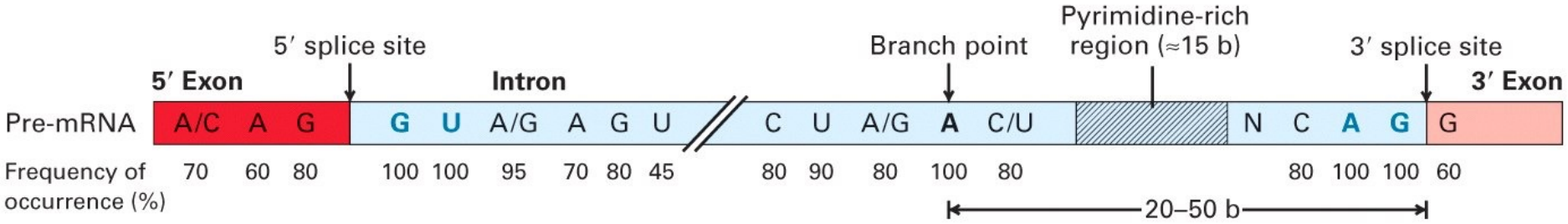
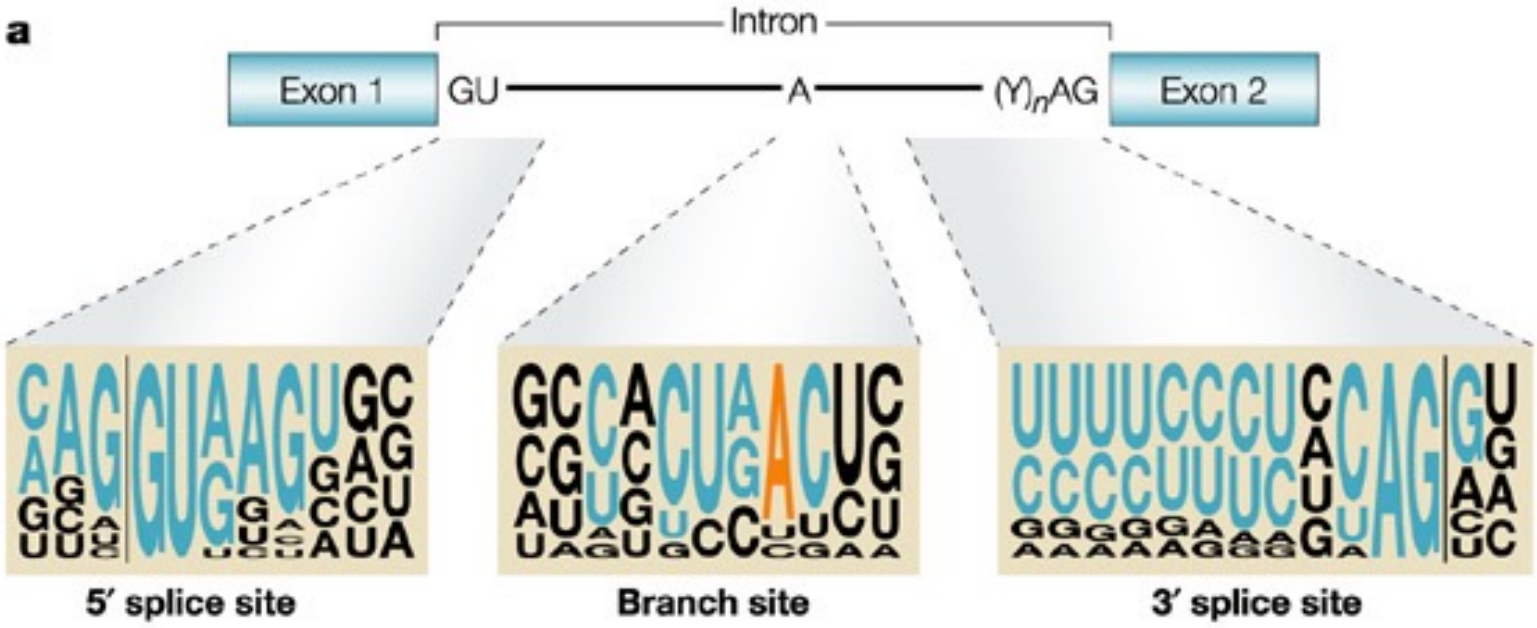
Alternative Splicing

Consensus Sequences Surrounding the 5′ and 3′ Splice Sites

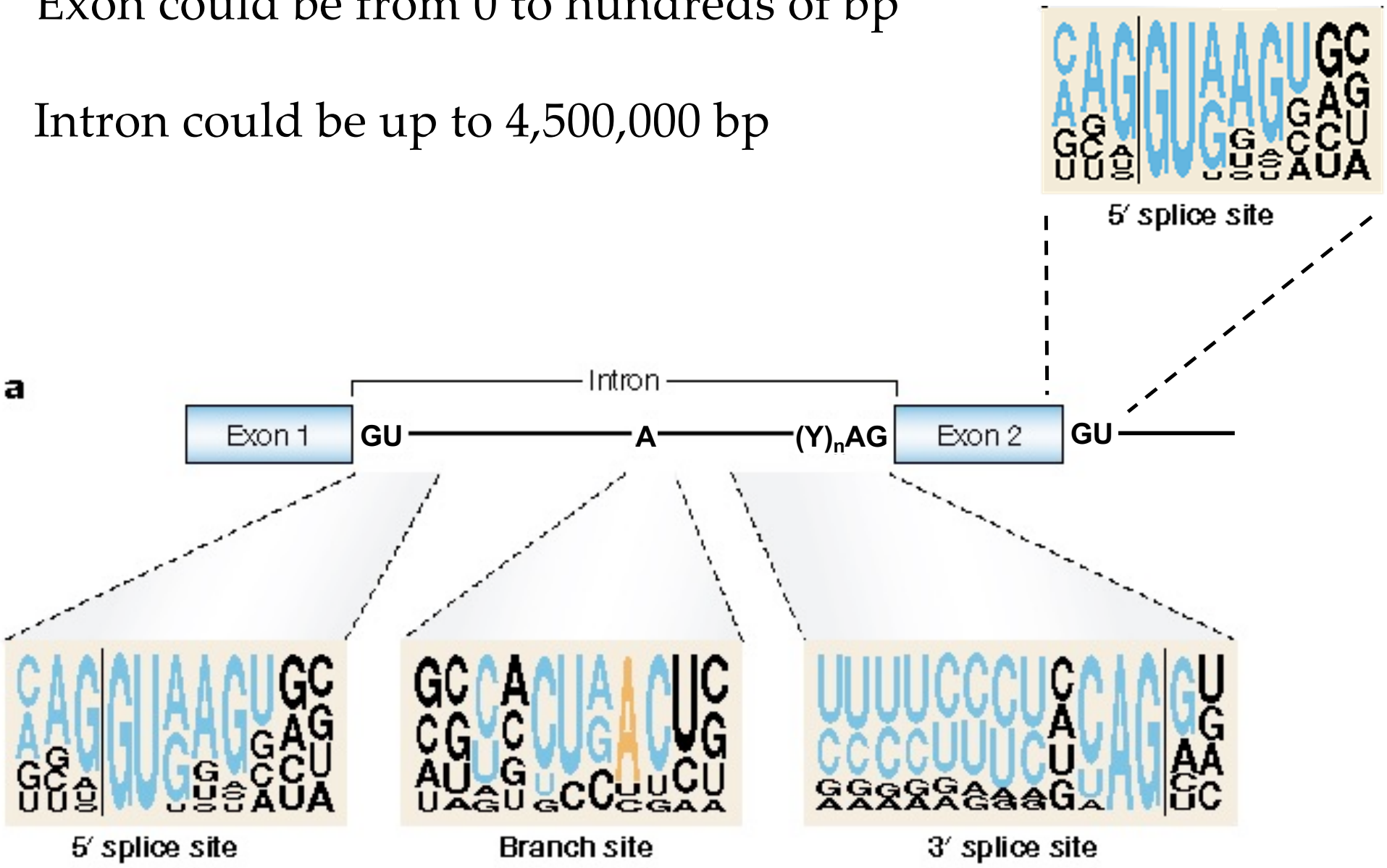




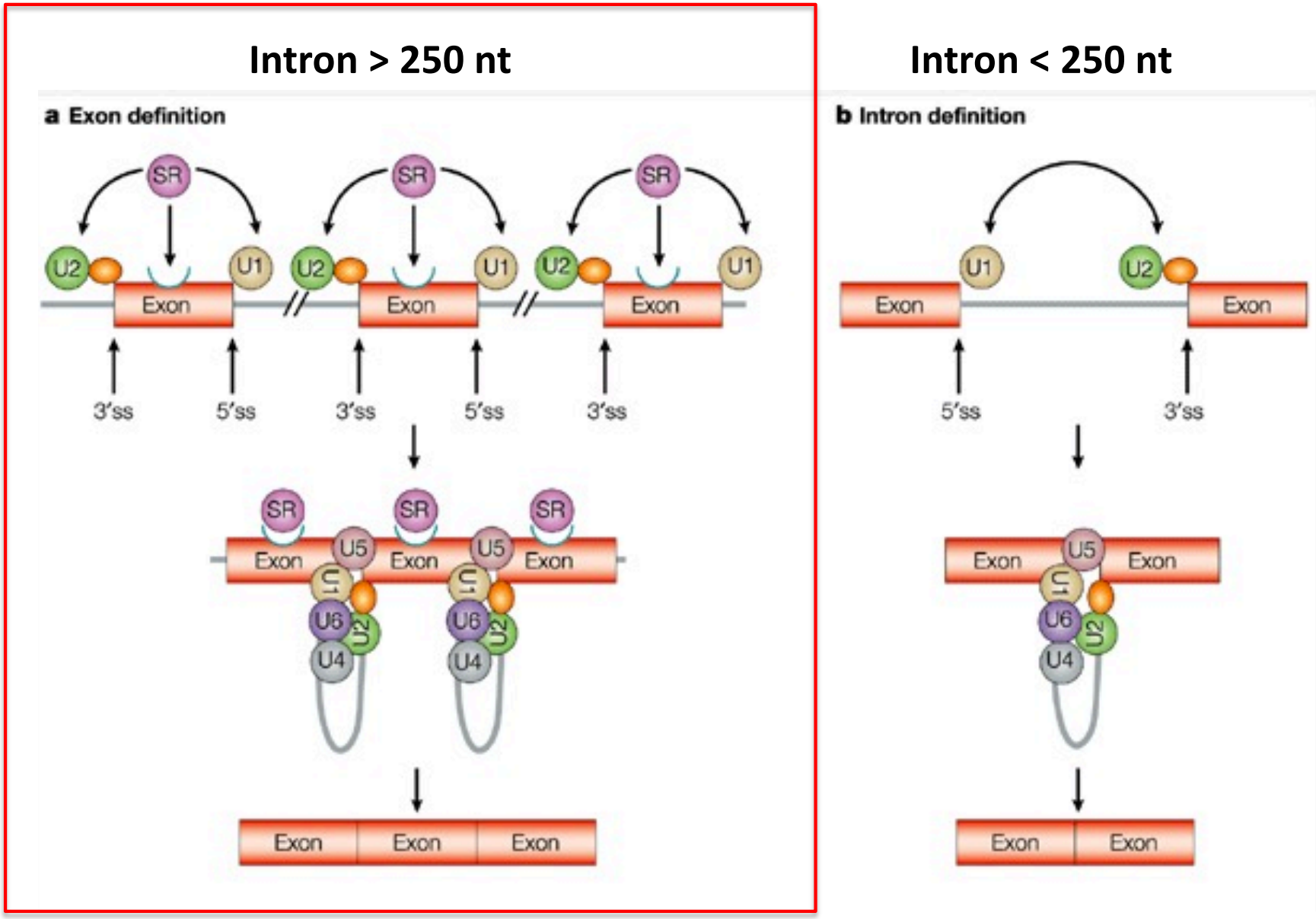
Problem

Exon could be from 0 to hundreds of bp

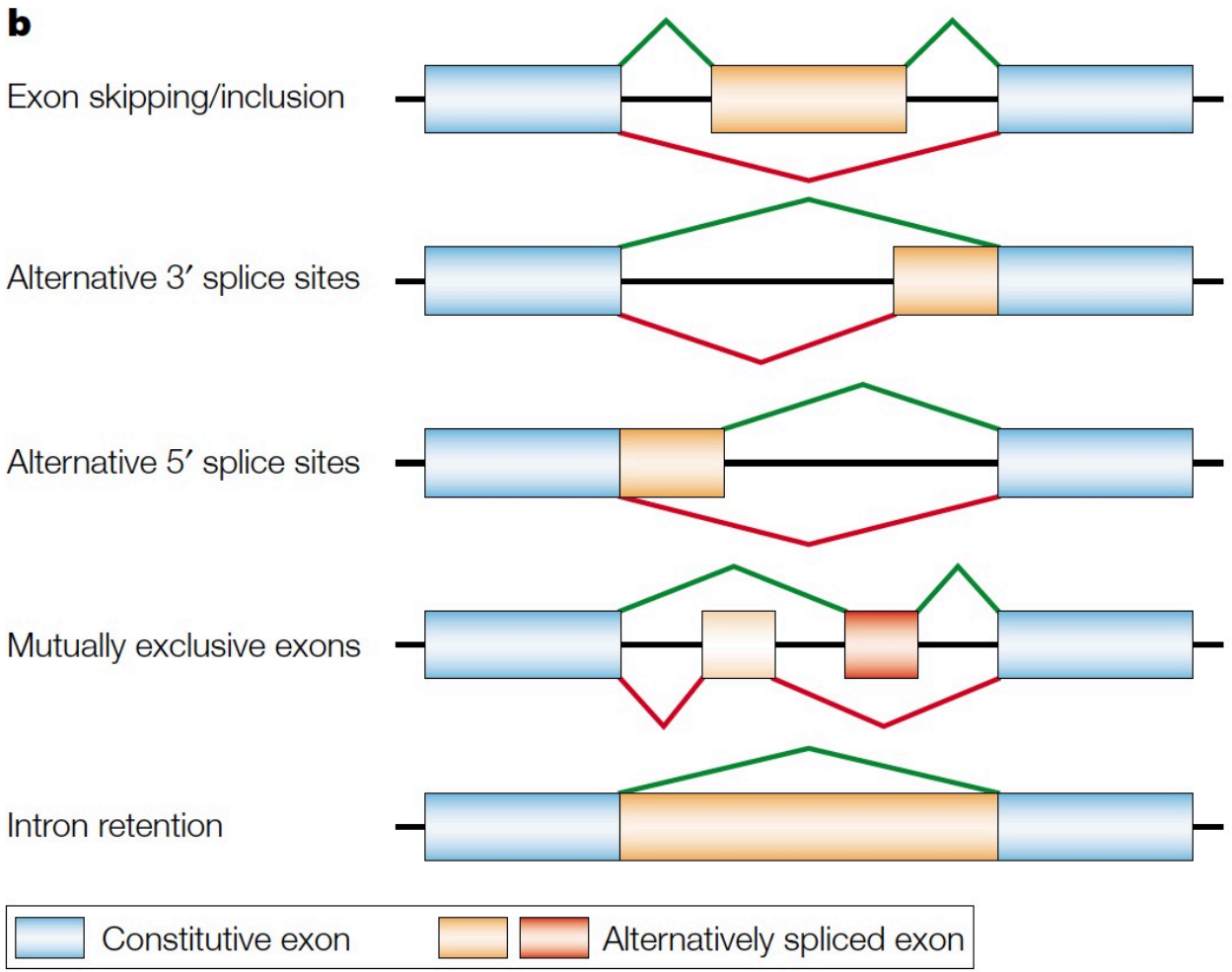
Intron could be up to 4,500,000 bp



How Are Short Exons Flanked by Long Introns Defined and Committed to Splicing?



Modes of alternative splicing



The human transcriptome is composed of a vast RNA population that undergoes further diversification by splicing

- **Alternative slicing** is an important mechanism for the production of different forms of proteins, called isoforms, by different types of cells.

- Alternative splicing can generate mRNAs that differ in their 3'-UTR or coding sequence.

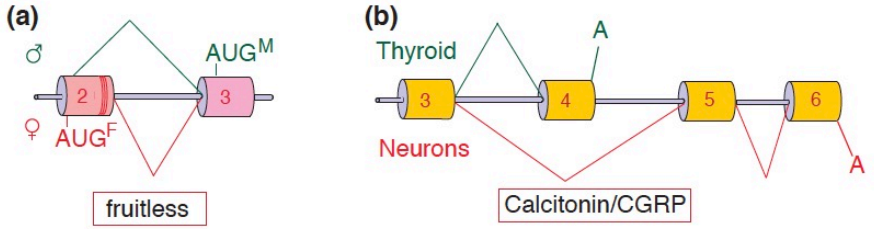
- These differences might affect mRNA stability, localization or translation. Furthermore, some splicing mRNA isoforms could change the reading frame, resulting in the generation of different protein isoforms with diverse functions and/or localizations.

