Chodavarapu, et al. (2010) *Nature*

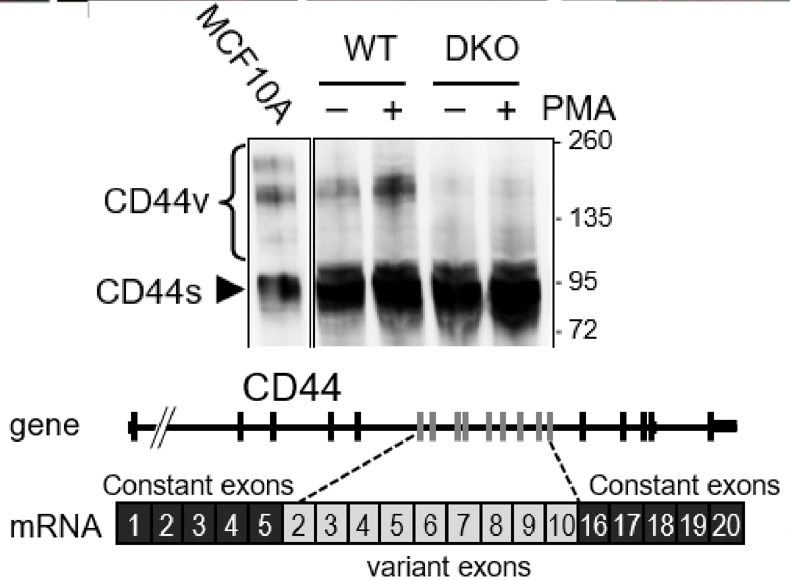
- DNA methylation increases on nucleosome DNA, and nucleosomes are enriched on exons.
- DNA methylation is also increased on exon DNA compared to intron DNA. Conserved in other organisms: plants, insects, animals
- This suggests that DNA methylation, which is most often found in transcriptionally active genes, may play a role in defining exons or regulating splicing.
- This also reinforces the observation that the position of nucleosomes plays an important role in the distribution of DNA methylation in the genome.

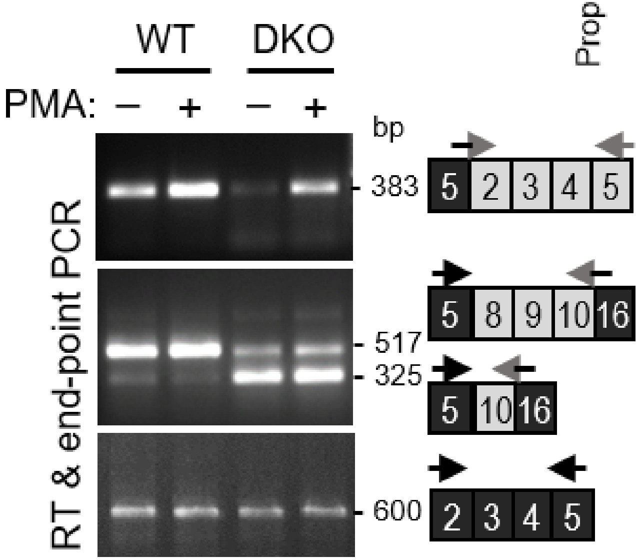
Inactivation of DNMT1 and DNMT3b in HCT116 cells leads an EMT resulting in modified CD44 alternative splicing



The meta-analysis of RNA-seq from 4 previous studies confirmed the modifications of CD44 splicing.

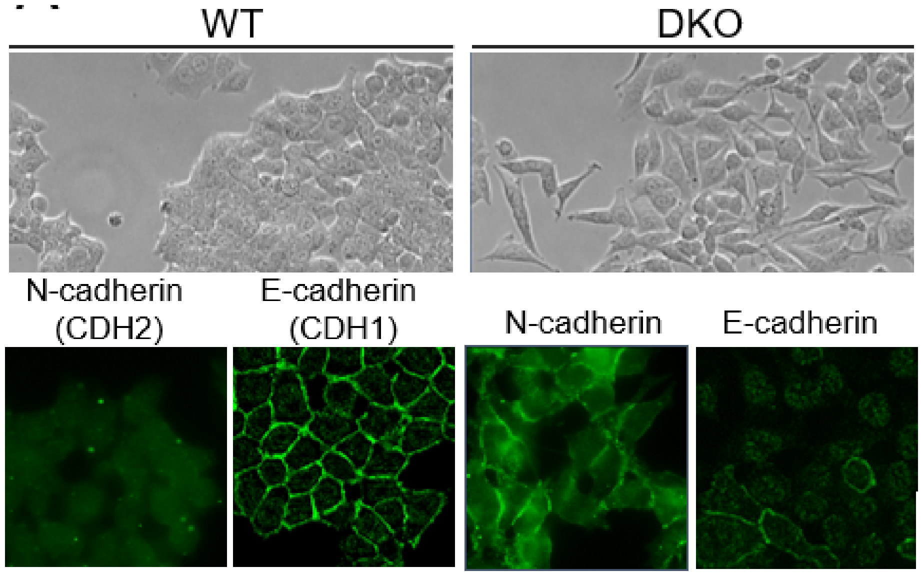


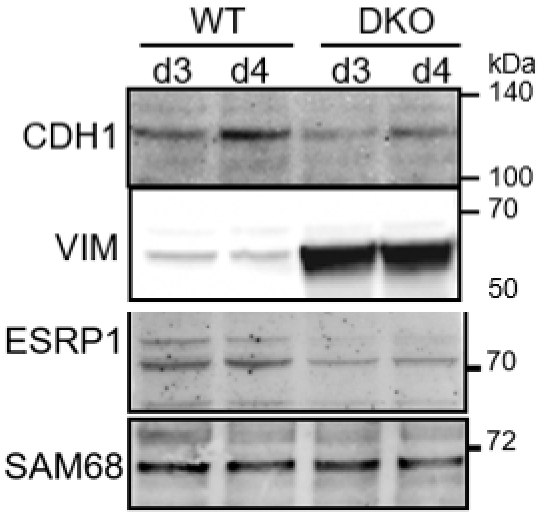




Batsché et al.
Nuc Ac Res 2021

Inactivation of DNMT1 and DNMT3b in HCT116 leads to an EMT

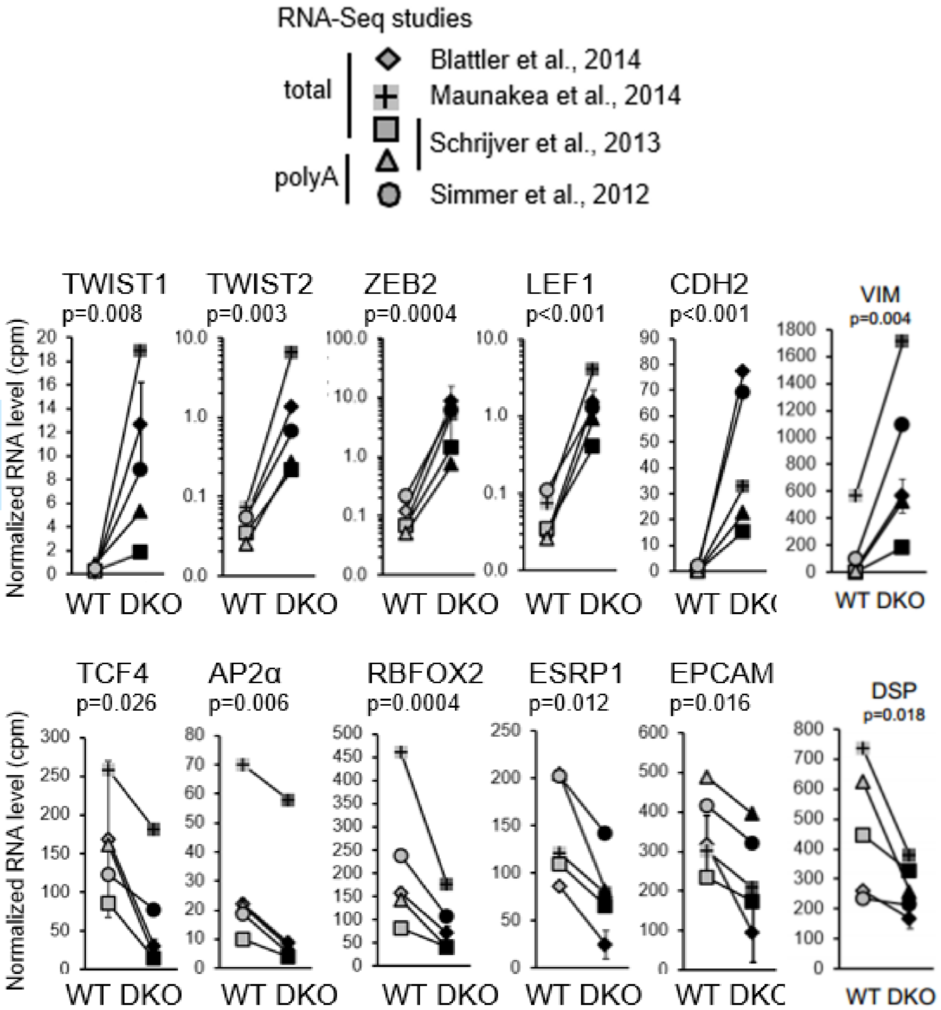




The meta-analysis of RNA-seq from 4 previous studies confirmed the EMT in DKO cells.

mesenchym markers

Epithelial markers



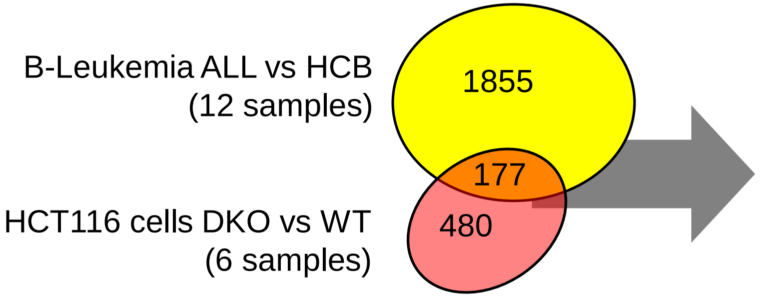
Correlation between the CD44 alternative splicing and the intragenic DNA methylation in 4 different cellular systems

Cellular model	**HCT116**	**HeLa**	**MCF10A carcinogenesis**	**B-ALL leukenogenesis**
DNMTs levels	DKO DNMT1 & DNMT3b ↓	siRNA DNMT1 ↓	Increase 4x ↑	Increase 3x ↑
Global DNA methylation	↓	↓**?**	change	change
DNA methylation in the CD44 body	↓	↓	↑	↓
SUV39H1	↓ 3x	no change	↑ 3x	↓ 3x
EMT (Twist1, Vim,…)	E→M	no	E→M	Not applicable
ESRP1 splicing factor	↓	Not expressed	↑	Not expressed
CD44 variant exons	↓	↓	↑	↓

Common alternative splicing events in different cell types

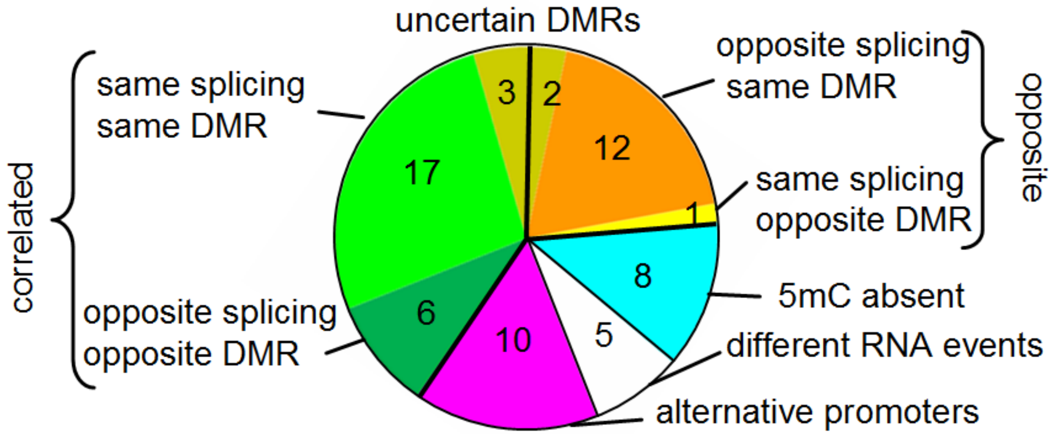
DNA methylation has a very poor predictive power of the outcome of alternative splicing for the host genes under scrutiny