- 90–95% of human genes undergo some level of alternative splicing. Out of the ~20,000 human protein coding genes, high resolution mass spectrometry analyses revealed that ~37% of them generate multiple protein isoforms, indicating that alternative splicing contributes to proteome complexity.

Different modes of alternative splicing

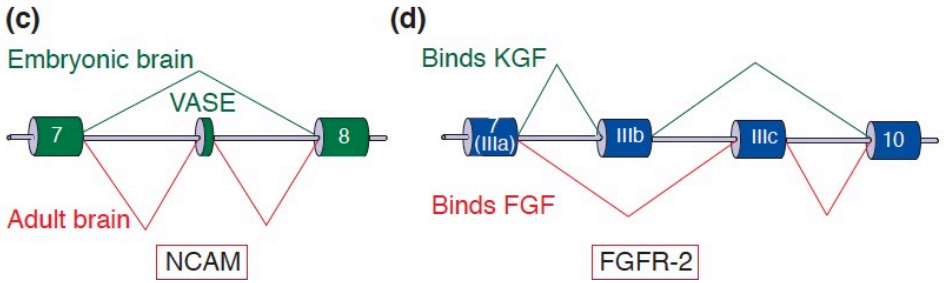
(a) Alternative spicing in the Drosophila gene *fruitless* governs sexual orientation and behaviour.

(b) Alternative 3′-splice-site usage, associated with differential use of polyadenylation sites in the vertebrate gene for calcitonin and calcitonin-gene-related peptide (CGRP) generates a calcium homeostatic hormone in the thyroid gland or a vasodilator neuropeptide in the nervous system.



(c) Differential inclusion or skipping of the variable alternatively spliced exon (VASE) in the gene for neural cell adhesion molecule (NCAM) in embryonic (green) versus adult (red) rat brain, represses or promotes axon outgrowth during development.

(d) Mutually exclusive use of exons IIIb and IIIc in mammalian fibroblast growth factor receptor 2 (FGFR-2) changes its binding specificity for growth factors during prostate cancer progression. The pattern of splicing represented in green generates an mRNA encoding a receptor with high affinity for keratinocyte growth factor (KGF), whereas that in red generates a receptor with high affinity for fibroblast growth factor (FGF).



Alternative splicing as a regulator of development and tissue identity

- It is now established that alternative splicing contributes to cell differentiation and lineage determination, tissue identity acquisition and maintenance, and organ development.

% of alternative spliced genes

- The biological importance of alternative splicing is highlighted by the large number of human diseases caused by mutations in *cis acting* sequence elements in pre-mRNA (including 5' and 3' splice sites, and exonic and intronic enhancer or silencer sequences), *trans acting* splicing factors or other components of the spliceosome.

Alternative splicing regulation

Alternative splicing outcomes are influenced by several factors:

1. **splice site strength;**

2. ***cis* regulatory sequences in pre-mRNAs that favour or impair exon recognition;**

3. **the expression levels of *trans acting* factors** (RNA-binding proteins and splicing factors);

4. **m^6A modification.**

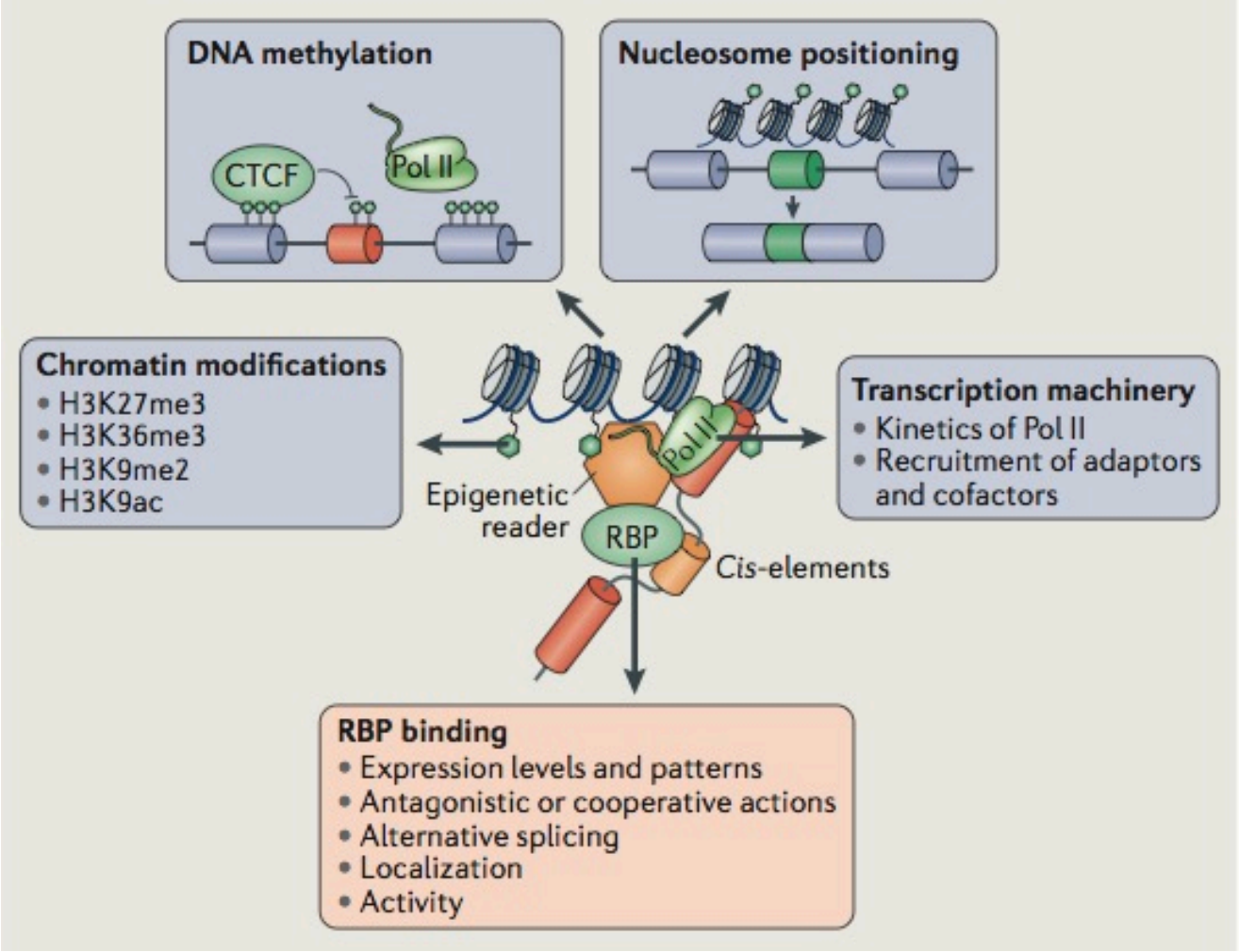
Alternative splicing regulation

To predict splicing outcomes in specific cell types and conditions is very difficult, if not impossible, for several reasons:

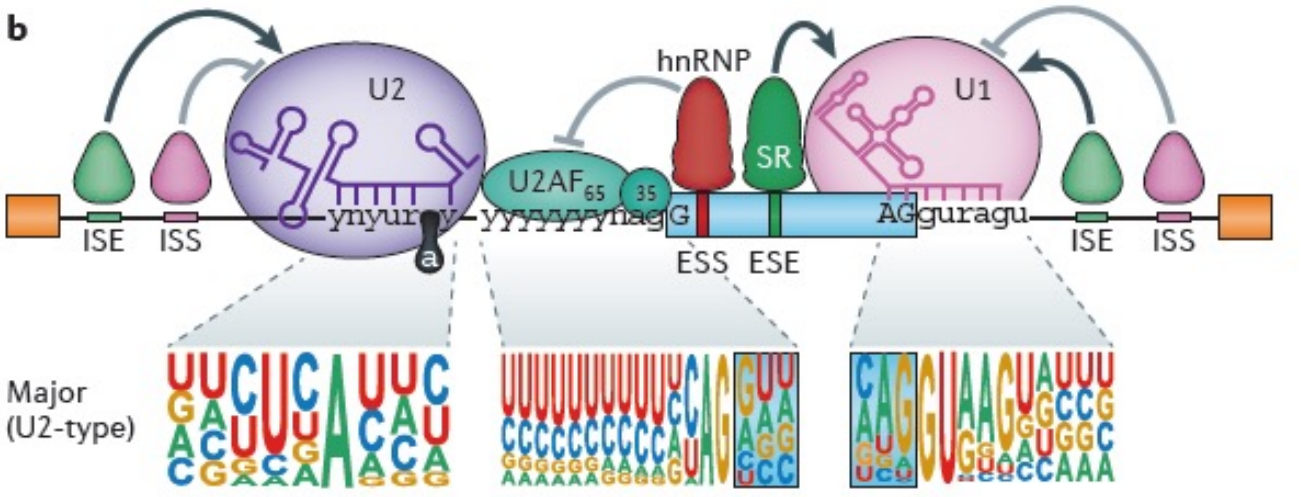
- The same regulatory sequences on different or the same transcripts can be recognized by different RBPs depending on cellular and specific sequence contexts;
- RBPs can be regulated by other RBPs or mRNA modifications;
- the same RBP can exert positive or negative regulatory effects on different splicing events depending on the location of the binding motif for the RBP relative to the alternatively spliced regions in the transcript;
- we still lack a complete list of RBPs and their binding sites.

Alternative splicing regulation

Alternative splicing outcomes can be affected by RNA polymerase II (Pol II) kinetics, which can be controlled by chromatin modifications, nucleosome occupancy, DNA methylation and the composition of transcription machinery.



RNA splicing regulation



***Cis*-acting regulatory elements:**

ESE Exonic Splicing Enhancer
ESS Exonic Splicing Silencer
ISS Intronic Splicing Enhancer
ISE Intronic Splicing Silencer
CERES Composite Exonic Regulatory Elements of Splicing (represent a physical overlap of enhancer/silencer activity)

***Trans*-acting factors:**

SR proteins Serine arginine rich proteins (SF2/ASF)
hnRNPs heterogeneous nuclear RiboNucleoprotein Particles (hnRNPA1)
snRNPs

RNA-binding proteins regulating alternative splicing

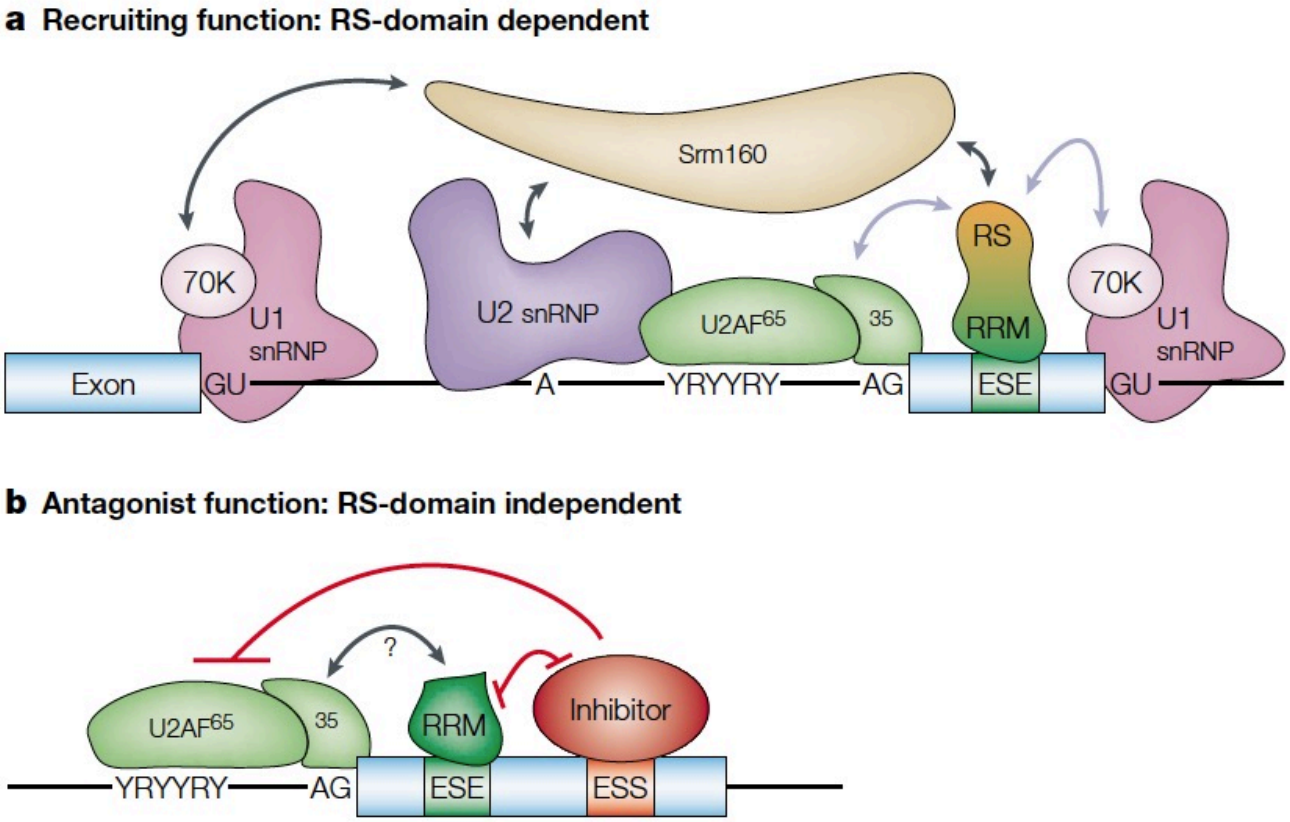
RBP	Context where RBP regulates alternative splicing	Binding motif from CLIP-sequencing experiments / alternative splicing effect
CELF1	- Heart development[1–3] - Skeletal muscle development[3] - Myoblast differentiation (C2C12 cells)[4]	UGUU motifs / skipping or inclusion (position dependent)[3,5,6]
CELF2	- T-cell activation[7–9] - Heart development[3]	UGU-rich motifs[8]
ELAVL	- Brain development[10]	U- and AU-rich motifs[10–12]
ESRP1 **ESRP2**	- Liver development (ESRP2)[13] - Epithelial-mesenchymal transition (ESRP1/2)[14] - Stomach smooth muscle development (ESRP1)[15] - Epidermis development (ESRP1/2)[16] - Kidney development (ESRP1/2)[17]	GU-rich motifs / skipping or inclusion (position dependent)[18]
HNRNPL	- T-cell development[19–21]	CA-rich motifs / skipping or inclusion [20,22]
HNRNPLL	- T-cell activation[23] - B-cell into plasma cell differentiation[24]	CA-rich motifs / skipping or inclusion [24]
MBNL1 **MBNL2**	- Heart development (MBNL1)[1–3] - Brain development (MBNL2)[25] - Erythropoiesis (MBNL1)[26]	YGCY (preferred: UGCU) / skipping or inclusion (position dependent)[25,27]
NOVA1 **NOVA2**	- Brain development (NOVA1/2)[28,29] - Vascular development (endothelial cells) (NOVA2)[30]	YCAY motifs / skipping or inclusion (position dependent)[31]
PTBP1 **PTBP2**	- Brain development[32–34] - Male germ cell development (PTBP2)[35] - Myoblast differentiation (C2C12 cells)[36] - Primary smooth muscle cell (aorta, bladder) differentiation (PTBP1)[37]	CU-rich motif / skipping[33,38]
QK	- Myoblast differentiation (C2C12 cells)[36] - Vascular smooth muscle development and cell de-differentiation[37,39]	UAA-rich motifs / skipping or inclusion (position dependent)[40]
RBFOX1 **RBFOX2**	- Brain development[34,41–44] - Heart development[45,46] - Skeletal muscle development[46,47] - Myoblast differentiation (C2C12 cells)[48,49]	UGCAUG / inclusion (downstream binding) or skipping (upstream binding or within alternative exon)[50,51]
RBM4	- Pancreas development and pancreatic cell differentiation (AR42J cells)[52] - Neuronal differentiation (P19 cells)[53]	CGG or GTAACG / skipping or inclusion (position dependent)[54,55]
RBM20	- Heart development[56]	UCUU / skipping[56]
RBM24	- Heart and skeletal muscle development[57]	G(A/U)GUG[58]
SAM68	- Spermatogenesis[59] - T-cell activation[60] - Brain development[61,62] - Adipogenesis[63]	AU-rich motifs
SRRM4	- Brain development and synaptogenesis[64,65]	UGC-rich motifs between polypyrimidine tract and 3'ss / inclusion[66]
SRSF1	- Heart development[67]	GGAGGA / inclusion[68]
SRSF10	- Heart development[69,70] - Skeletal muscle development, myoblast differentiation (C2C12 cells)[70] - Adipogenic differentiation[71]	GA-rich motifs / skipping or inclusion (position dependent)[69,72]

SR protein action in ESE-dependent splicing

Exonic enhancers serve as binding sites for specific **serine/arginine-rich (SR) proteins**

SR protein family is composed by a structurally related and highly conserved splicing factors that are characterized by the presence of RNA recognition motifs (RRM) and by a distinctive carboxy terminal domain that is highly enriched in Arg/Ser dipeptides (the RS domain).

SR proteins that are bound to ESEs can promote EXON DEFINITION carrying suboptimal splice sityes by directly recruiting the splicing machinery through their RS domain and/or by antagonizing the action of nearby silencer elements



RNA motifs recognized by human SR proteins

Protein	High-affinity binding site	Ref.	Functional ESE
SRp20	WCWWC CUCKUCY	112 14	GCUCCUCUUCC CCUCGUCC
SC35	AGSAGAGUA GUUCGAGUA UGUUCSAGWU GWUWCCUGCUA GGGUAUGCUG GAGCAGUAGKS AGGAGAU	32 32 112 112 112 112 112	GRYYMCYR* UGCYGYY
9G8	$(GAC)_n$ ACGAGAGAY WGGACRA	112 112 14	
SF2/ASF	RGAAGAAC AGGACRRAGC	32 32	CRSMSGW*
SRp40	UGGGAGCRGUYRGCUCGY	114	YRCRKM*
SRp55			YYWCWSG*
TRA2ß	$(GAA)_n$	115	

The SR proteins

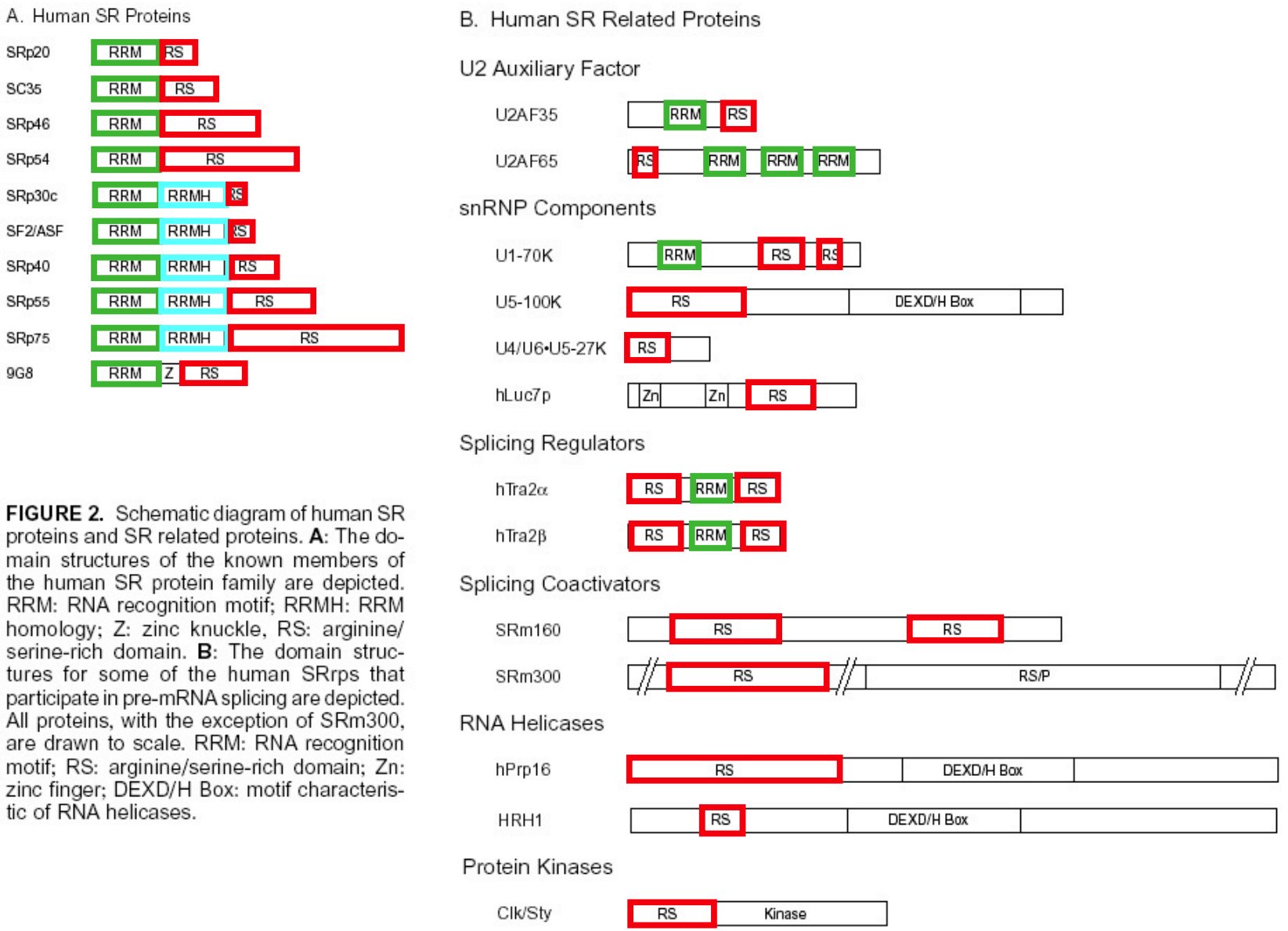
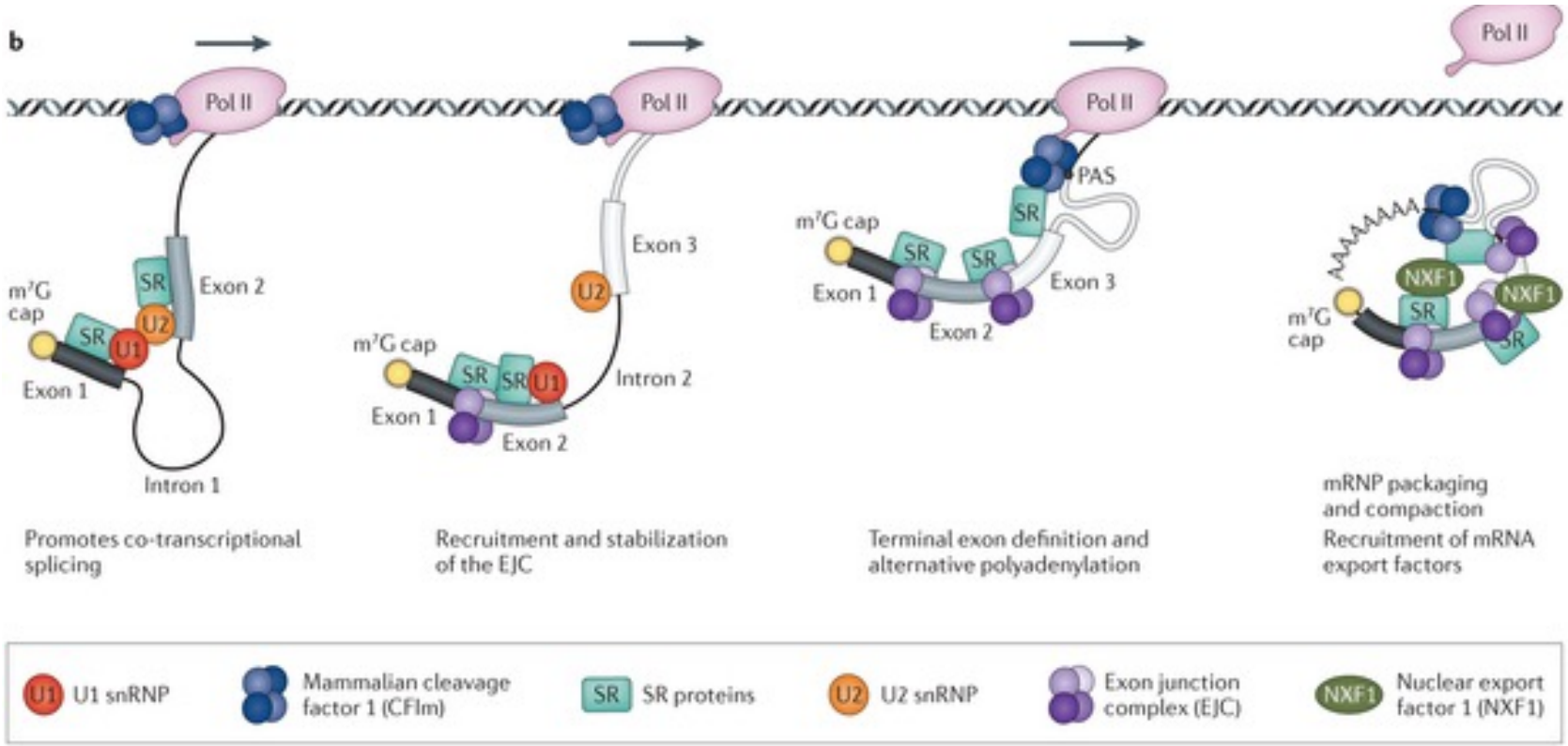


FIGURE 2. Schematic diagram of human SR proteins and SR related proteins. **A**: The domain structures of the known members of the human SR protein family are depicted. RRM: RNA recognition motif; RRMH: RRM homology; Z: zinc knuckle, RS: arginine/serine-rich domain. **B**: The domain structures for some of the human SRrps that participate in pre-mRNA splicing are depicted. All proteins, with the exception of SRm300, are drawn to scale. RRM: RNA recognition motif; RS: arginine/serine-rich domain; Zn: zinc finger; DEXD/H Box: motif characteristic of RNA helicases.

From: B. Graveley (2000). RNA 6, 1197-1211

SR proteins couple co-transcriptional splicing to mRNA export

SR proteins bind to specific binding sites within nascent transcripts and aid in the recruitment of the U1 and U2 snRNPs, thereby promoting co-transcriptional splicing. SR proteins help in the packaging and compaction of mRNPs through tight interactions with EJC components and other SR proteins bound to the same transcript. Ultimately, they recruit nuclear RNA export factor 1 (NXF1) to the compact, mature mRNP to allow efficient nuclear export.



Splicing silencers

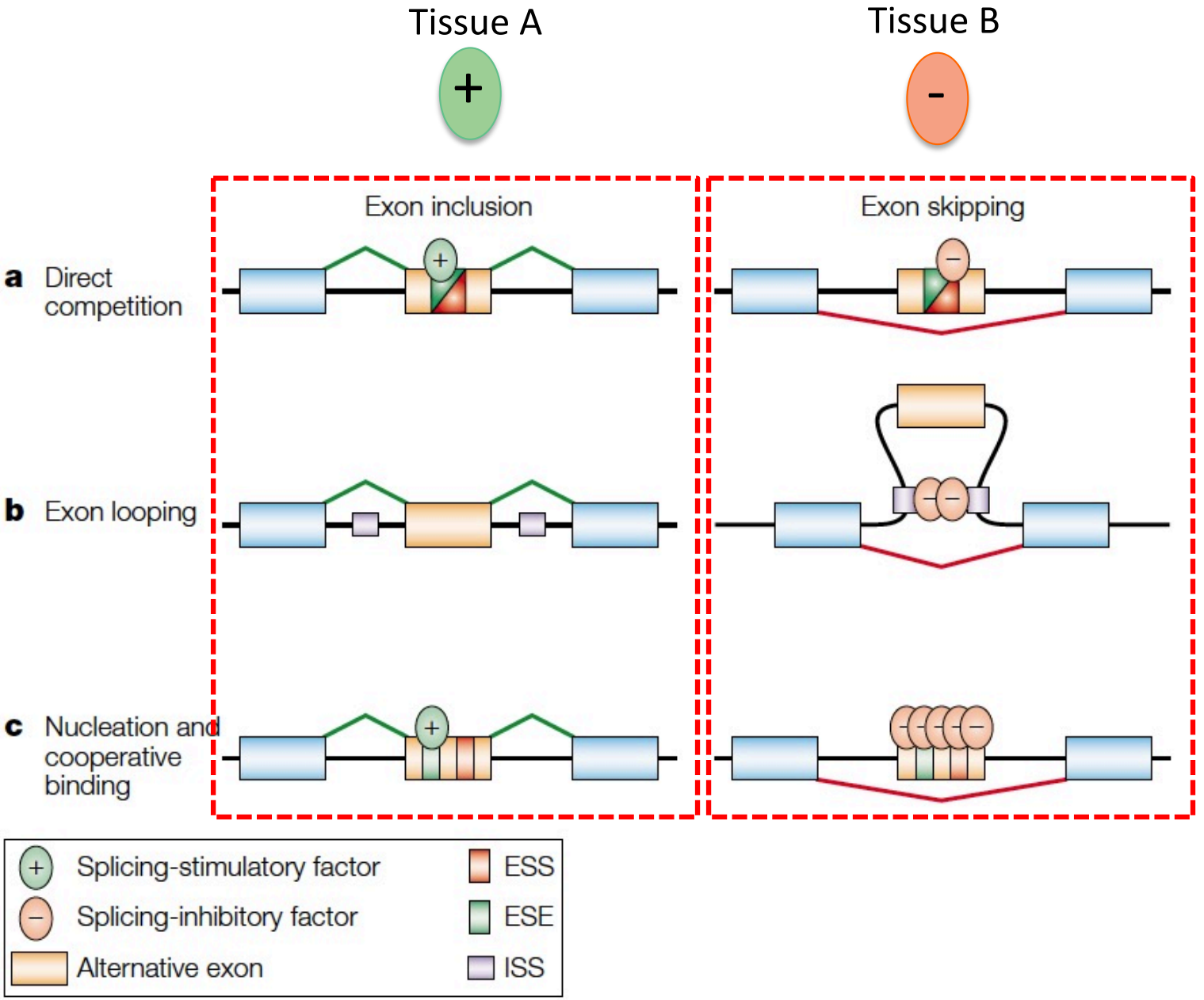
Splicing silencers are less well characterized than splicing enhancer.

Most described silencers are intronic elements, but several ESS elements have also been reported

Their mechanisms of action are still not fully understood. Silencers seem to work by interacting with negative regulators, which often belong to the **heterogeneous nuclear ribonucleoprotein (hnRNP**) family

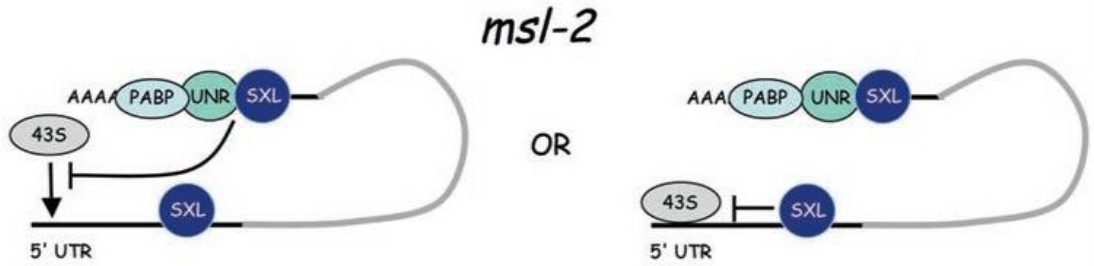
Similar to SR proteins, hnRNP proteins have a modular structure, which consists of one or more RNA-binding domains associated with an auxiliary domain that is often involved in protein–protein interactions.