Differentially spliced genes
(MAJIQ analysis)



64 common
RNA events

Differentially spliced sites in ALL and in DKO



56% (23/41) of them showed modified splicing consistent with the change in 5mC levels in ALL and in DKO cells

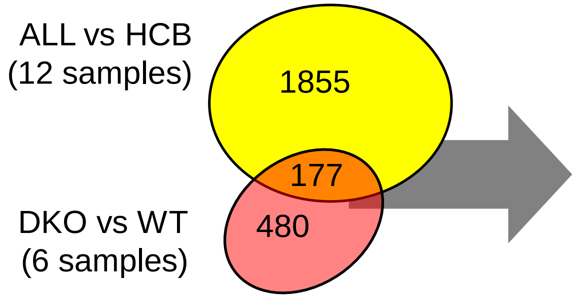
26% (13/41) of them showed modified splicing inconsistent with the change in 5mC levels in ALL and in DKO cells

12% (5/41) of them showed modified splicing with uncertain change in 5mC levels in ALL and in DKO cells

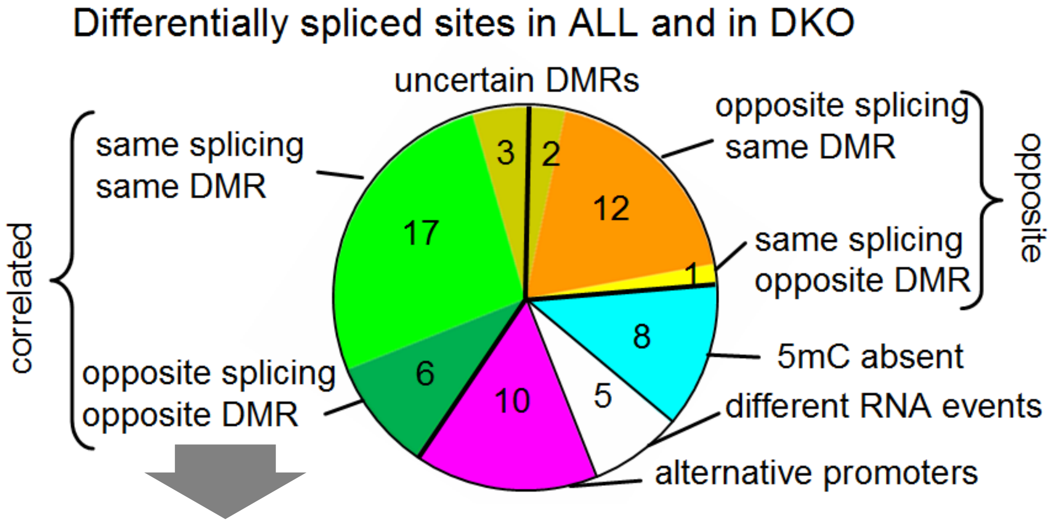
Evidence for indirect effects of DNA methylation on alternative splicing regulation

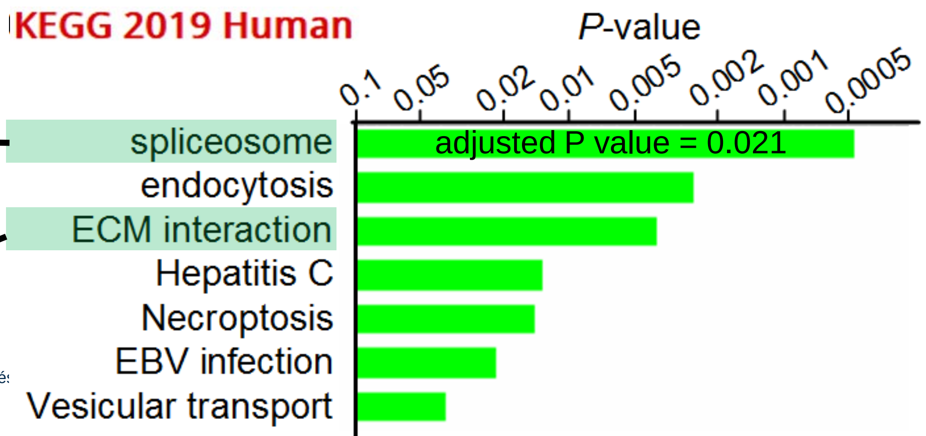
A small number of differentially spliced genes (enriched in splicing factors) may function as sensors of DNA methylation, and thereby causing the more global changes in alternative splicing.

Differentially spliced genes (MAJIQ analysis)



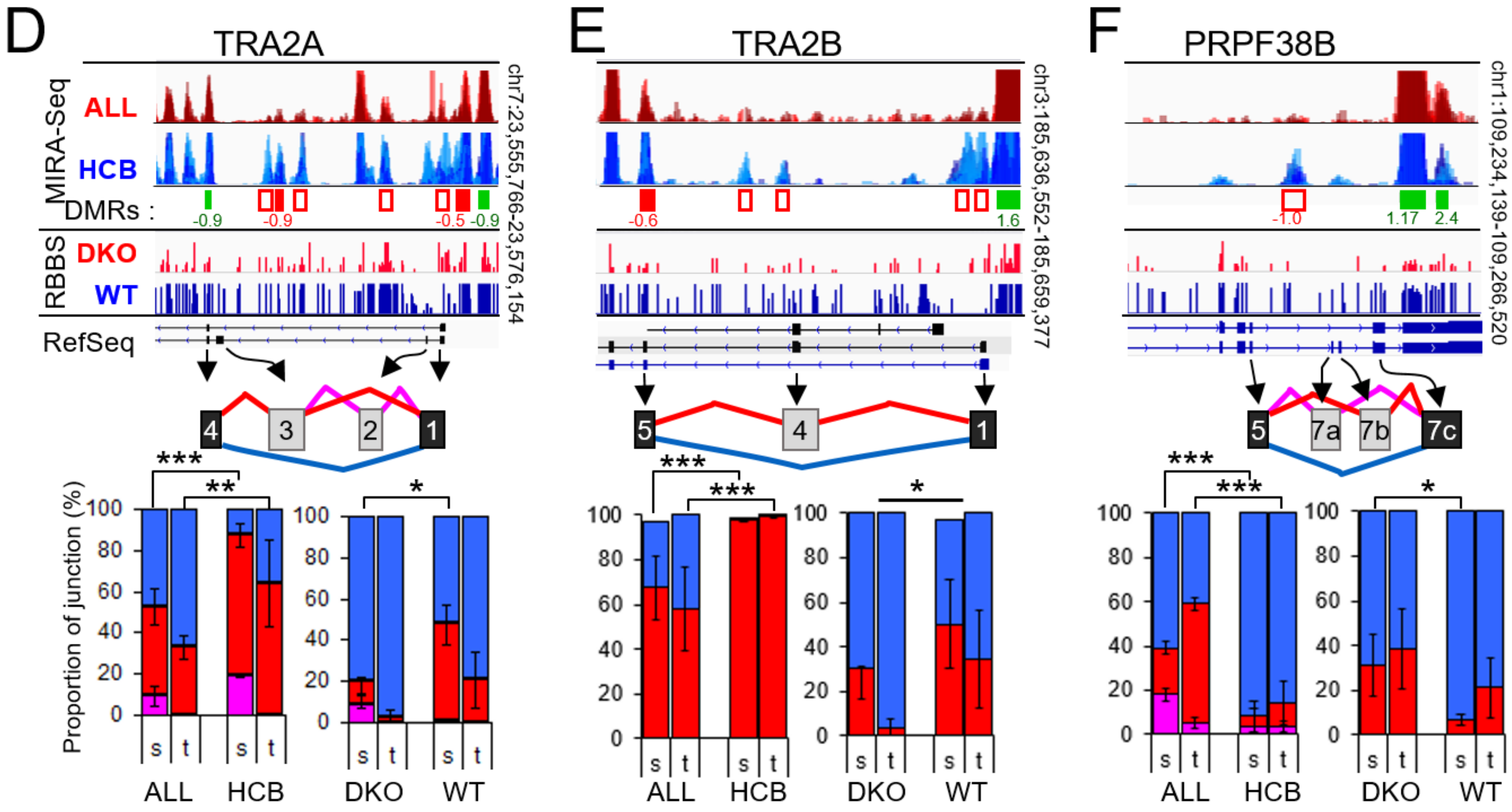
64 common RNA events





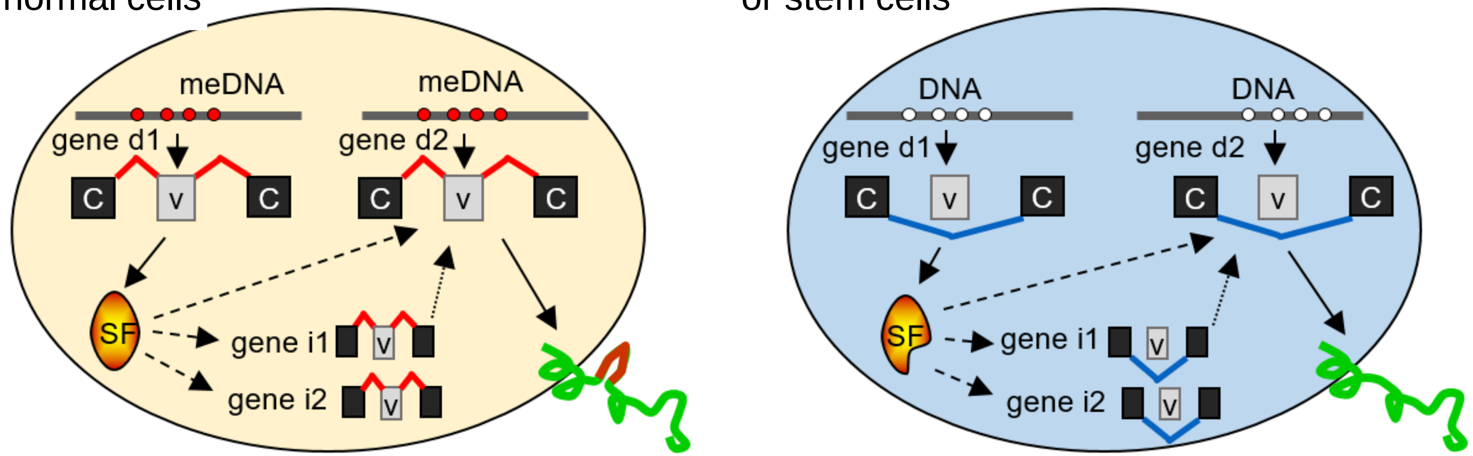
3 splicing factors among one has been described as a regulator of CD44, ...

Splicing factors differentially spliced



DNA methylation exerts direct and indirect effects on splicing decisions.

Only a few genes could serve as direct detectors of DNA methylation levels.



Differentiated or normal cells

Cancer cells or stem cells

Certain genes, including those involved in RNA splicing, can have their function altered by DNA methylation. This could help the cell adjust when methylation levels change.

Batsché et al., 2021 *Nuc. Ac. Res.*

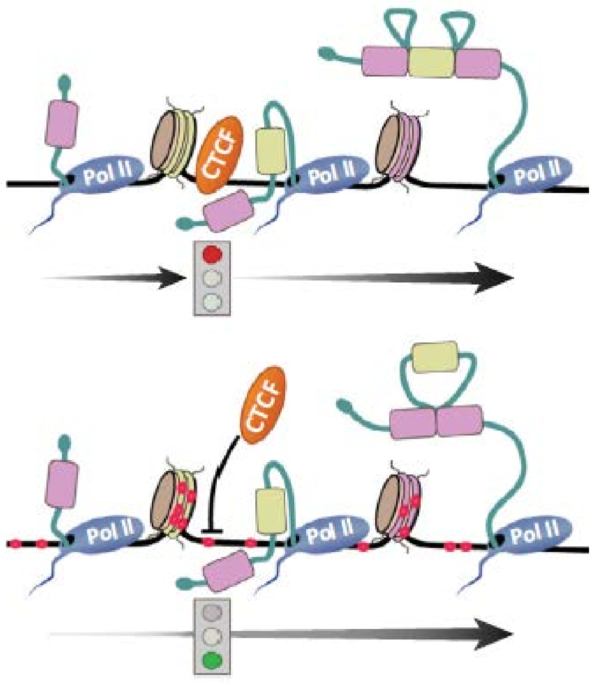
How DNA methylation could directly influence alternative splicing ?