Models of splicing silencing

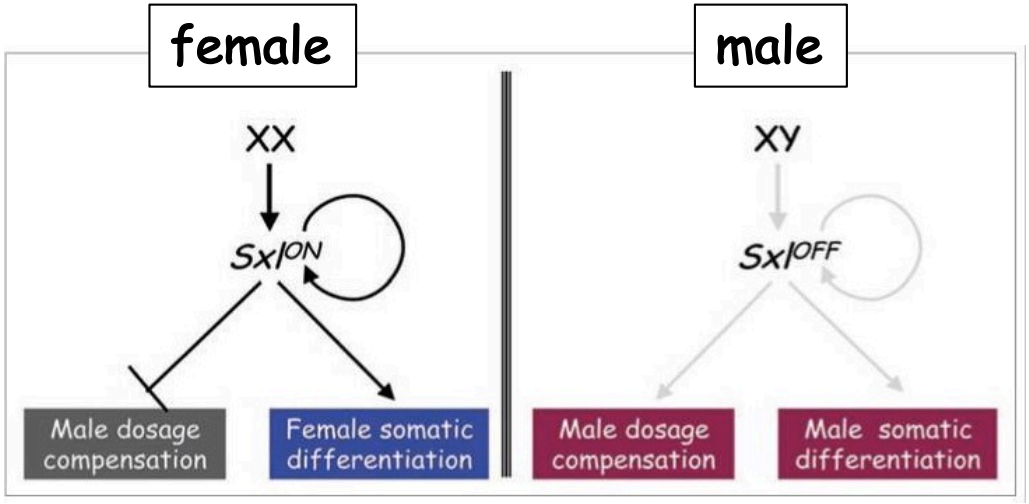


Sex determination in *Drosophila*

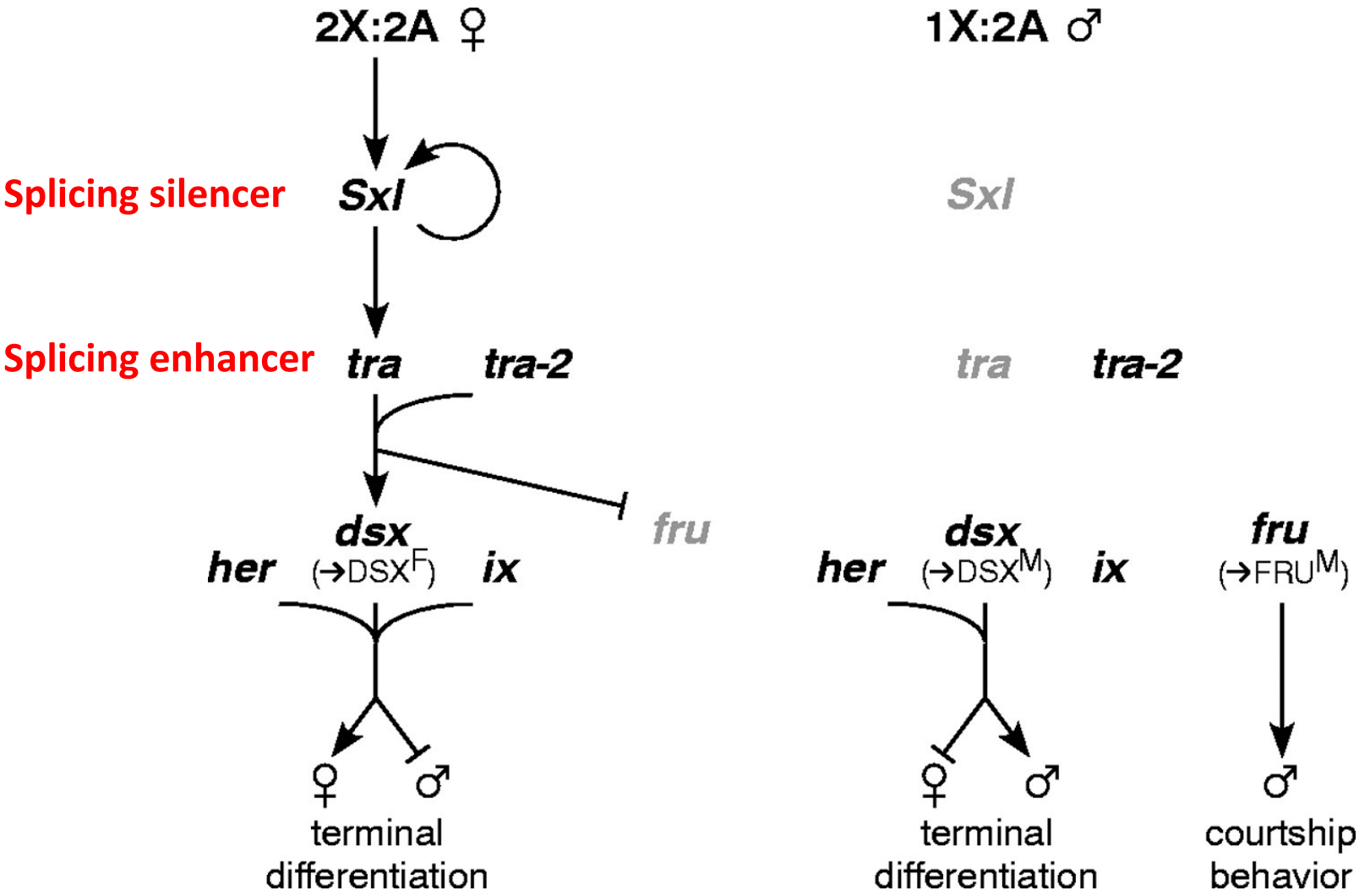
In *D. melanogaster* males, dosage compensation is orchestrated by the **MSL2** protein. Translation of ***msl2*** is specifically **repressed** in females since the binding of the sex regulator ***sex lethal*** **(SXL)** and ***Upstream of N-ras*** **(UNR)** to the 5'- and 3'-UTR of the *msl2* mRNA inhibit the recruitment of the small Ribosome subunit



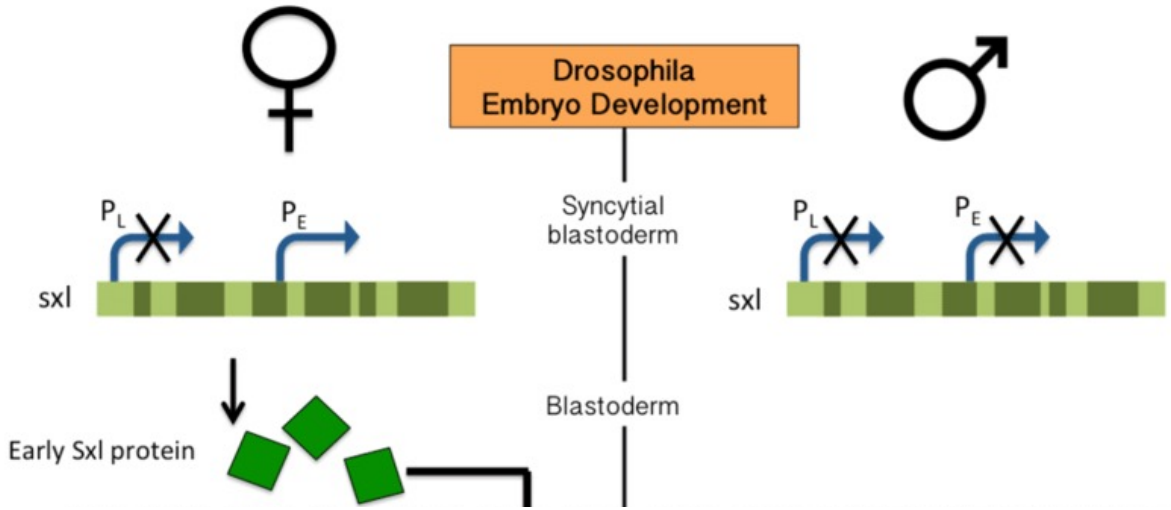
By contrast, in males, an **alternative splicing cascade** prevents the expression of a functional SXL protein leading to translation of MSL2



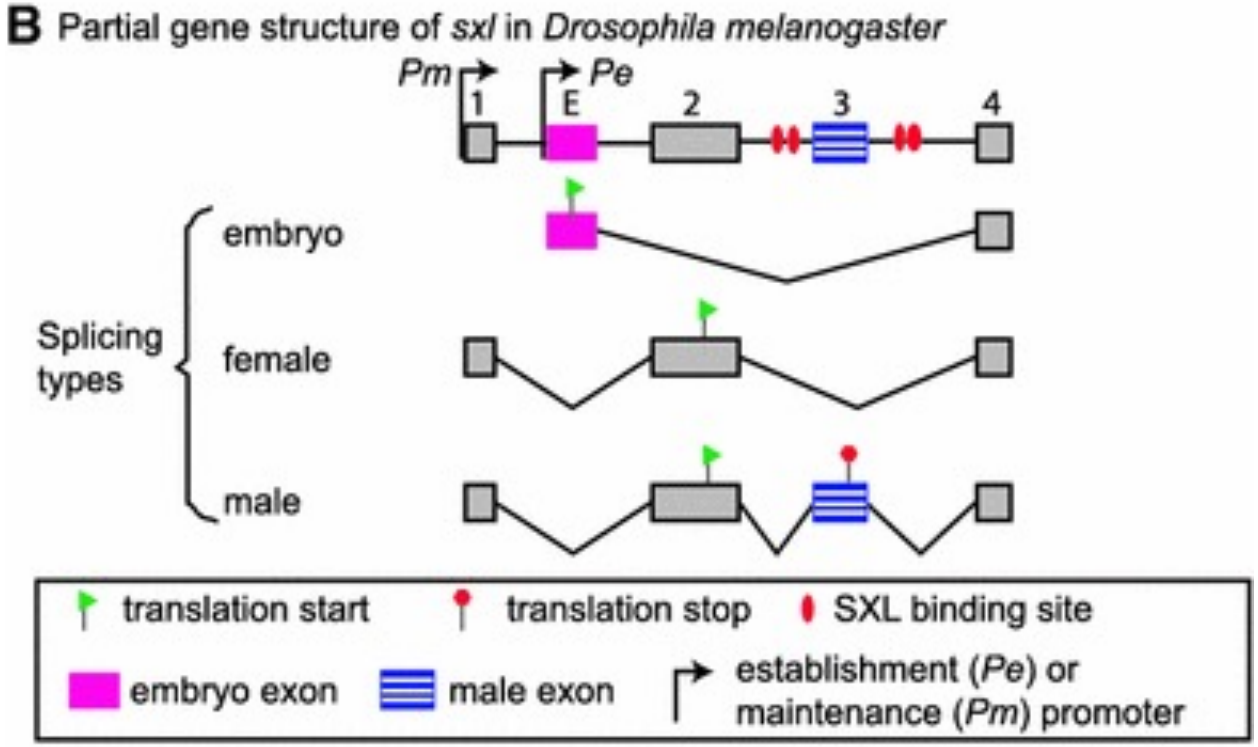
Sex determination in *Drosophila*



DSX-F and DSX-M are transcription factors that determine somatic sexual characterization



Promoter Early (P_E) is active only in female while Promoter maintaince (P_M) in both sexes but Sxl mRNA maturation required Sxl protein for correct splicing



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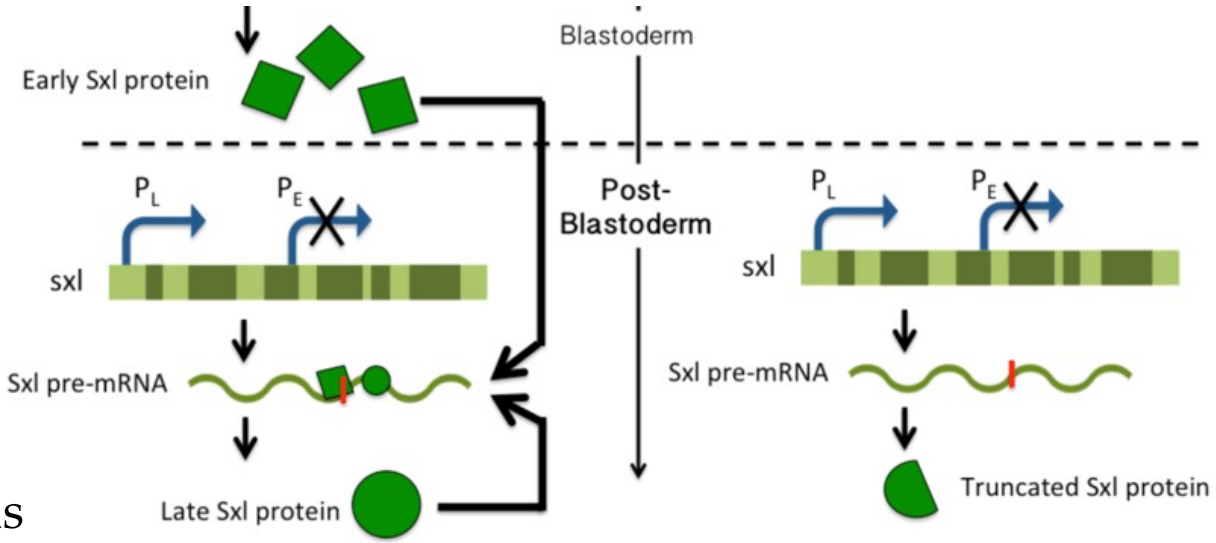
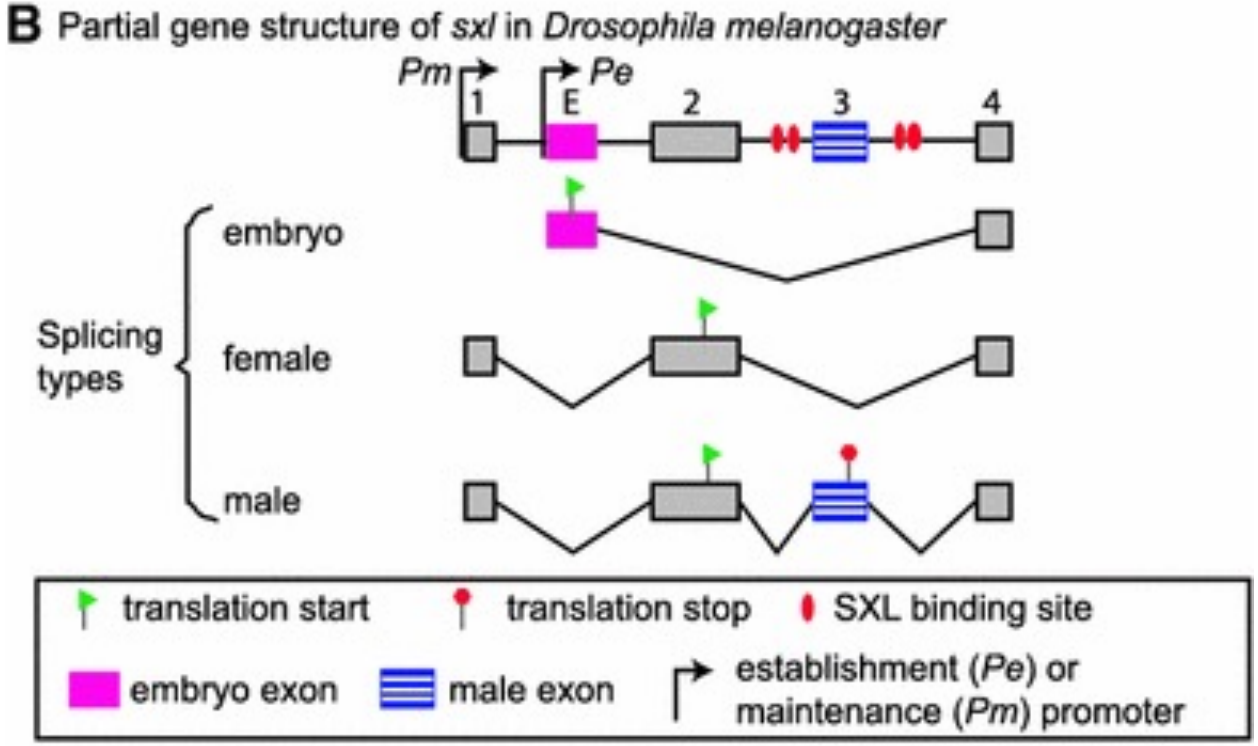
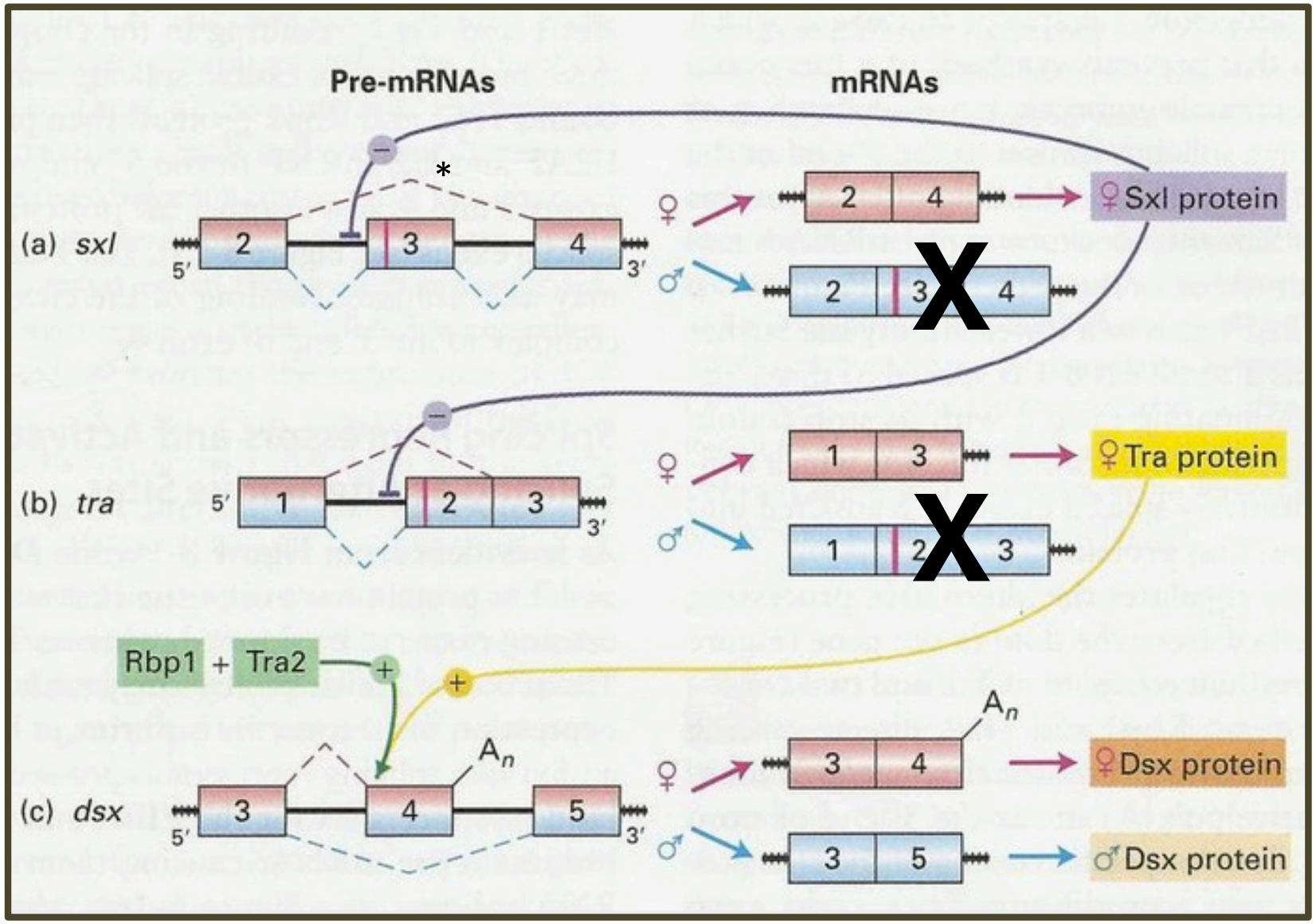