> DNA methylation itself affects RNAPII elongation on a reporter Lorincz, et al. (2004)

> Readers that interpretes the methylome

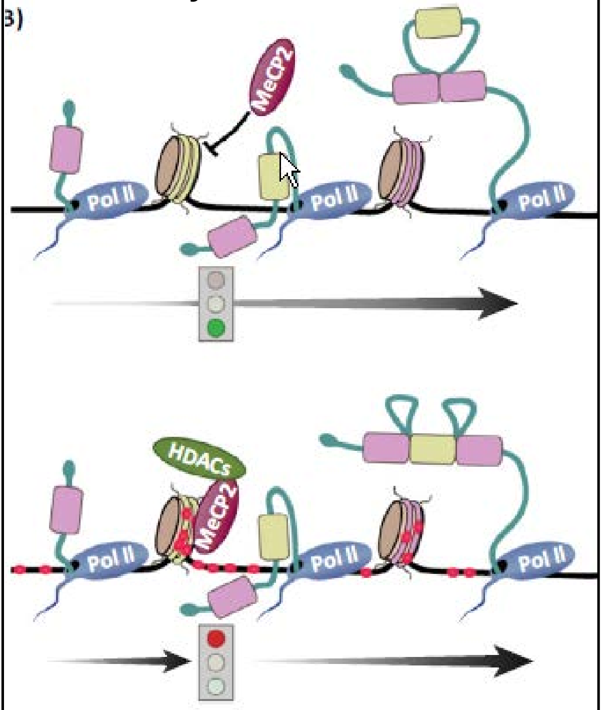
Key:
- ▭ Constitutive exon
- ▭ Alternative exon
- ● Methylated CpG
- ● H3K9me3

CTCF recognizes unmethylated DNA



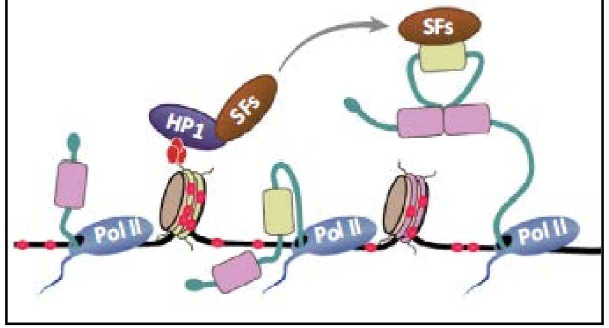
Shukla et al. 2011 *Nature*

MeCP2 recognizes methylated DNA



Young et al. 2005 *PNAS*,
Maunakea et al. 2013 *Cell Research*

HP1s contributes to methylated DNA effects



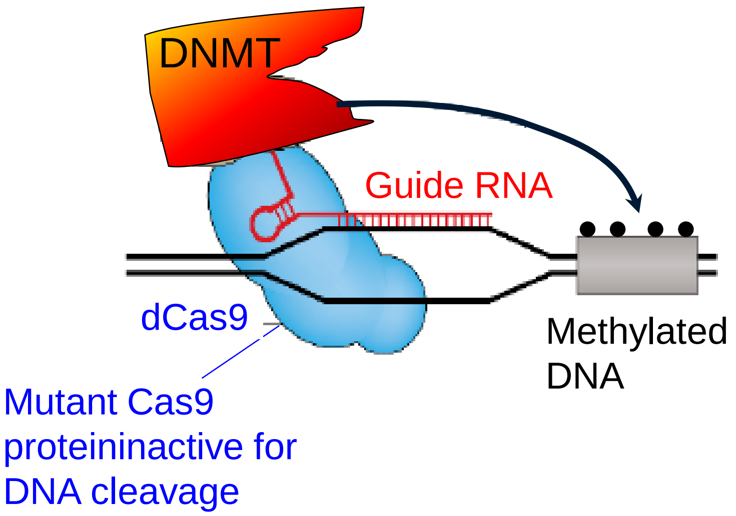
Yearim et al. 2015 *Cell*

Other readers of DNA methylation ?
The Methyl-binding proteins.

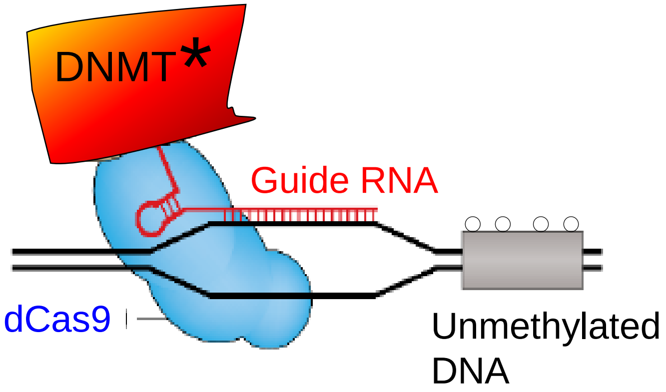
Direct effects of epigenetic modifications?

DNA methylation can be targeted at specific loci by the CRISPR/dCas9-DNMT chimera.

Catalytic domain of DNA methyltransferase



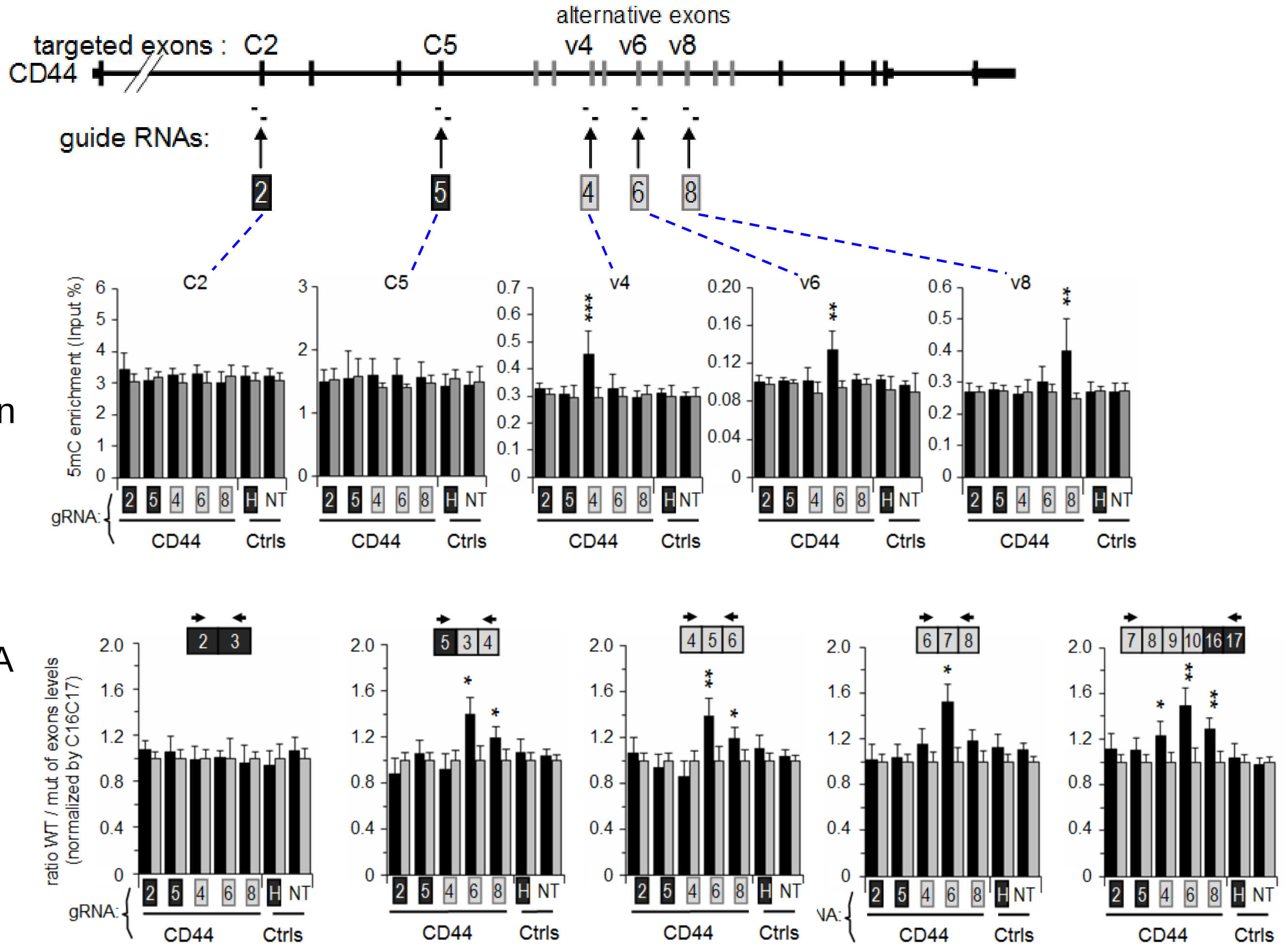
Mutant catalytic domain of DNA methyltransferase



In cells where there is very little DNA methylation due to the invalidation of the DNMT1 and DNMT3b genes

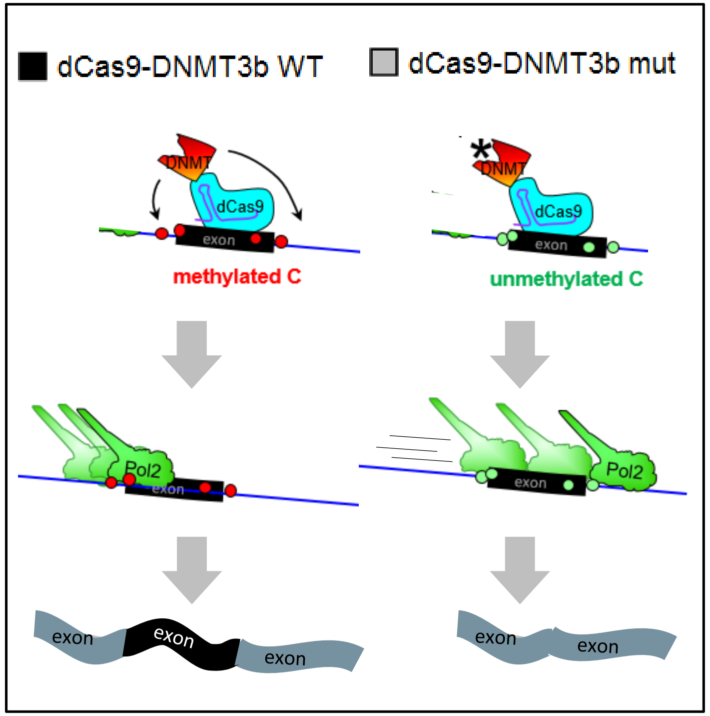
Batsché et al., 2021 *Nuc. Ac. Res.*

Effets directs des modifications épigénétiques ?