Sex determination in *Drosophila*

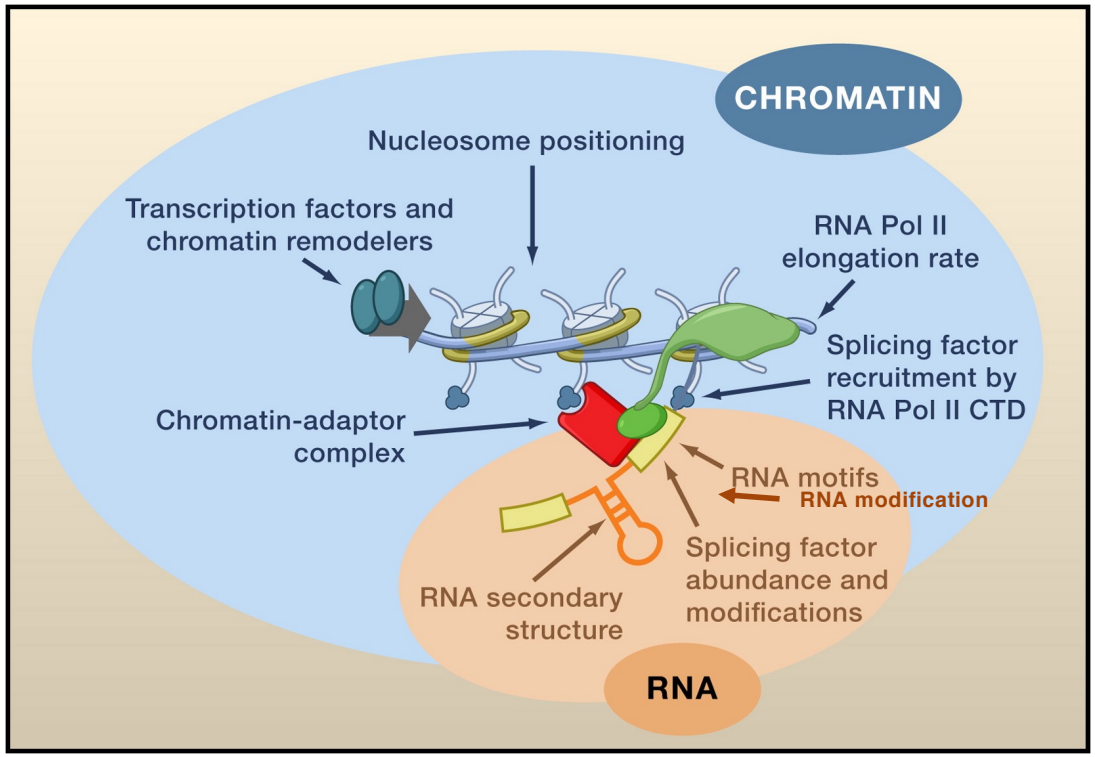


*m6A modification is required for female-specific alternative splicing of Sxl

Splicing is coupled to transcription and RNA modification

The decision of whether to include an exon reflects the intrinsic strength of the flanking splice sites and the combinatorial effects of positive and negative elements.

However, the situation is more complex because splicing is coupled to transcription and m^{6}A RNA modification. Factors that regulate transcription also affect alternative splicing. Moreover, splicing can influence transcription elongation.



The pre mRNA splicing pathology

- A considerable number of disease-causing mutations in exons or introns may disrupt previously un-recognised splicing regulatory elements

- Variability in the basal splicing machinery among different cell types cause cell-specific sensitivities to individual splicing mutations

- Exon sequence variation at CERES elements (Composite Exonic Regulatory Elements of Splicing, which represents a physical overlap of enhancer/silencer activity) may represent a frequent disease-causing mechanism

- Even the most benign looking polymorphism in an exon (or in an intron) cannot be ignored as it may affect the splicing process

Pathologies resulting from aberrant splicing can be grouped in two major categories

- Mutations affecting proteins that are involved in splicing

  Examples: **Spinal Muscular Atrophy**
  Retinitis Pigmentosa
  Myotonic Dystrophy

- Mutations affecting a specific messenger RNA and disturbing its normal splicing pattern

  Examples: ß-Thalassemia
  Duchenne Muscular Dystrophy
  Cystic Fibrosis
  Frasier Syndrome
  Frontotemporal Dementia and Parkinsonism

- **Single point mutations in coding exons**

	Probability of effect on encoded protein	Probability of nonsense-mediated decay	Probability of effects on splicing
Nonsense mutations	+	+	+/-
Missense mutations	+/-	-	+/-
Silent mutations	-	-	++

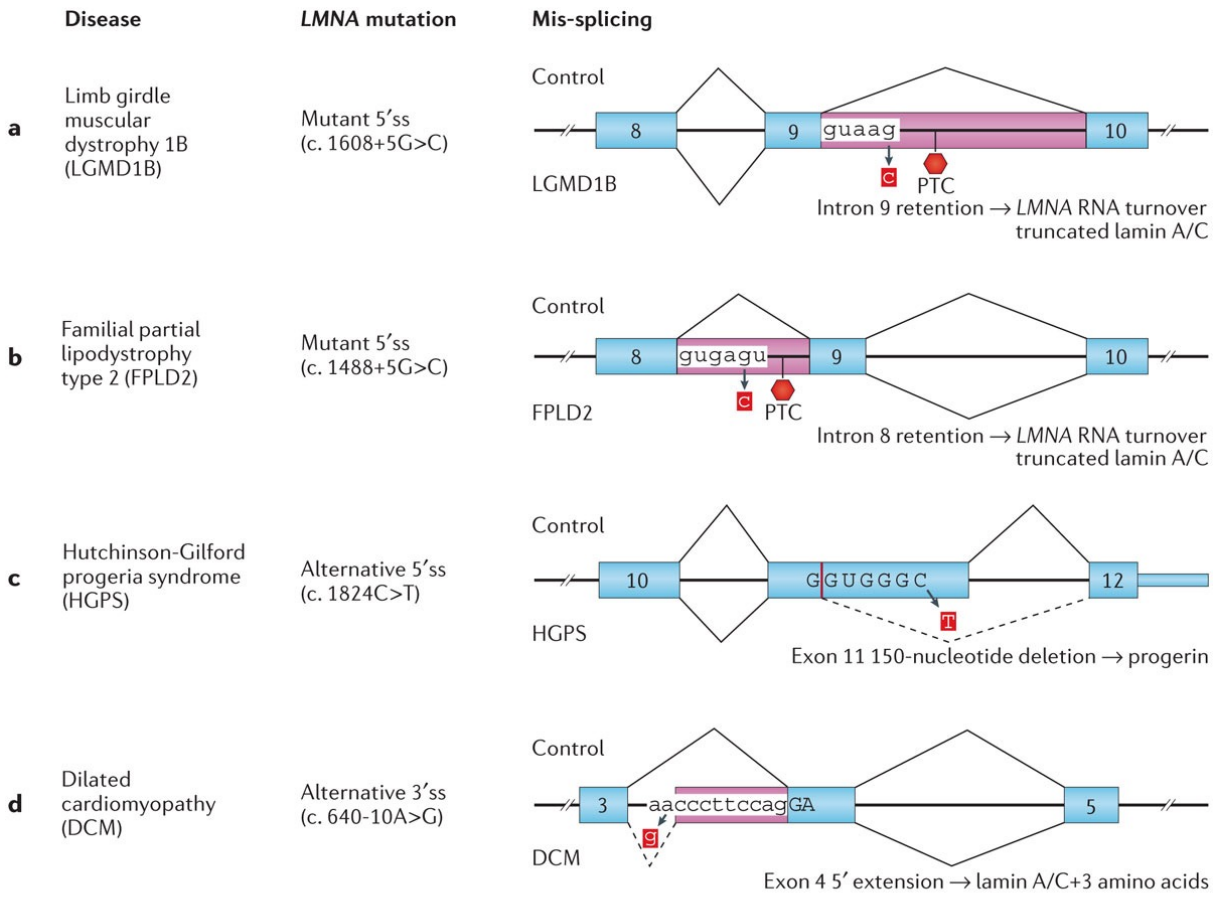
Table 1 | **Disease-associated splicing alterations**

Disease	Gene (mutation)	Mechanism	Splicing effect	Inheritance
Cis				
Limb girdle muscular dystrophy type 1B (LGMD1B)	*LMNA*[24] (c.1608 + 5G>C)	5′ss mutation	Intron 9 retention resulting in NMD	Dominant
Familial partial lipodystrophy type 2 (FPLD2)	*LMNA*[25] (c.1488 + 5G>C)	5′ss mutation	Intron 8 retention resulting in NMD	Dominant
Hutchinson–Gilford progeria syndrome (HGPS)	*LMNA*[26] (c.1824C>T)	Alternative 5′ss	150 nt deletion in exon 11, resulting in progerin generation	Dominant
Dilated cardiomyopathy (DCM)	*LMNA*[28] (c.640-10A>G)	Alternative 3′ss	Extension of exon 4 adding 3 amino acids to lamin A/C	Dominant
Familial dysautonomia (FD)	*IKBKAP*[128] (c.2204 + 6T>C)	Decreased U1 recruitment	Exon 20 skipping	Recessive
Duchenne muscular dystrophy (DMD)	*DMD*[129] Exon 45–55 deletions are common	Exon deletions and skipping	Frameshift resulting in NMD	X-linked
Becker muscular dystrophy (BMD)	*DMD*[130] (c.4250T>A)	ESS creation	Exon 31 partial in-frame skipping	X-linked
Early-onset Parkinson disease (PD)	*PINK1* (REF. 131) (c.1488 + 1G>A)	U1 5′ss mutation	Cryptic splice site usage, resulting in exon 7 skipping	Recessive
Frontotemporal dementia with parkinsonism chromosome 17 (FTDP-17)	*MAPT*[132] (c.892A>G)	ESS mutation	Increased exon 10 inclusion	Dominant
X-linked parkinsonism with spasticity (XPDS)	*ATP6AP2* (REF. 133) (c.345C>T)	Novel ESS creation	Increased exon 4 exclusion	X-linked
Spliceosome				
Retinitis pigmentosa (adRP)	*PRPF6* (REF. 134) (c.2185C>T)	Abnormal nuclear localization	Decreased U4/U6 interaction affecting spliceosome assembly and recycling	Dominant
	SNRNP200 (REF. 135) (c.3260C>T), (c.3269G>T)	• Decreased helicase activity • Decreased proof-reading	Compromised splice site recognition, leading to mis-spliced mRNAs	Dominant
Myelodysplastic syndromes (MDS)	*U2AF1* (REF. 46) (c.101G>A)	Altered 3′ss preference	Increased alternative 3′ss usage	Somatic
Microcephalic osteodysplastic primordial dwarfism type 1 (MOPD I)	*RNU4ATAC*[54–56] (g.30G>A), (g.50G>A), (g.50G>C), (g.51G>A), (g.53C>G), (g.55G>A), (g.111G>A)	5′ and 3′ stem loop mutations & secondary structure disruption	Compromised minor spliceosome activity	Recessive
Trans				
Spinal muscular atrophy (SMA)	*SMN1* (REFS 136,137) (c.922 + 6 T/G), deletion	Loss of SMN full-length protein	Altered RNP biogenesis[98]	Recessive
Amyotrophic lateral sclerosis (ALS)	*TARDP*[77] (c.991C>A), (c.1009A>G)	C-terminal mutations alter protein-protein interactions	TDP-43 target mis-splicing	Sporadic and Dominant
	FUS[138] (c. 1566C>T), (c. 1561T>G)	• Decreased U1 interaction • Increased SMN binding	FUS target mis-splicing	Dominant
Dilated cardiomyopathy (DCM)	*RBM20* (REF. 139) (c.1962T>G)	Altered R/S RNA binding domain	*TTN* mis-splicing	Dominant
Limb-girdle muscular dystrophy 1G (LGMD1G)	*HNRPDL*[140] (c. 1667G>A), (c. 1667G>C)	Altered import of HNRPDL into nucleus	HNRPDL target mis-splicing	Dominant
Autosomal dominant leukodystrophy (ADLD)	*LMNB1* (REF. 141) duplication	Increased *RAVER2* expression	PTBP1 target mis-splicing mediated by RAVER2	Dominant

ATP6AP2, ATPase, H+ transporting, lysosomal accessory protein 2; *DMD*, dystrophin; ESS, exonic splicing silencer; *HNRPDL*, heterogeneous nuclear ribonucleoprotein D-like; *IKBKAP*, inhibitor of κ-light polypeptide gene enhancer in B cells, kinase complex-associated protein; *LMNA*, lamin A; *MAPT*, microtubule-associated protein tau; NMD, nonsense-mediated decay; *PRPF6*, pre-mRNA processing factor 6; PTBP1, polypyrimidine tract binding protein 1; RNP, ribonucleoprotein; *SMN1*, survival of motor neuron 1; ss, splice site.

Mis-splicing of a single gene results in different diseases

Lamins are type V intermediate filament proteins of the nucleus that have crucial roles in differentiated cell nuclear architecture and gene expression. Laminopathies comprise a heterogeneous group of over 14 diseases, including cardiomyopathies, hereditary peripheral neuropathies, lipodystrophies, muscular dystrophies and premature ageing (progeroid) syndromes.



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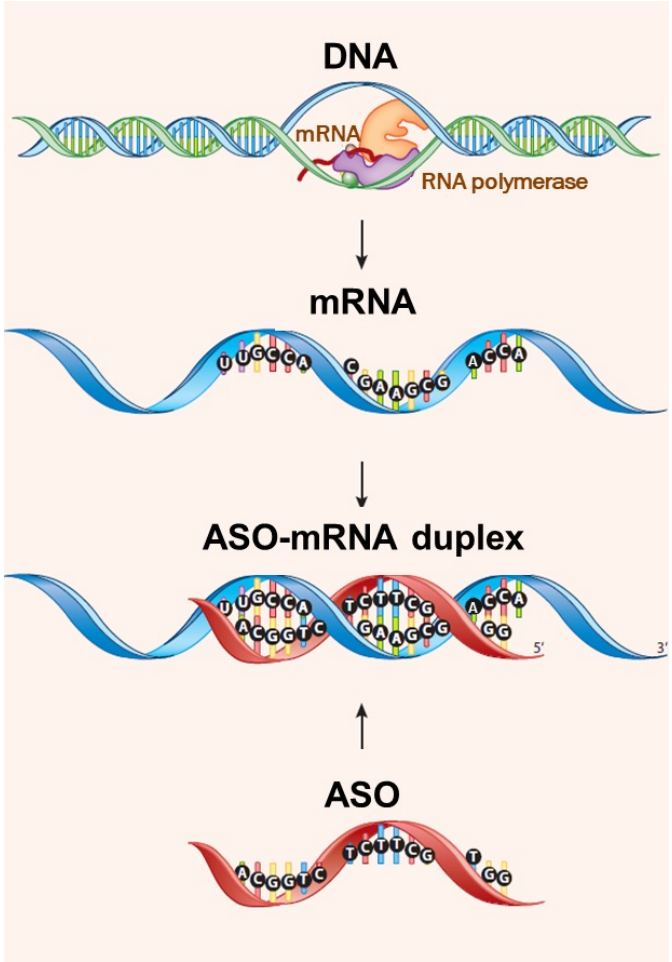
Antisense oligonucleotides (ASOs)

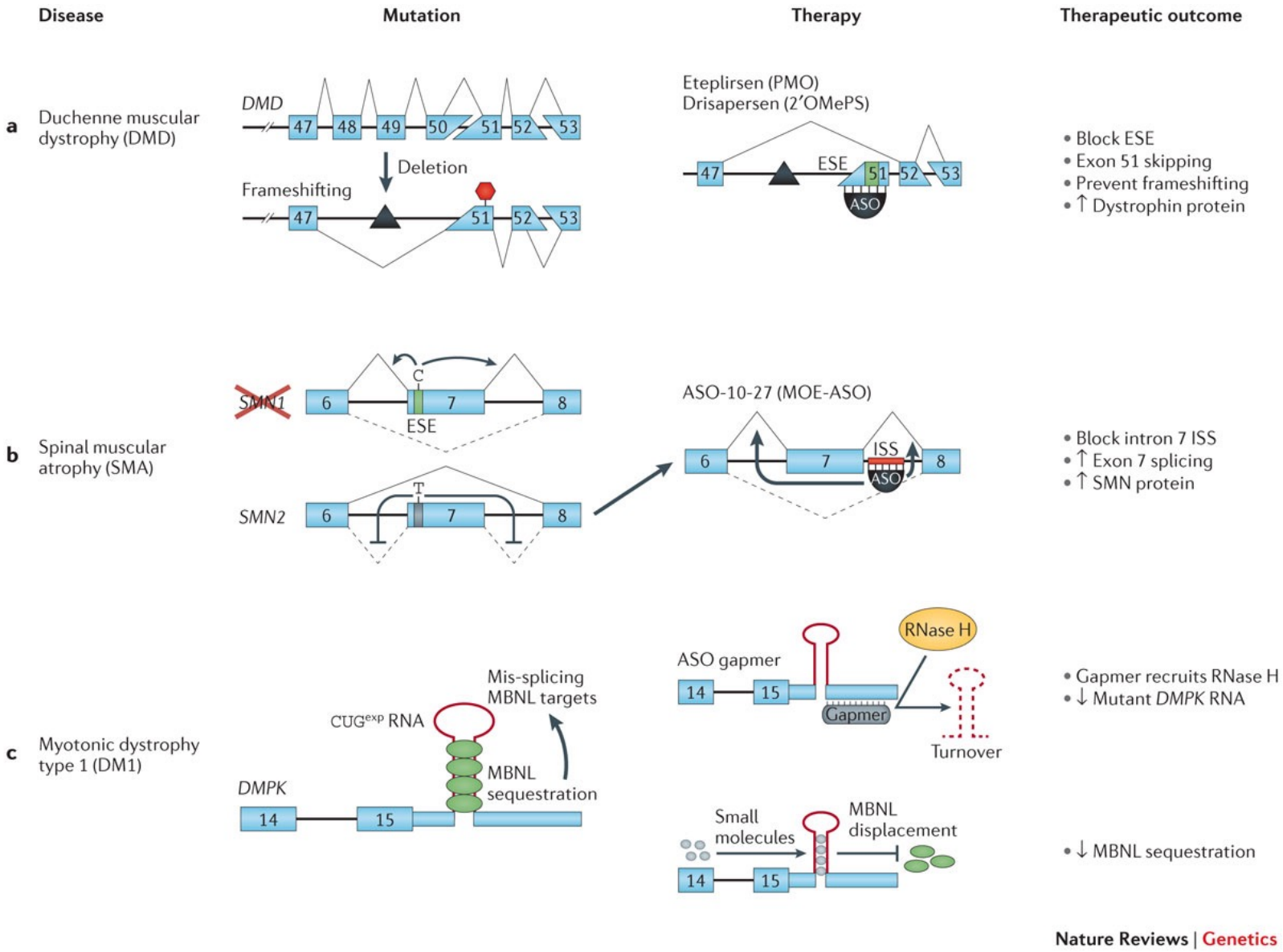
- ASOs are short, synthetic, single-stranded nucleic acids that bind to mRNA by base pairing and can modulate protein levels through several mechanisms
- Numerous chemical classes; most comprise a phosphorothioate backbone, plus one or more ribose-sugar modifications and a base modification
- The sequence of an ASO determines what RNA it will bind, and where along the RNA sequence
- By binding to a pre-mRNA in the nucleus, an ASO can affect the resulting splicing pattern
- The size and chemical characteristics of ASOs prevent blood-brain barrier penetration



Bennett (2019) *Annu Rev Med* 70: 307-32.

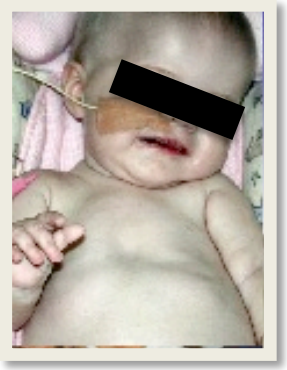
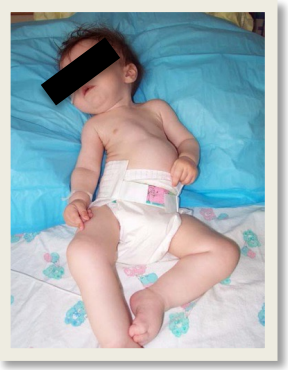
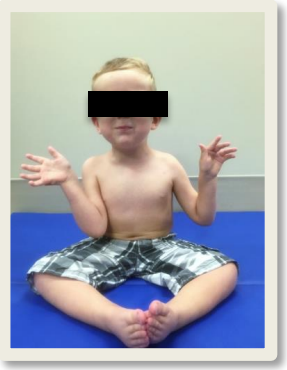
Therapeutic strategies

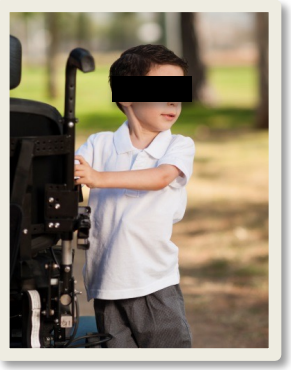
Examples of therapies based on antisense oligonucleotide (ASO) and small molecule approaches:



Spinal Muscular Atrophy

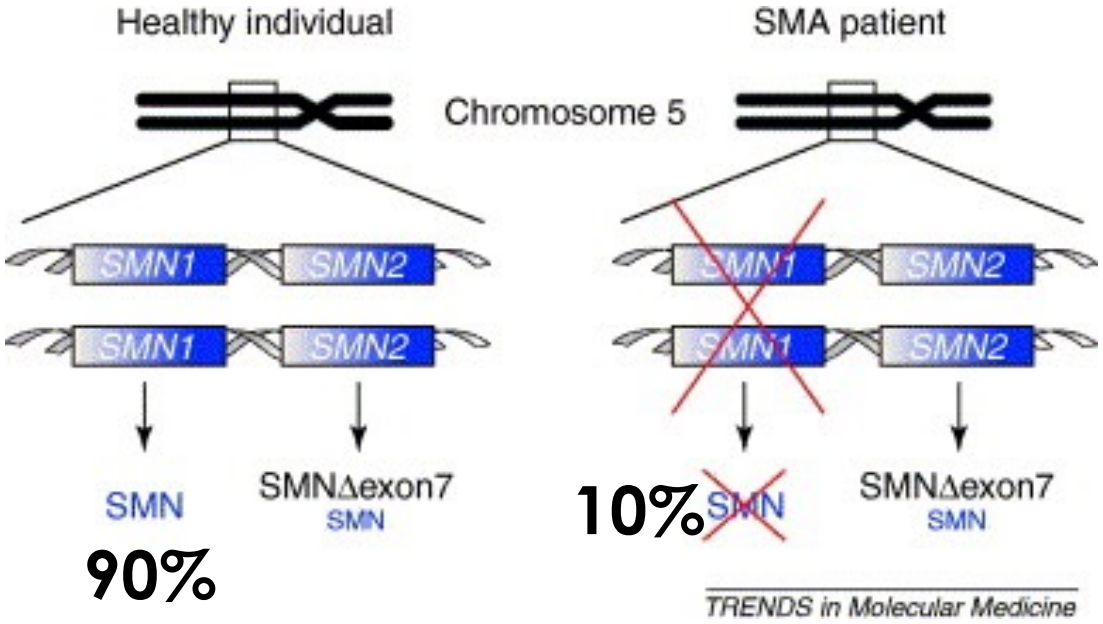
- **Pediatric neuromuscular disorder, autosomal recessive**
- **Degeneration of α-motor neurons in the spinal cord and lower brainstem**
- **1 in ~10,000 newborns**
- **Loss-of-function mutations in the *SMN1* gene, which codes for SMN protein**
  - SMN functions in snRNP assembly and axonal mRNA transport
- **Closely related *SMN2* gene (unique to humans) provides partial function**
- **Variable severity (SMA type 1-4) inversely proportional to *SMN2* copy number**



Spinal muscular atrophy: the RNP connection

Degenerated motor neurons in the spinal cord are the pathological hallmark of **spinal muscular atrophy (SMA)**. SMA is caused by mutations in the ubiquitously expressed **survival motor neuron 1** (*SMN1*) gene, which lead to reduced levels of functional SMN protein. Two nearly identical copies of the *SMN* gene are located on the long arm of chromosome 5 (5q13). Functional SMN protein is predominantly produced from *SMN1*, whereas the major product of *SMN2* is a truncated and non-functional protein (SMNΔexon7). Mutations that cause disease inactivate *SMN1*, leaving *SMN2* as the only source of functional protein.

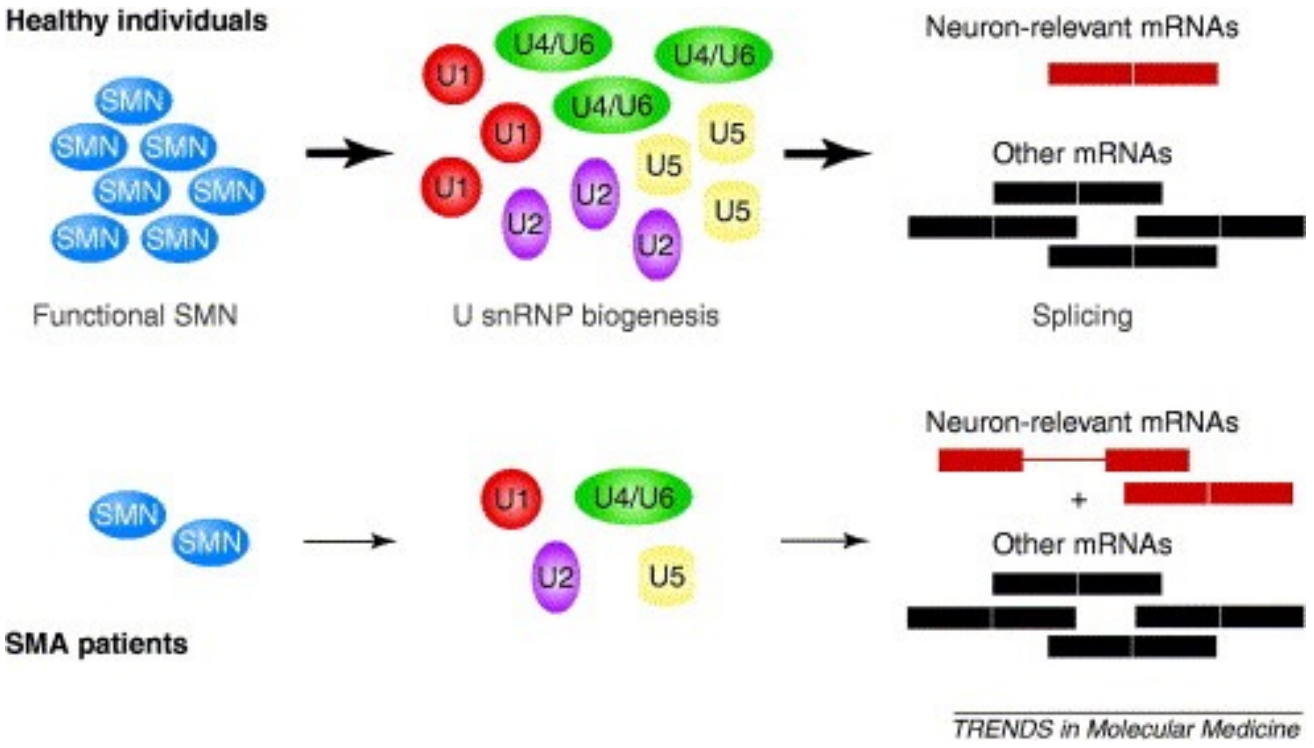


SMA is classified based on age of onset and severity:

- Type I: severe (1 or 2 copies of SMN2)
- Type II: intermediate (3 copies of SMN2)
- Type III: mild (4 copies of SMN2)

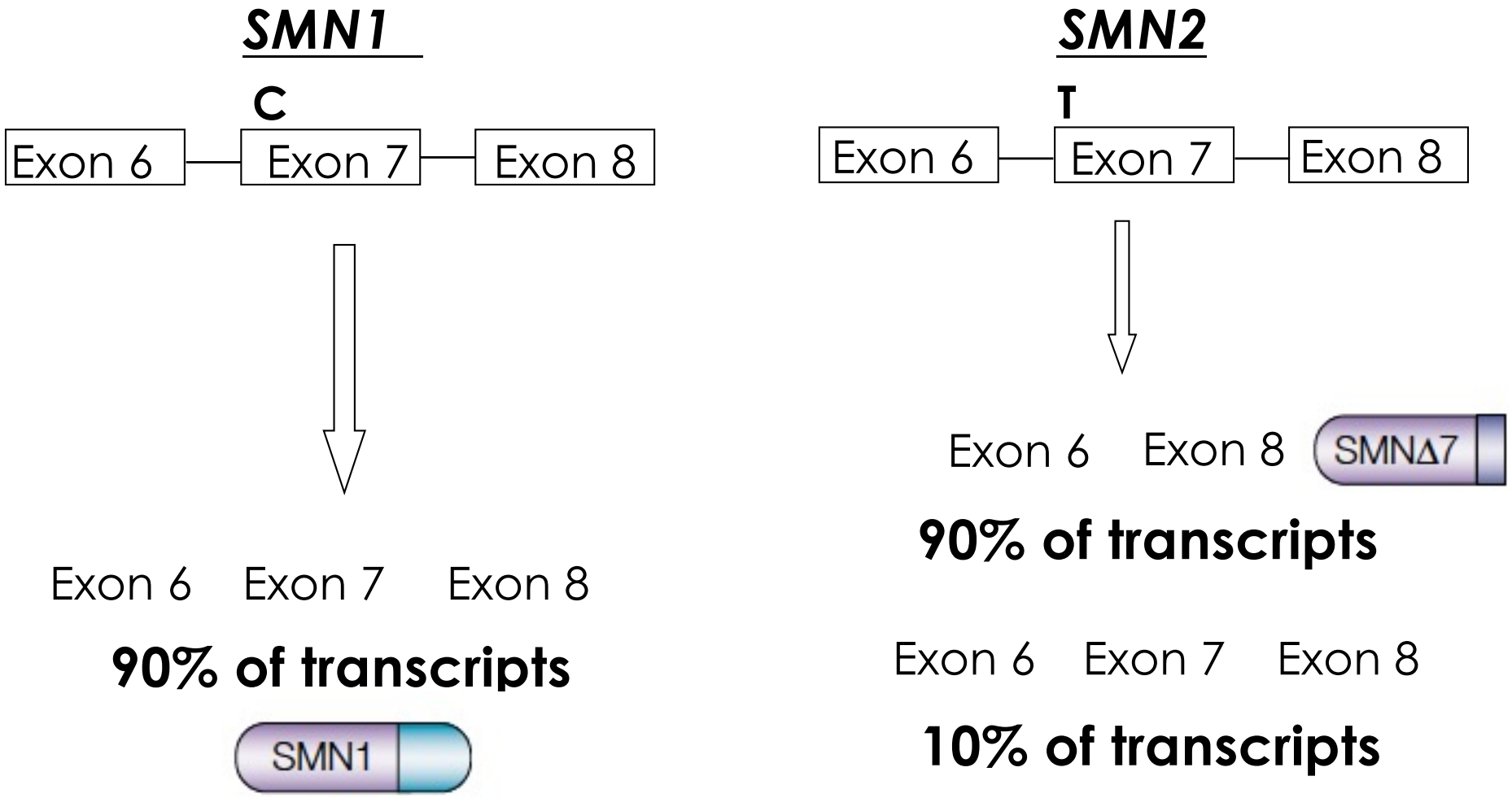
Spinal muscular atrophy: the RNP connection

SMN deficiency results in reduced production of spliceosomal U snRNPs. As a consequence, processing of mRNAs with sub-optimal splice sites (e.g. tissue-specific transcripts) would be compromised. Therefore, inefficient splicing of neuron-relevant mRNAs is the basis for the tissue-specific phenotype in SMA.