Level of DNA methylation (MeDIP)

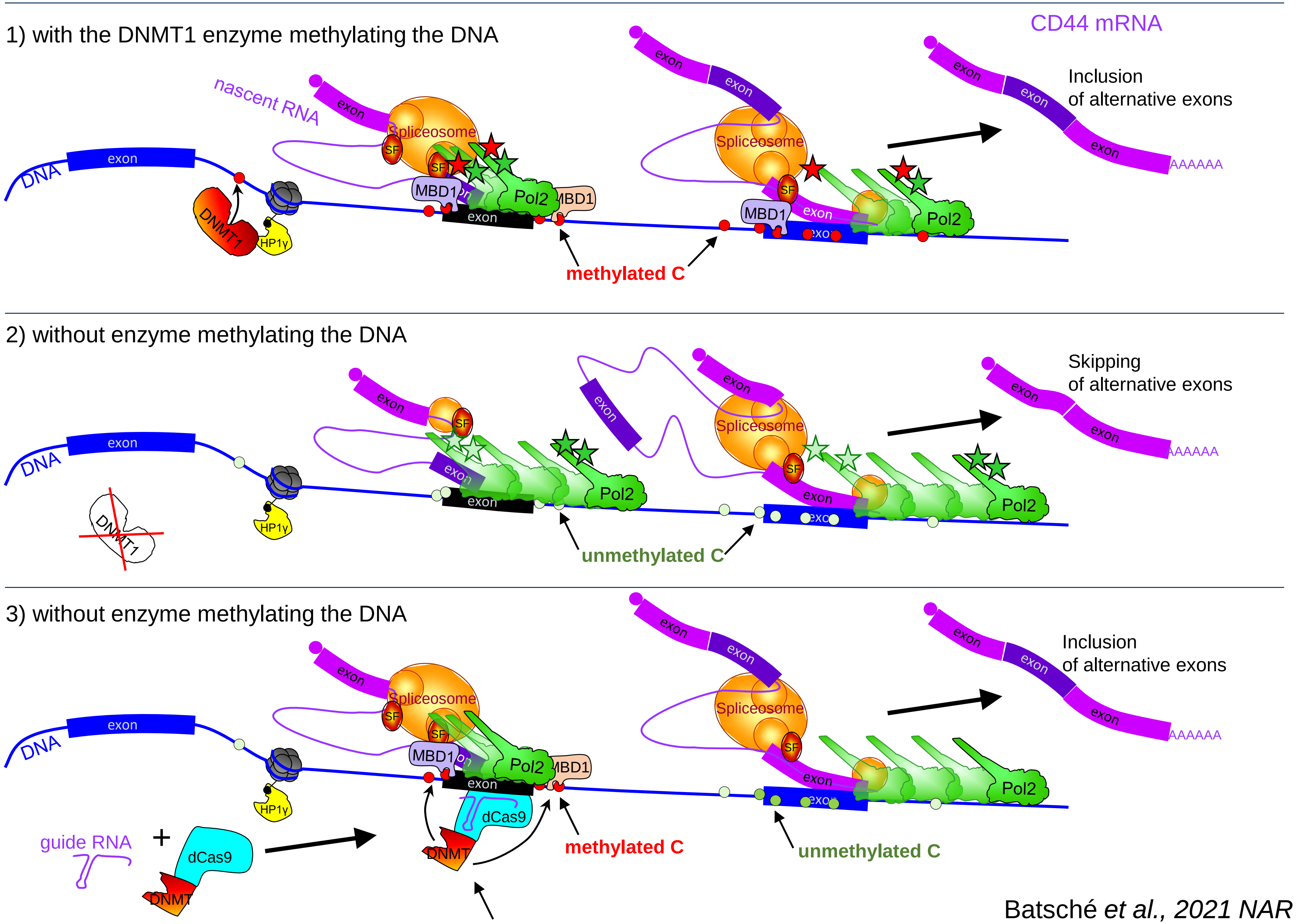
Change in RNA levels



The functional catalytic domain of DNMT promoted the inclusion of variant exons when specifically targeted to the variant region of CD44.

Batsché et al., 2021 *Nuc. Ac. Res.*

DNA can be locally remethylated by a chimeric protein providing the catalytic DNA methylation domain via the inactivated endonuclease Cas9 and a guide RNA complementary to the targeted region.



1) with the DNMT1 enzyme methylating the DNA

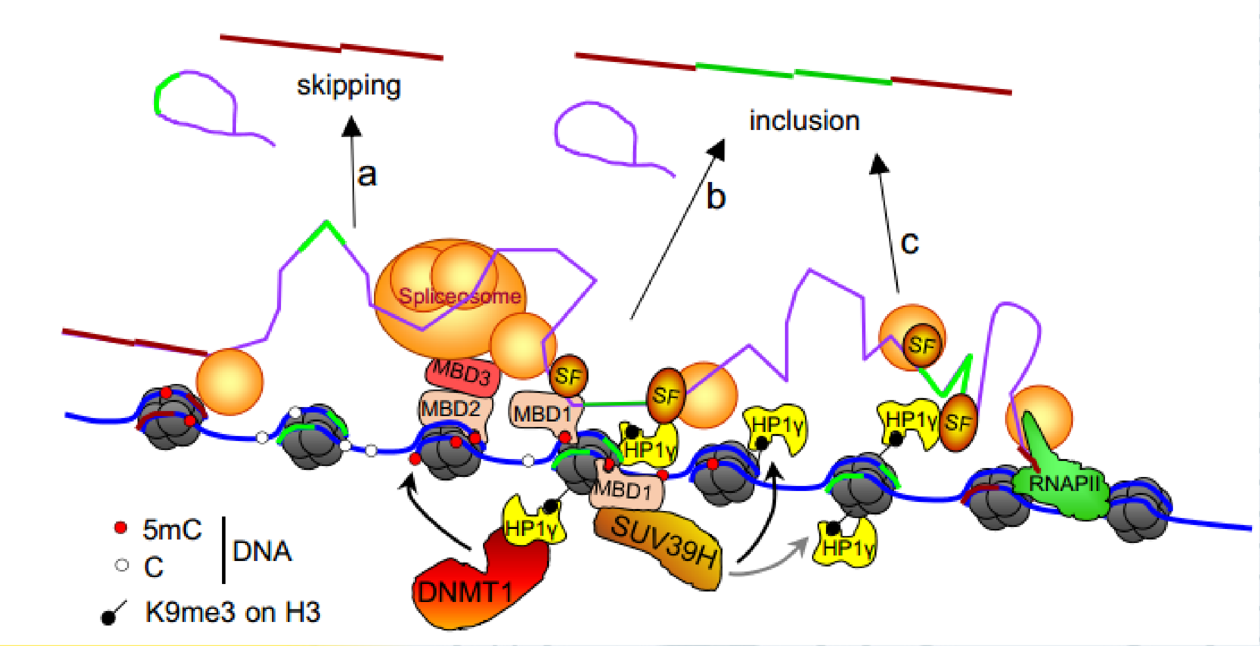
2) without enzyme methylating the DNA

3) without enzyme methylating the DNA

Batsché *et al., 2021 NAR*

Conclusions

- ➔ CRISPR-dCas9 chimeric proteins are valuable tools to study epigenetic transcription and alternative splicing regulation
- ➔ Direct contribution of DNA methylation to the alternative splicing decisions is mediated by MBD1/2/3 and HP1γ proteins



Oriane Mauger
PhD
T. Johnson

Ben Hopkins
Erasmus

Charly Hanmer-Llyod
Erasmus

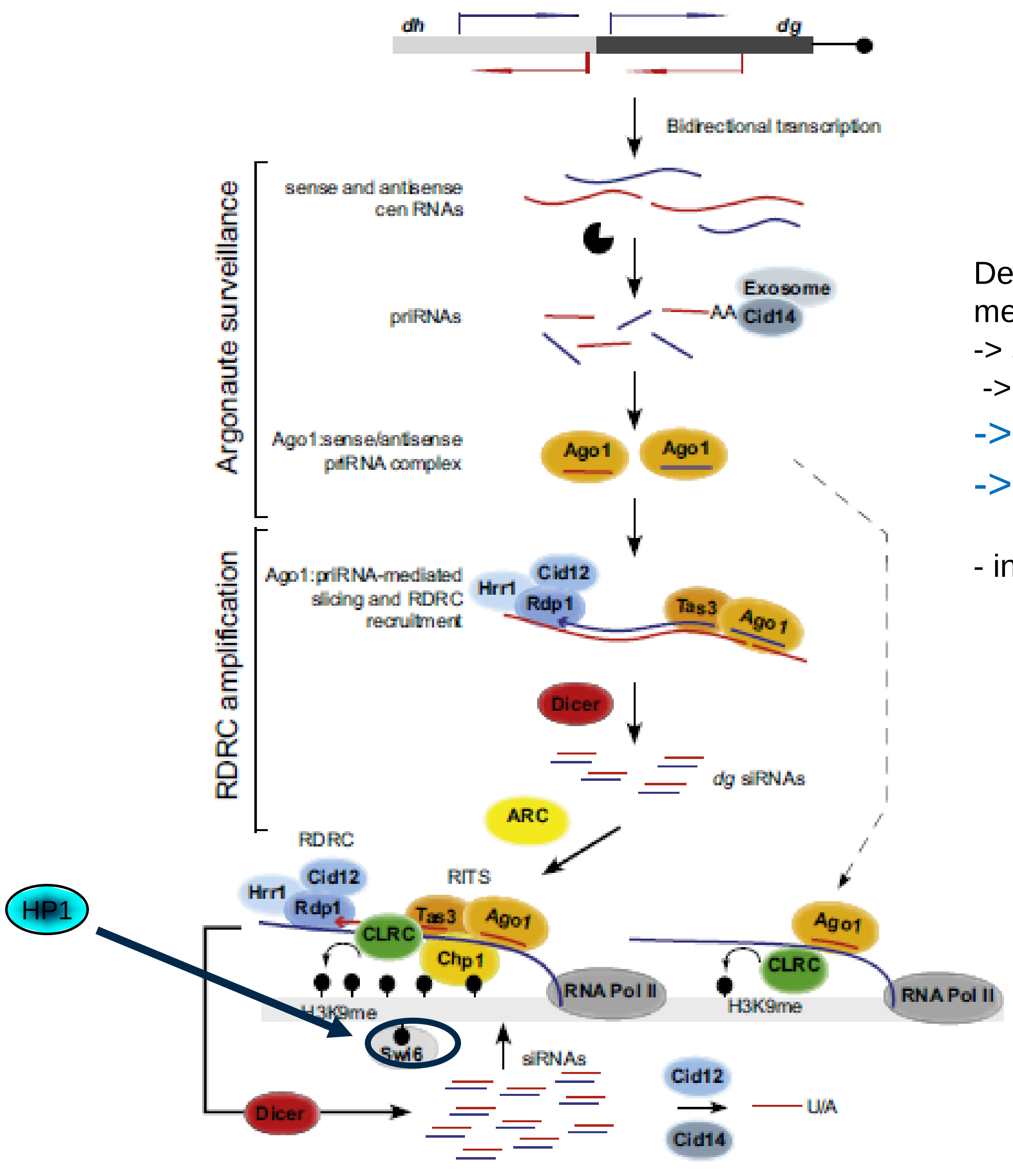
Etienne Kornobis
bioinfo

Jia Yi
PhD

→ 10/10/2025

Nuclear RNA interference and alternative splicing

Role of Nuclear Argonaute : chromatin silencing



Heterochromatin dependent amplification

Demonstration of a nuclear transcriptional gene silencing mechanism by the Argonaute proteins :
-> small RNA (Dicer dependent)
-> recruitment through base-pairing with the native transcript
-> methylation of H3K9me3, DNA methylation
-> recruitment of HP1

- in Vertebrates : evidences of nuclear functions exist
but still discussed

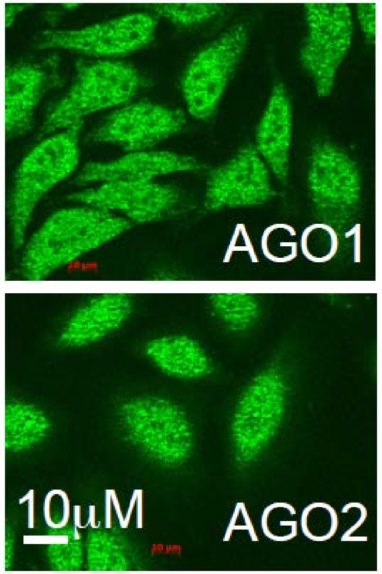
In *S. pombe*:
Laboratories of
-Danesh Moazed
-Shiv Grewal
-Rob Allshire

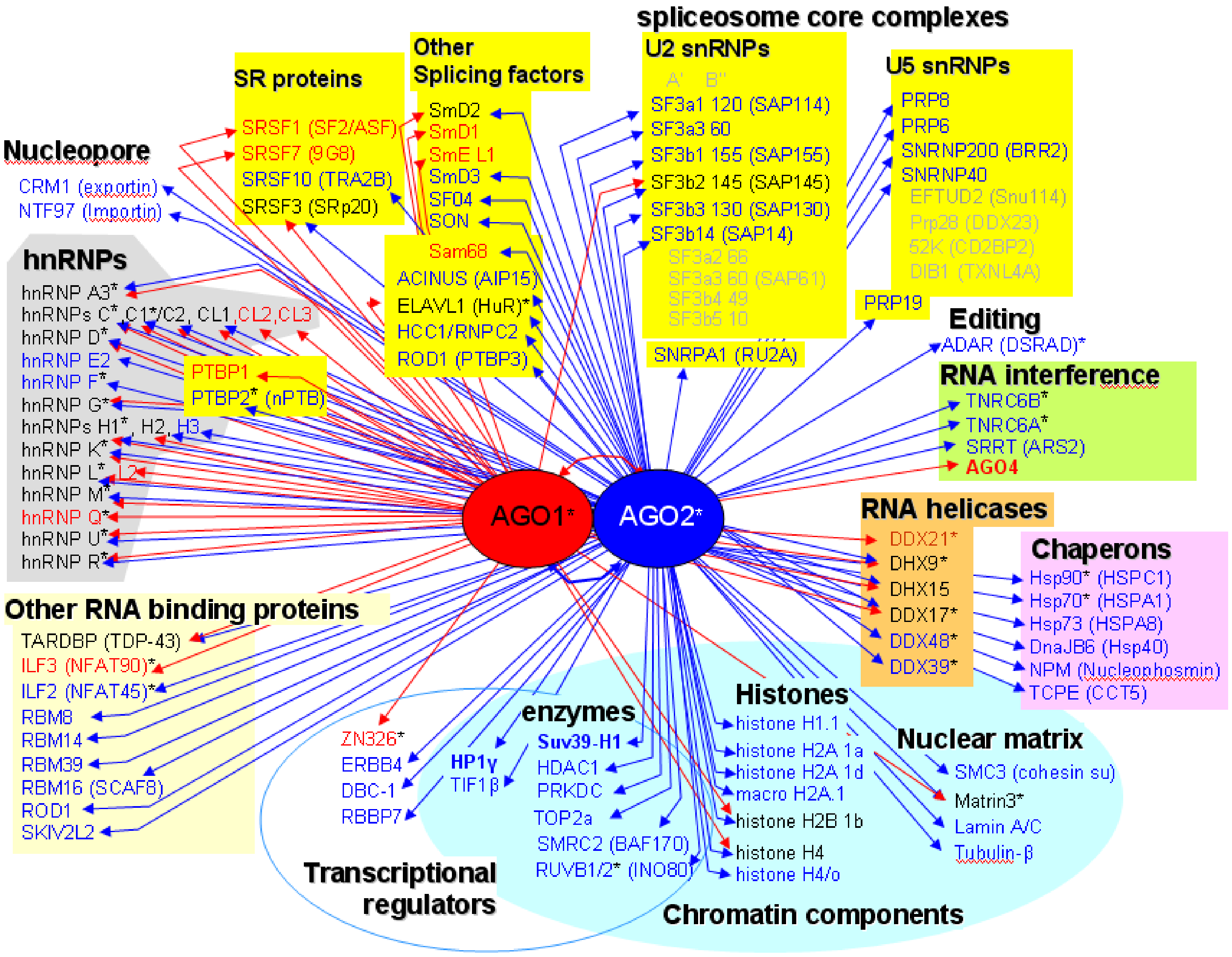
In S. *pombe* from Halic & Moazed (Cell 2010)

Interactome of AGO1/2 in the chromatin

AGO proteins are also nuclear in mamalian somatic cells

HeLa cells



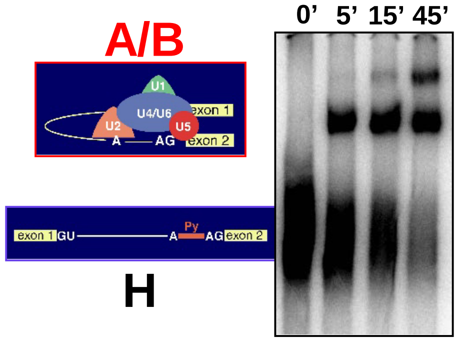


Maya Ameyar

Annick Harel-Bellan

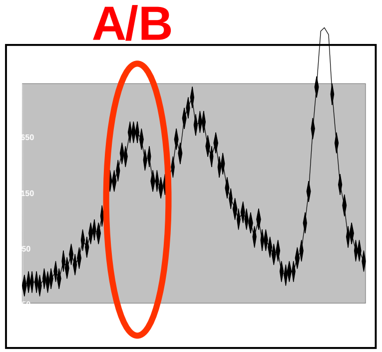
Ameyar-Zazoua et al. 2012 *NSMB*

Spliceosome purified with U2 snRNP



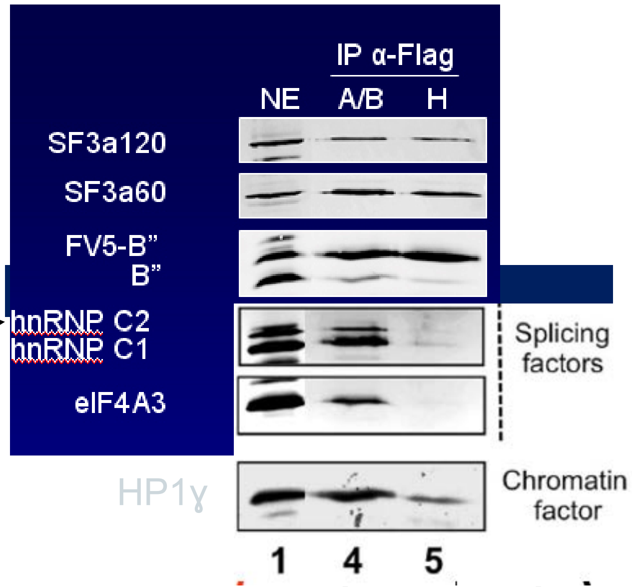
Spliceosomal complexes

chromatography →



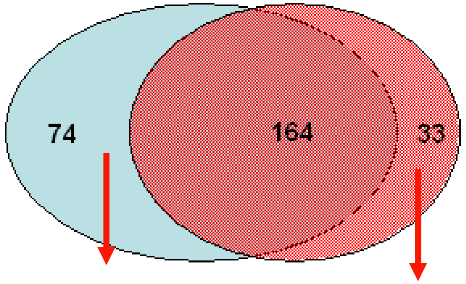
Gel filtration

IP of Flag-B"



Western blot analysis

Mass spectrometry analysis



New factors associated to Spliceosome

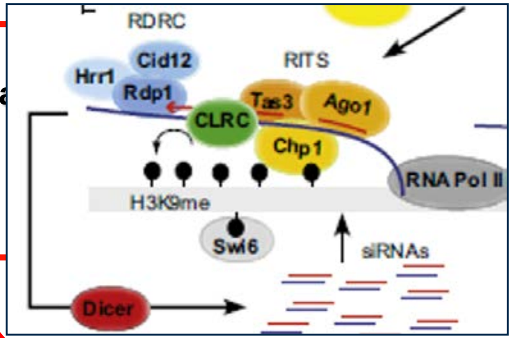
Factors previously described

BAF200
Brm / Brg1
BAF170
BAF155
Snf5/Ini1
BAF60a/b/c
BAF57
mSIN3A
HDAC1
HDAC2
RbAP48