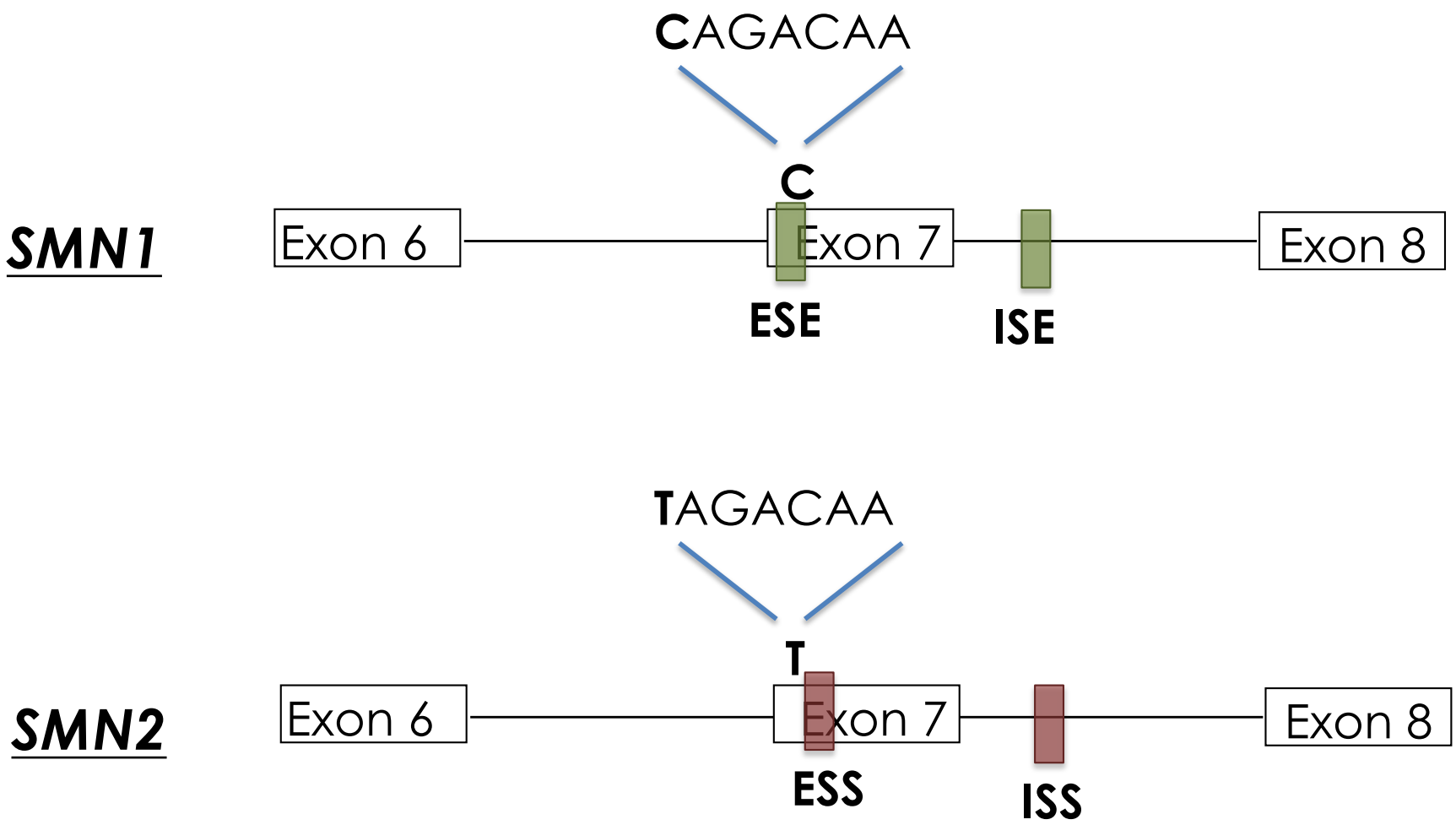


Alternative Splicing of the *SMN* Genes

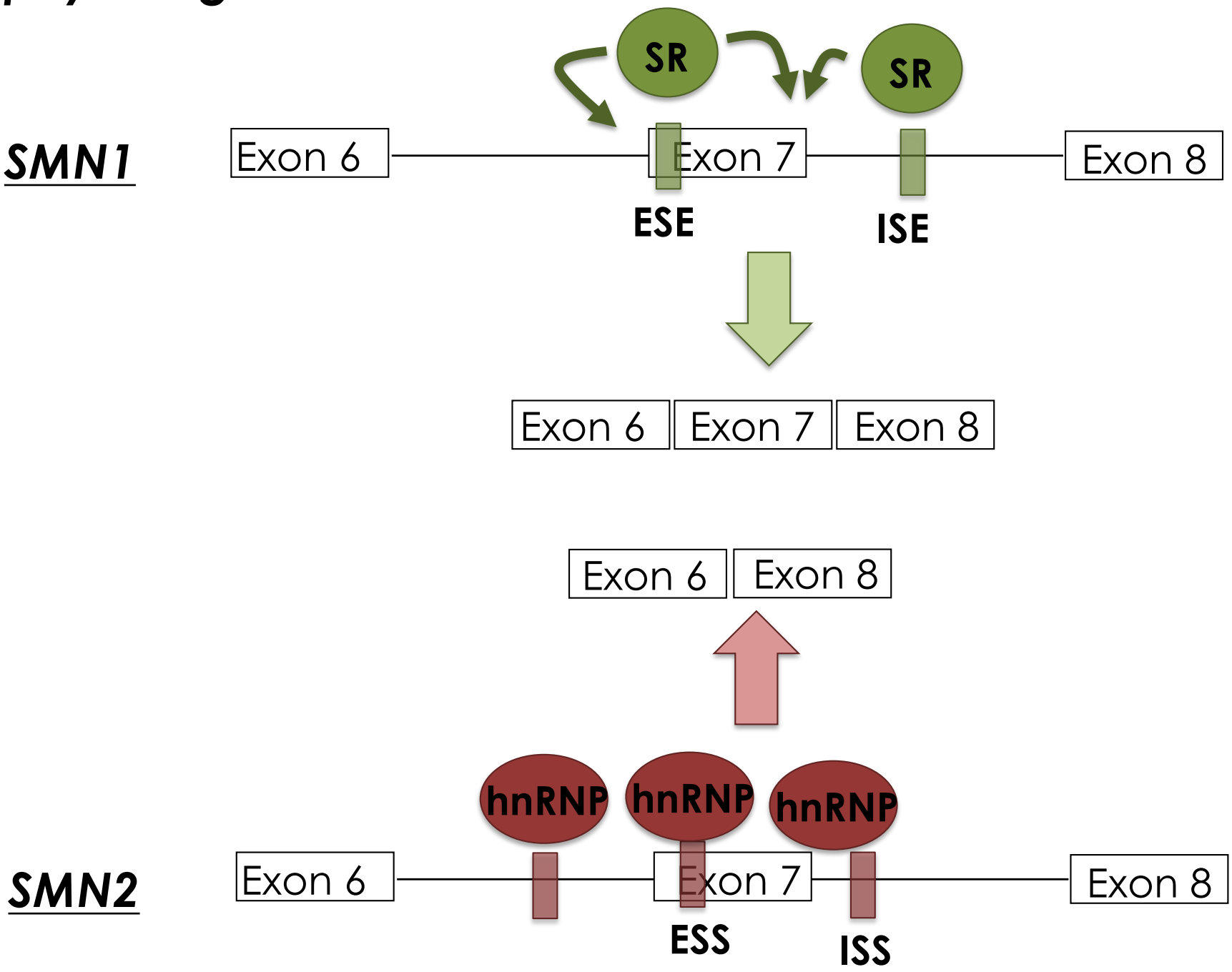
SMN2, a nearly identical copy of *SMN1*, fails to compensate for *SMN1* loss due to a critical C to T transition at the 6th position in exon 7 that leads to exon 7 skipping during pre-mRNA splicing of *SMN2* (9); as a consequence, a truncated, dysfunctional and rapidly degraded protein (SMNΔ7) is produced



Differences between SMN1 e SMN2 genes

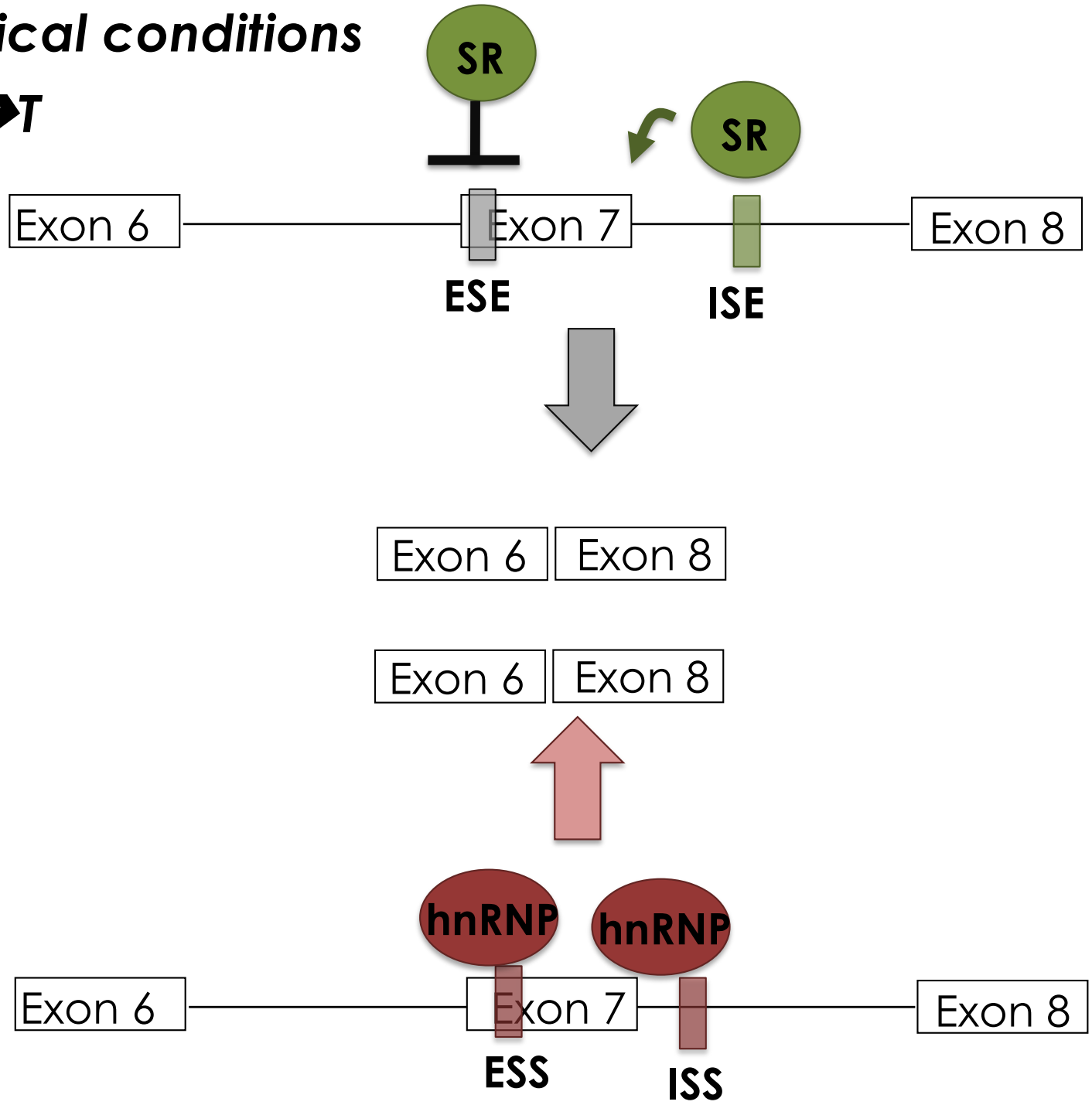


physiological conditions



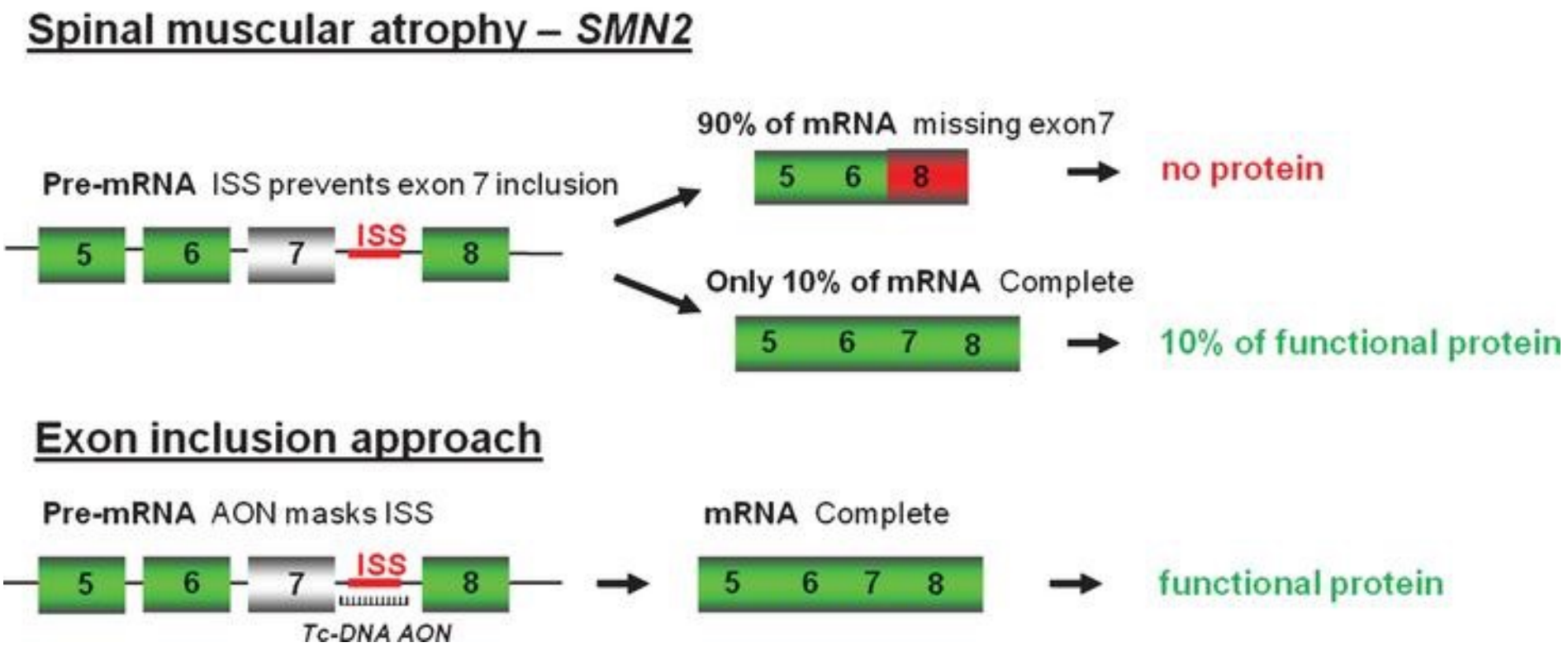
pathological conditions

SMN1 C ➔T



Targeting RNA-Splicing for SMA Treatment

Modulation of alternative splicing (exon 7 inclusion) in SMN2 by antisense oligonucleotide is targeted for SMA treatment. Systemic administration of an antisense oligonucleotide (ASO-10-27) to neonates with SMA robustly rescues mice with severe SMA and extended median lifespan of animals with SMA 25-fold.