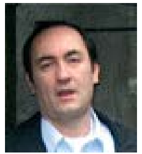
Ehmt1
Ehmt2 (G9a
AGO1
AGO2
DICER

MRG15

several histones
…

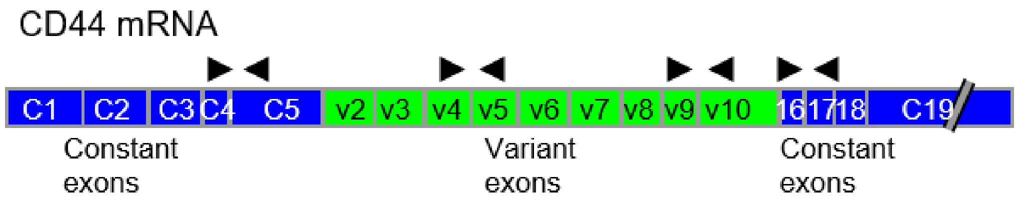


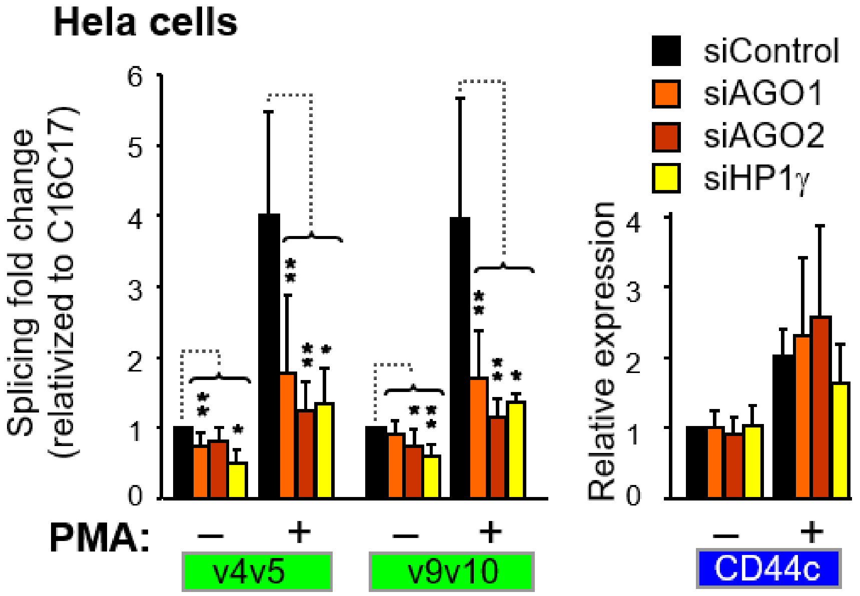
RITS factors Associated to Spliceosome

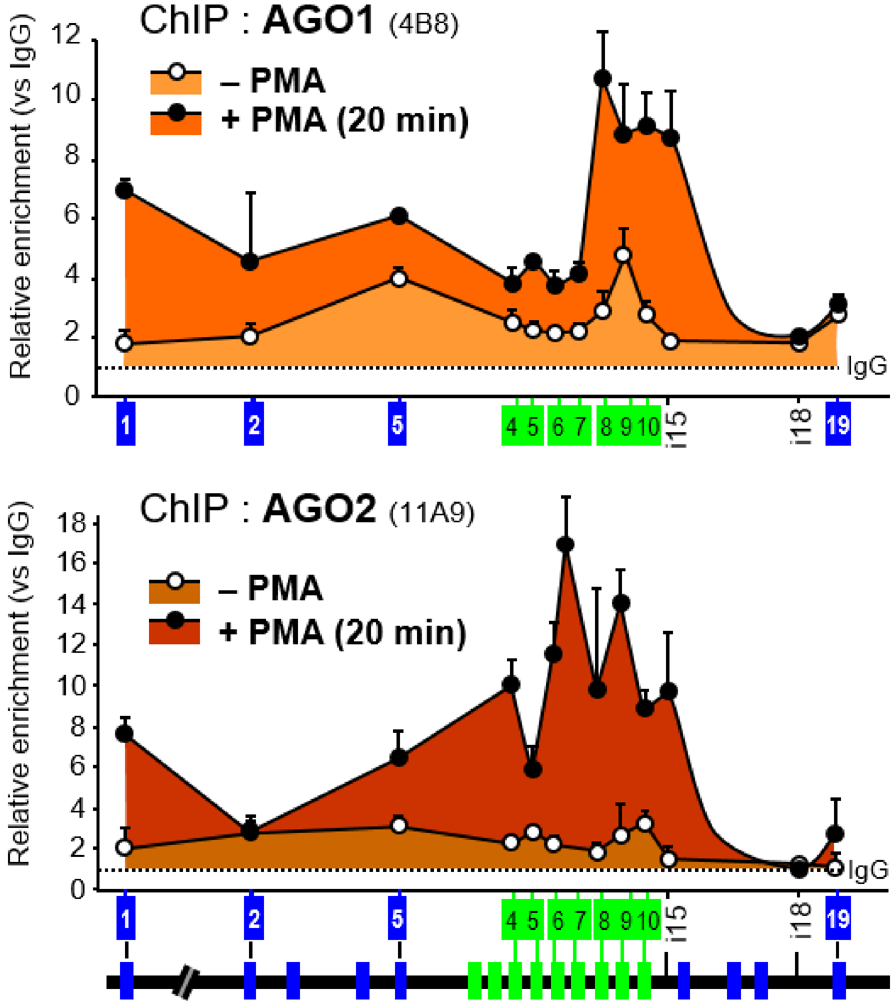


E. Allemand (2016) PloS Genet

AGO1 and AGO2 regulate the alternative splicing of CD44

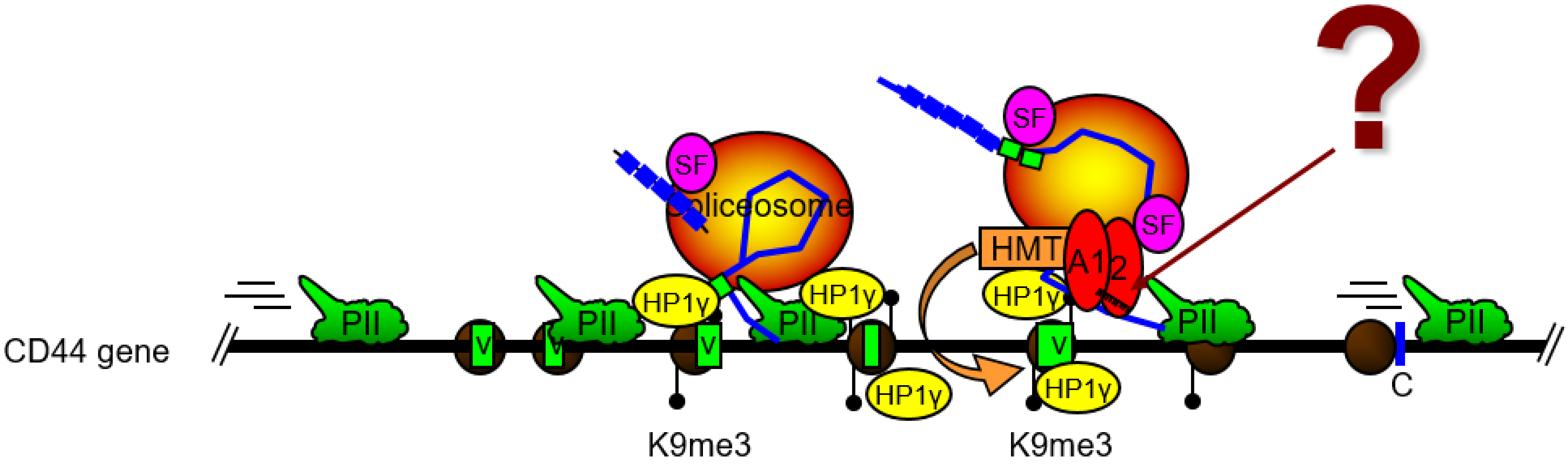






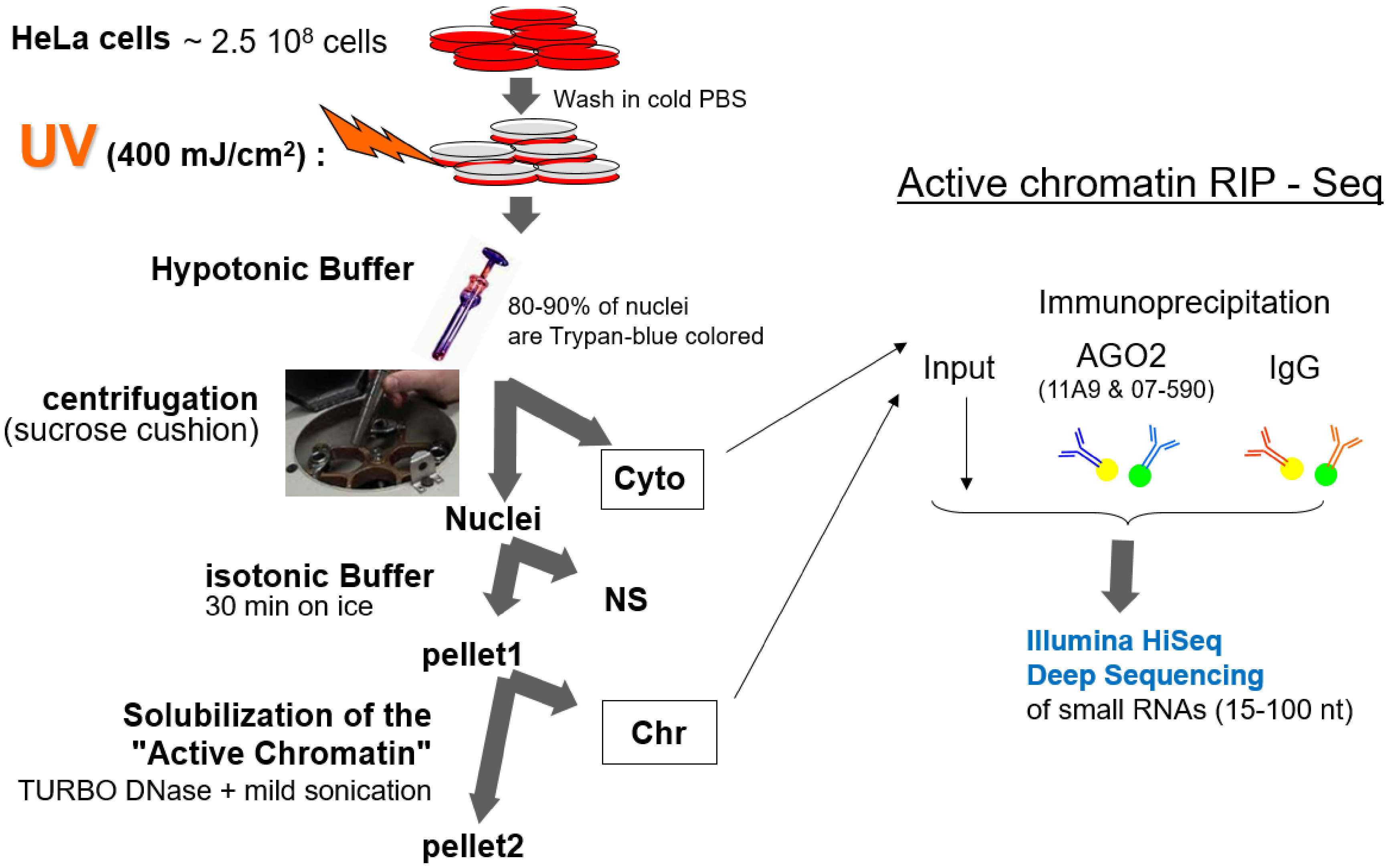
Ameyar-Zazoua et al. 2012 *NSMB*

What are the endogenous small RNAs that target the recruitment of the AGO2.ca ?



RNAi-dependent Inducing Transcriptional Slowdown
and alternative splicing regulation

Small RNAs bound to the chromatin complex associated to endogenous AGO2 proteins



HeLa cells ~ 2.5 10^8 cells

Wash in cold PBS

UV (400 mJ/cm^2) :

Hypotonic Buffer

80-90% of nuclei
are Trypan-blue colored

centrifugation
(sucrose cushion)

Cyto

Nuclei

isotonic Buffer
30 min on ice

NS

pellet1

Solubilization of the "Active Chromatin"
TURBO DNase + mild sonication

Chr

pellet2

Active chromatin RIP - Seq

Immunoprecipitation

Input

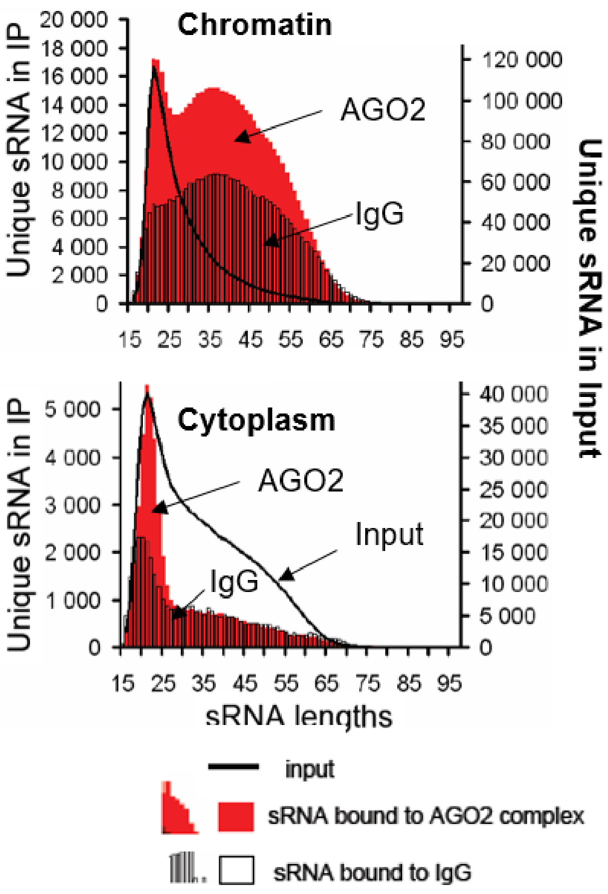
AGO2
(11A9 & 07-590)

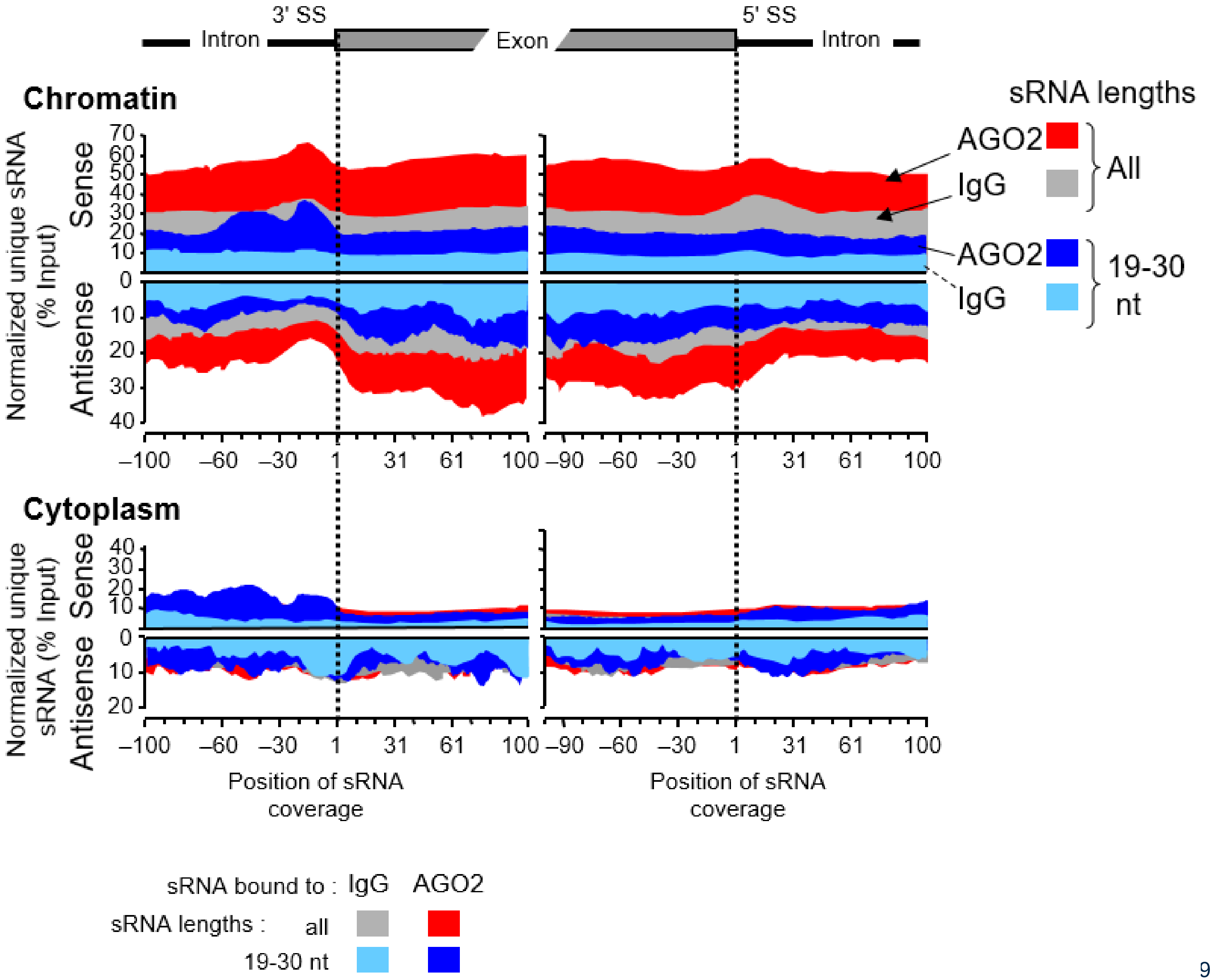
IgG

Illumina HiSeq
Deep Sequencing
of small RNAs (15-100 nt)

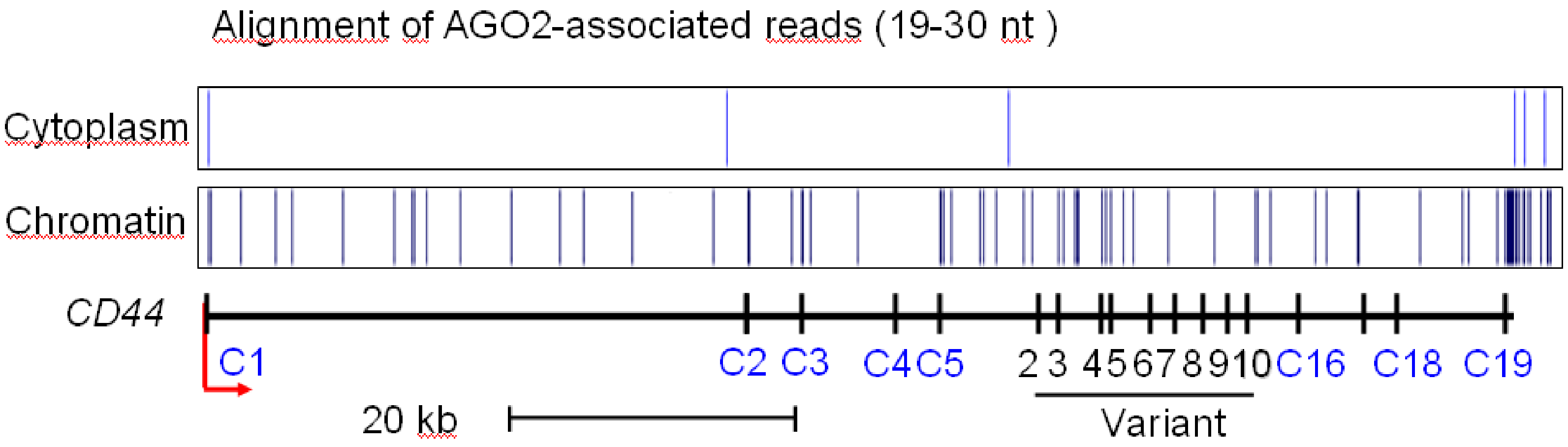
small RNAs bound to chromatin associated AGO2 are mainly in SENSE

Alignments on Protein-coding genes





AGO2-bound small RNAs on the CD44 gene

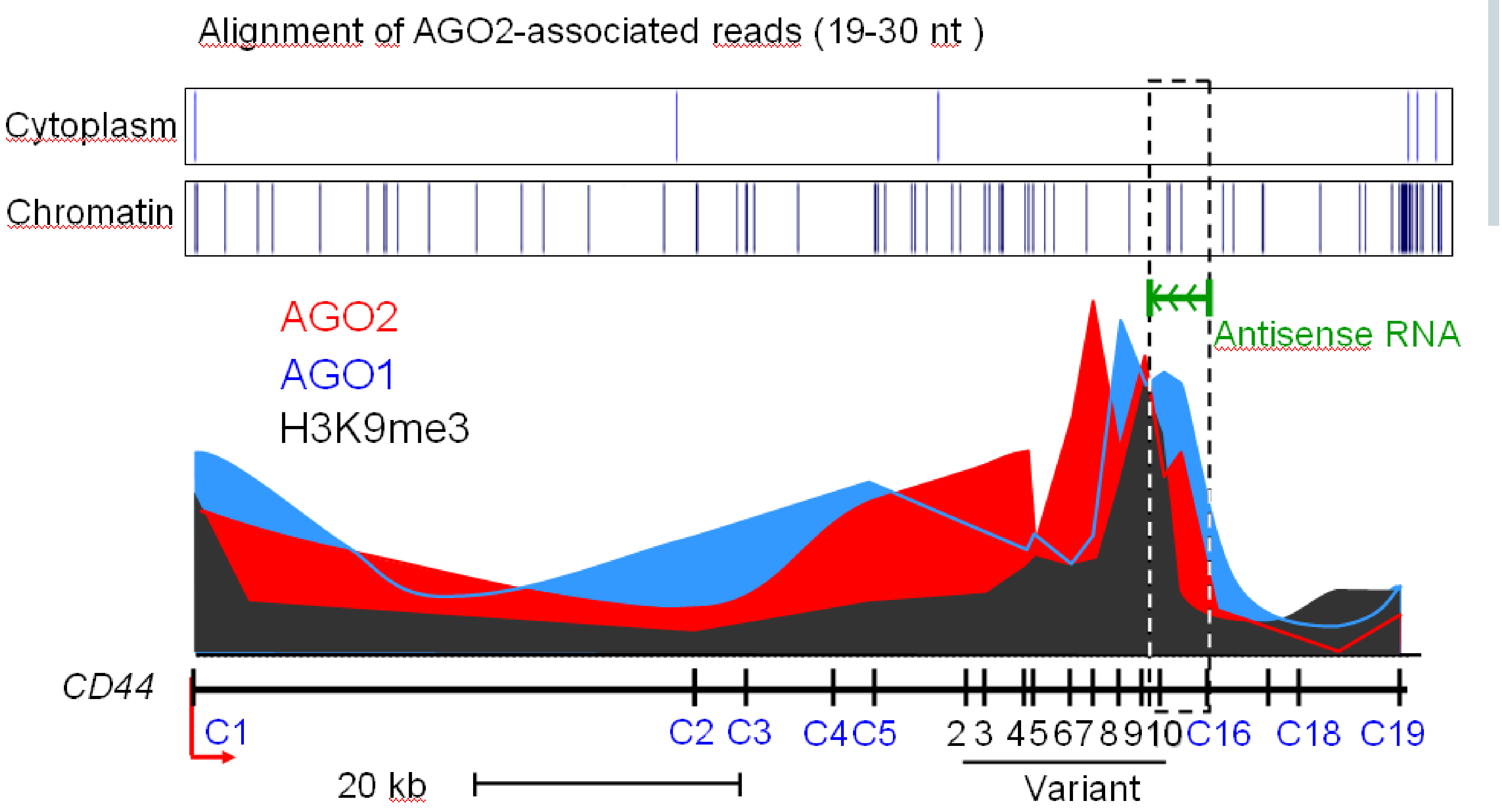


The distribution of sRNAs associated with AGO2 in the CD44 gene does not show any particular enrichment in exons or intron-exon junctions

There is no **particular antisense sRNA** that would ‘simply’ explain the recruitment of AGO2.ca

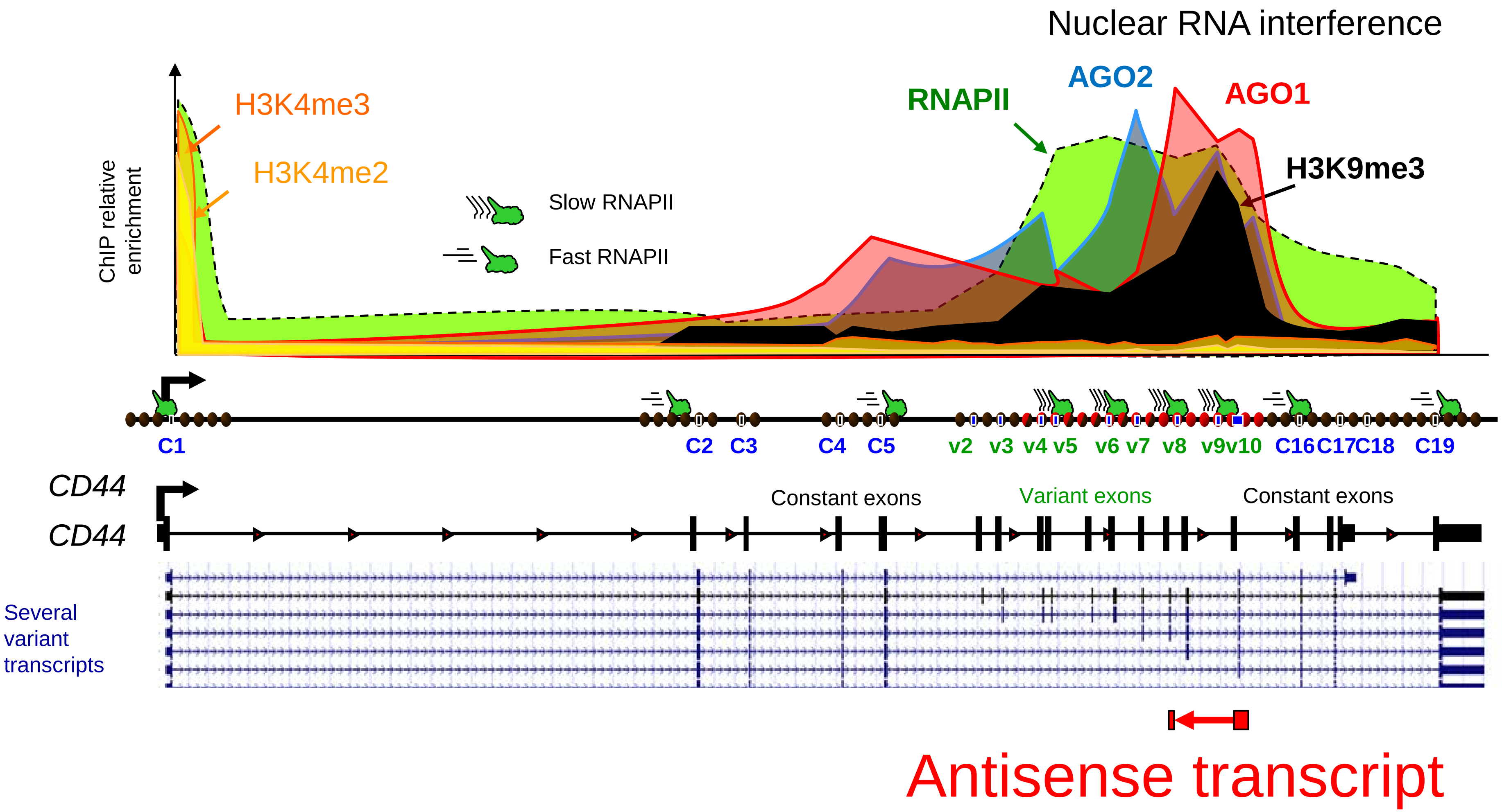
This distribution suggests a role for AGO2 in chromatin ‘surveillance’.

AGO2-bound small RNAs on the CD44 gene



Le recrutement des proteines AGO1/2 et le maximum d'activité des HMT associés peuvent s'expliquer par la présence d'un transcrit antisens à la fin de la région variante de CD44

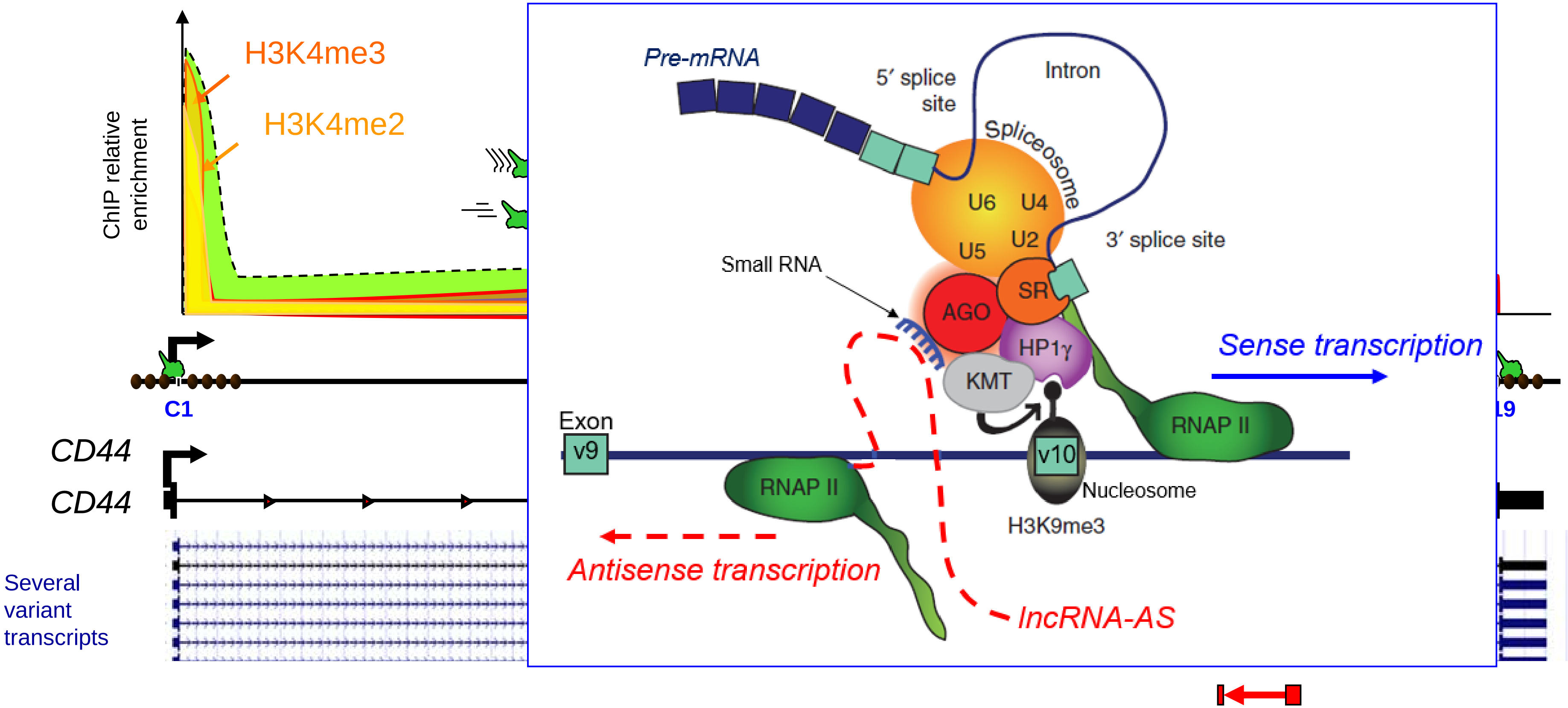
Epigenetic induction of RNAPII slowing down favors the inclusion of variant exons in the CD44 gene



Ameyar-Zazoua et al. 2012 *NSMB*

Epigenetic induction of RNAPII slowing down favors the inclusion of variant exons in the CD44 gene

Nuclear RNA interference



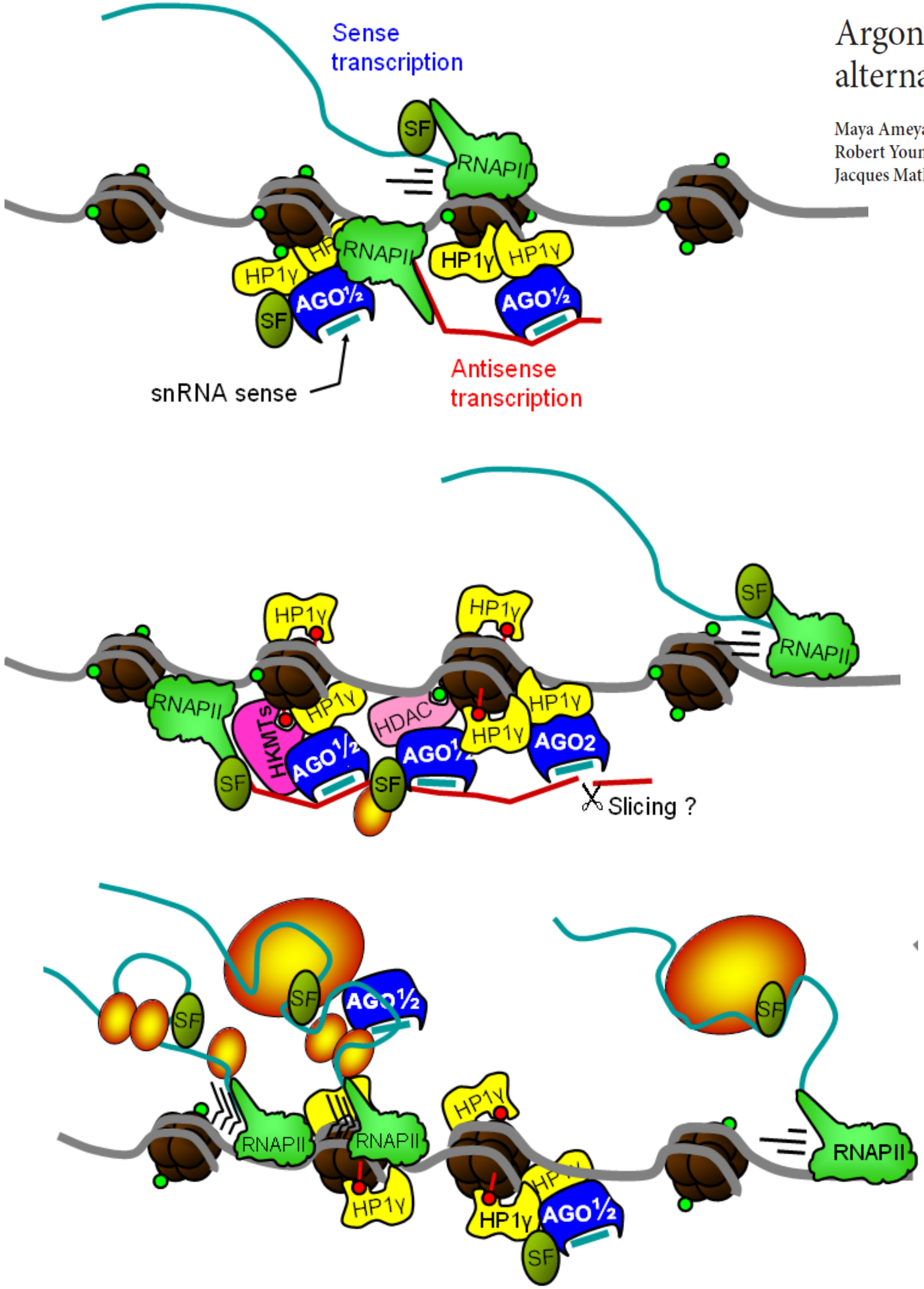
Antisense transcript

Ameyar-Zazoua et al. 2012 *NSMB*

9 September 2012
nature structural & molecular biology

Argonaute proteins couple chromatin silencing to alternative splicing

Maya Ameyar-Zazoua[1–4], Christophe Rachez[4], Mouloud Souidi[1–3], Philippe Robin[5], Lauriane Fritsch[5], Robert Young[6,11], Nadya Morozova[1–3], Romain Fenouil[7–9], Nicolas Descostes[7–9], Jean-Christophe Andrau[7–9], Jacques Mathieu[1,2], Ali Hamiche[10], Slimane Ait-Si-Ali[5], Christian Muchardt[4,12], Eric Batsché[4,12] & Annick Harel-Bellan[1–3]



Model:

The specificity of recruitment relies on properly localized intragenic antisense transcripts : asLincCD44

It is detected by the AGO2 complex by base-pairing mediated by sense short RNA sequences.

Consequently, H3K9me3 and HP1γ are increased locally.

These favor slowing-down of RNAPII and recruitment of splicing factors promoting inclusion of variant exons.

Chromatin modulation by RNA interference enhances the effect of ASO drugs that modify alternative splicing associated with diseases

Article

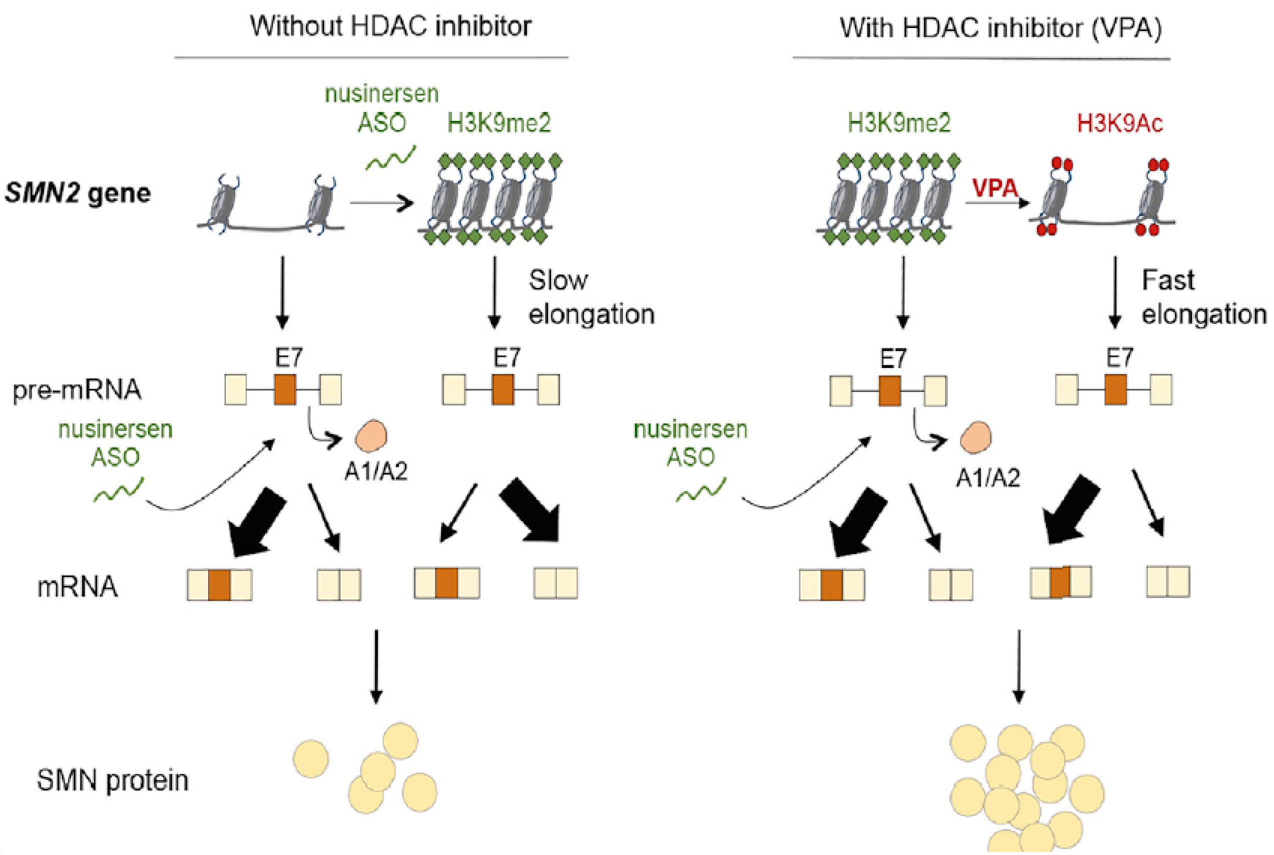
Counteracting chromatin effects of a splicing-correcting antisense oligonucleotide improves its therapeutic efficacy in spinal muscular atrophy

Cell

Cell *185*, 2057–2070, June 9, 2022

Luciano E. Marasco,[1,5] Gwendal Dujardin,[2,5] Rui Sousa-Luís,[3] Ying Hsiu Liu,[4] Jose N. Stigliano,[1] Tomoki Nomakuchi,[4] Nick J. Proudfoot,[2] Adrian R. Krainer,[4] and Alberto R. Kornblihtt[1,6,*]

Spinal muscular atrophy splicing-correcting ASO therapy



SMN1/2 proteins are involved in snRNP biogenesis. A mutation in SMN1 is responsible for SMA. The SMN2 paralogue can be used to restore SMN function by correcting exon 7 splicing.

The antisense oligonucleotide (ASO) nusinersen (Spinraza) is a drug targeting specific exon splicing that has been approved for clinical use in a therapy aimed at reducing the effects of spinal muscular atrophy.

Histone deacetylase (HDAC) inhibitors can promote transcription elongation to cooperate with antisense oligonucleotides (ASOs) that correct Smn2 gene splicing to restore functionality.

- SMN2 E7 is a class II exon whose inclusion is stimulated by rapid Pol II elongation.
- The Nusinersen-type ASO promotes the inclusion of E7 and the chromatin-inhibiting mark H3K9me2 via AGO1/AGO2
- The H3K9me2 epigenetic mark creates a barrier to Pol II elongation that inhibits E7 inclusion
- HDAC inhibitors remove the barrier and the inhibitory epigenetic mark