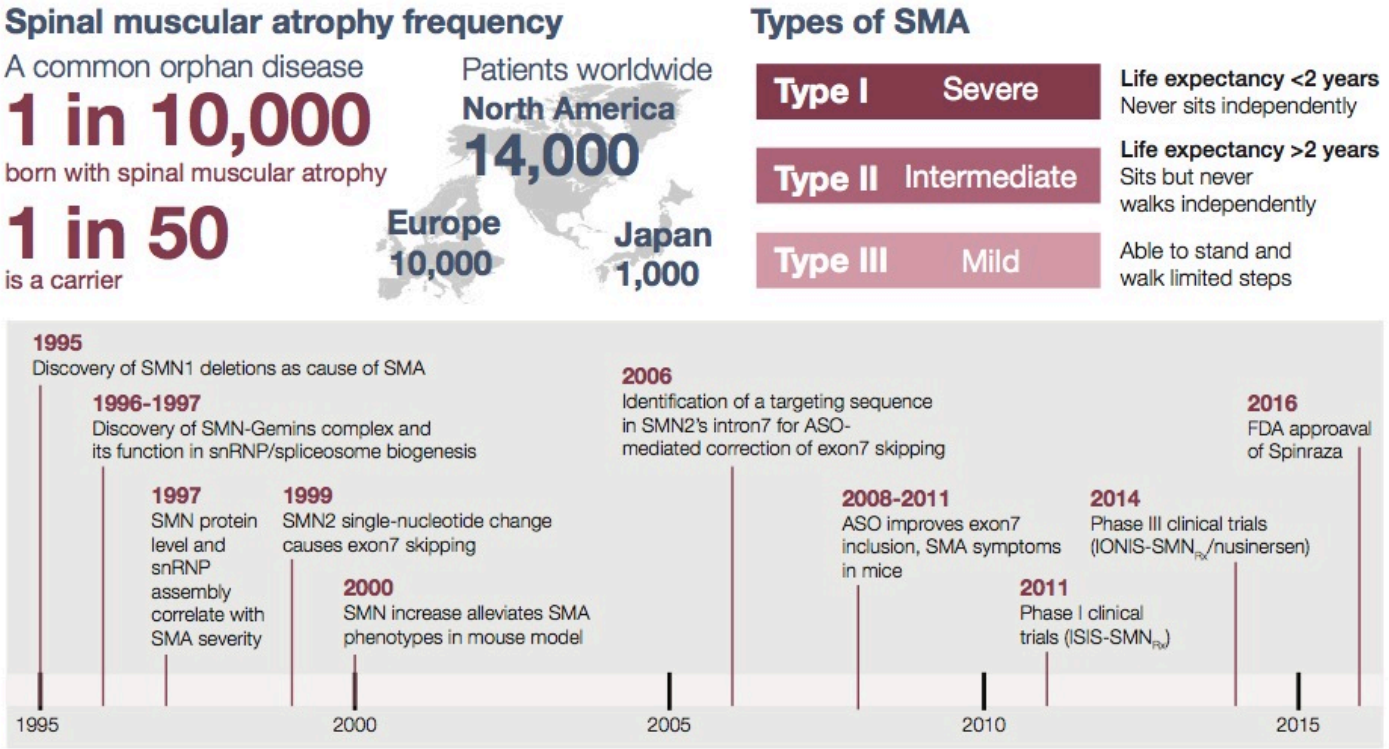


Clinical classification of SMA

SMA Type	Age of Onset	Highest Achieved Motor Function	Natural Age of Death	Typical Number of *SMN2* Copies
0	Prenatal/fetal	None	<6 months	1
I	<6 months	Sit with support only	<2 years	1-3
II	6–18 months	Sit independently	>2 years	2-3
III	>18 months	Walk independently	Adulthood	3-4
IV	Adult (20s-30s)	Walk through adulthood	Adult	≥4

Adapted from Table 1 of Verhaart et al. 2017.[6]

Number of SMN2 copies based on Calucho et al. 2018.[11]



NAME: **Spinraza** (nusinersen, IONIS-SMN, ISIS-SMN)

APPROVED FOR: Spinal muscular atrophy (SMA) in pediatric and adult patients

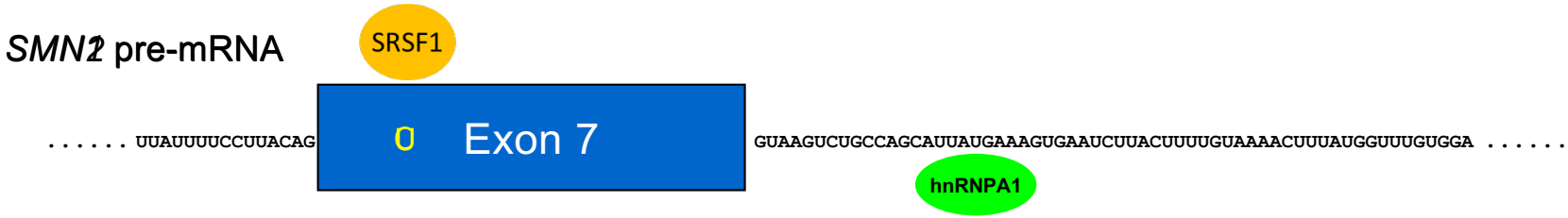
TYPE: 2'-O-methoxyethyl phosphorothioate-modified antisense oligonucleotide (ASO) delivered by intrathecal injection into the cerebrospinal fluid

MOLECULAR TARGETS: SMN2 pre-mRNA intron7 ISS

EFFECTS ON TARGETS: Masking SMN2 pre-mRNA intron7 ISS with ASO enhances exon7 splicing inclusion, boosting full-length SMN mRNA and protein level in the spinal cord, and improving achievement of motor milestones

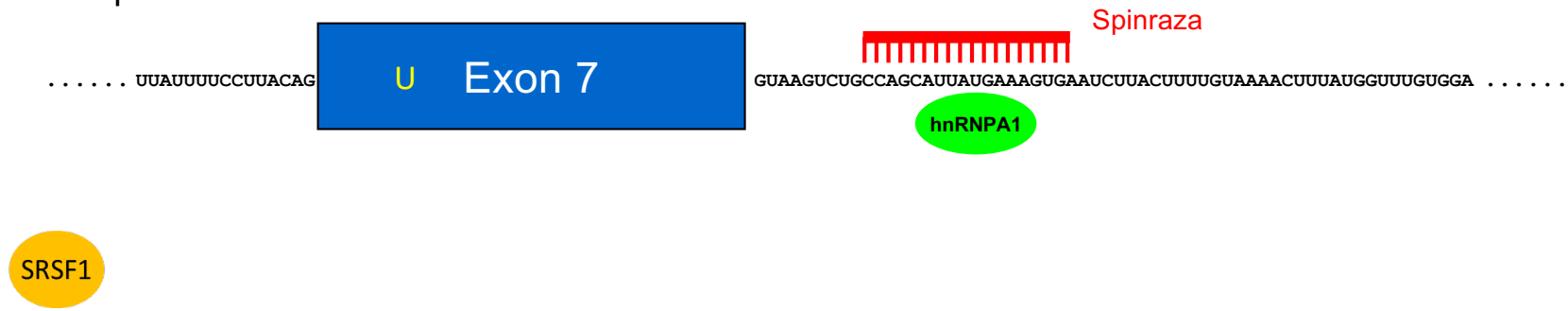
PRICE: $125,000 per injection, which adds up to $750,000 for the first year of treatment and $375,000 after that.

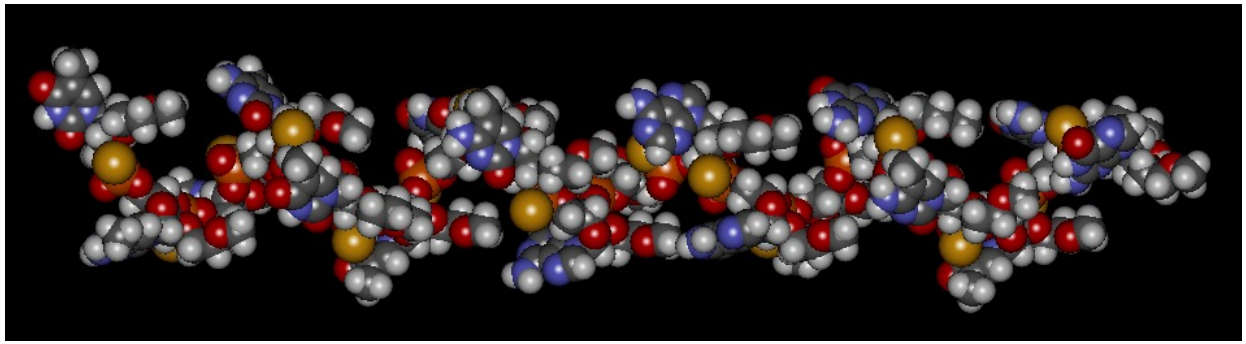
Nusinersen (Spinraza)



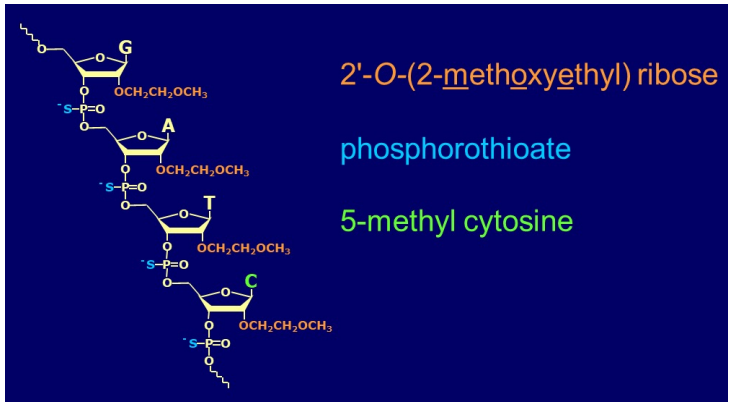
Hua, Vickers, Okunola, Bennett & Krainer (2008) *Am J Hum Genet* 82: 834

Nusinersen (Spinraza)



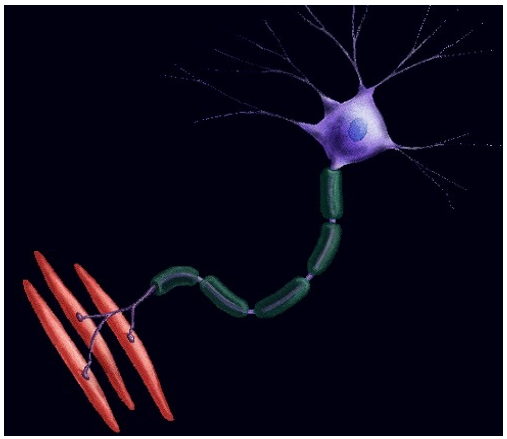


18mer ASO; MW 7127

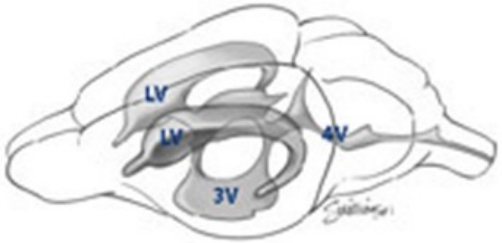


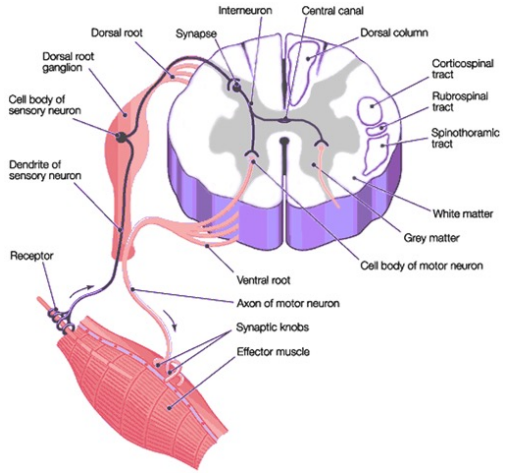
Hua, Vickers, Okunola, Bennett & Krainer (2008) *Am J Hum Genet* 82: 834

Intrathecal ASO delivery

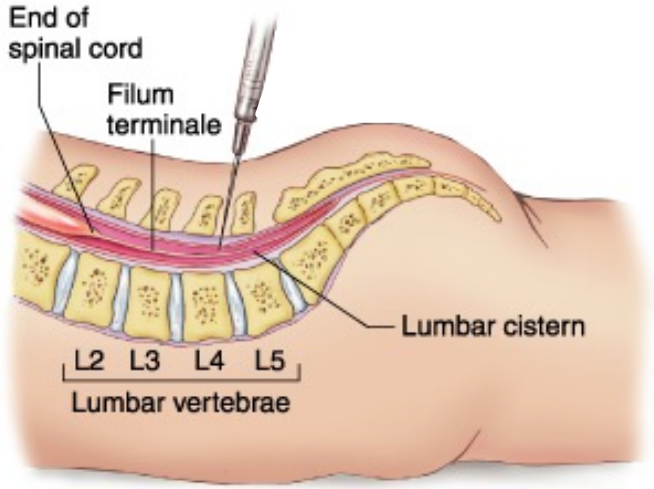


www.uofaweb.ualberta.ca





http://www.glittra.com/yvonne/neuropics.html



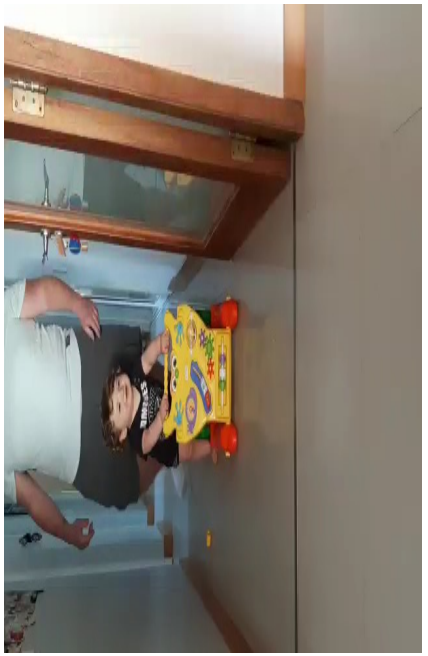
http://www.mdguidelines.com/lumbar-puncture

Long half-life of nusinersen in the CNS
Loading doses: 12 mg @ 2 weeks x 4
Maintenance doses: 12 mg @ 4 months

Spinraza updates

- September 30, 2022: >11,000 patients currently on nusinersen worldwide, including commercial, early-access, and clinical-trial settings
- Nusinersen approved in >50 countries; reimbursed in >30
- NURTURE trial: genetically diagnosed SMA infants treated before onset of symptoms show unprecedented survival and motor-function gains for up to 5 years
- 46 states in the U.S. currently do newborn screening for SMA: 97% of newborn babies; implementation studies in other countries
- New DEVOTE randomized clinical trial currently evaluating higher nusinersen doses

Biogen



Two other approved SMA therapies:
Zolgensma (Novartis 2019) & Evrysdi (Roche 2020)

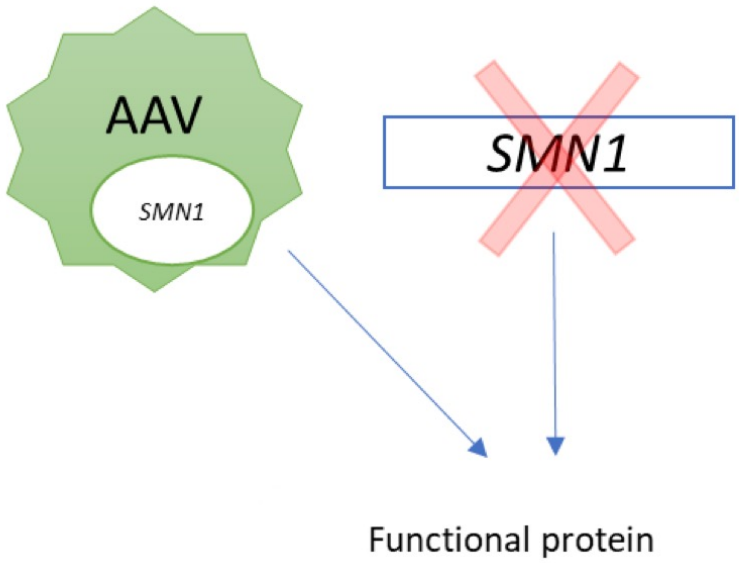
Novartis' gene therapy for SMA - Zolgensma

PRICE: $2.125 million

ZOLGENSMA is a prescription gene therapy used to treat children less than 2 years old with spinal muscular atrophy (SMA).

ZOLGENSMA is given as a one-time infusion into the vein.

ZOLGENSMA was not evaluated in patients with advanced SMA.



May-2020
approved in Europe

Table 3.13. Evidence Ratings for Spinraza and Zolgensma for SMA

Population	Spinraza	Zolgensma	Ability to Distinguish?
Type 0 SMA	I*	I*	I†
Infantile-Onset (Type I) SMA	A	A	I
Later-Onset (Type II and III) SMA	B+	I*	I†
Type IV SMA	I*	I*	I†
Presymptomatic SMA	B+	I*	I†

*No studies (e.g., RCTs, observational, etc.) identified.

†Comparison is based on lack of available evidence for Zolgensma.

Comparative Net Health Benefit

***A** = **"Superior"** - High certainty of a substantial (moderate-large) net health benefit*

***B** = **"Incremental"** - High certainty of a small net health benefit*

***C** = **"Comparable"**- High certainty of a comparable net health benefit*

***D** = **"Negative"**- High certainty of an inferior net health benefit*

***B+** = **"Incremental or Better"** - Moderate certainty of a small or substantial net health benefit, with high certainty of at least a small net health benefit*

***C+** = **"Comparable or Better"** - Moderate certainty of a comparable, small, or substantial net health benefit, with high certainty of at least a comparable net health benefit*

***P/I** = **"Promising but Inconclusive"** - Moderate certainty of a comparable, small, or substantial net health benefit, and a small (but nonzero) likelihood of a negative net health benefit*

***C-** = **"Comparable or Inferior"** - Moderate certainty that the point estimate for comparative net health benefit is either comparable or inferior*

***I** = **"Insufficient"** - Any situation in which the level of certainty in the evidence is low*

la Repubblica

12 Ottobre 2019

Per la prima volta messo a punto farmaco per un singolo paziente

Un'analisi del Dna ha rivelato che la causa era un'unica mutazione di un gene chiamato Cln7, indispensabile a produrre una proteina necessaria ai lisosomi, che nelle cellule hanno il ruolo di rimuovere o riciclare la 'spazzatura', le sostanze indesiderate prodotte dai processi cellulari. Una volta isolato il difetto, i ricercatori hanno ideato un oligonucleotide antisenso, un piccolo frammento di Dna in grado di 'mascherare' il difetto. Una volta testato sugli animali il farmaco, che è stato chiamato 'milasen', è stato infuso nella bimba, dopo l'approvazione dell'Fda per il test, con esiti positivi.

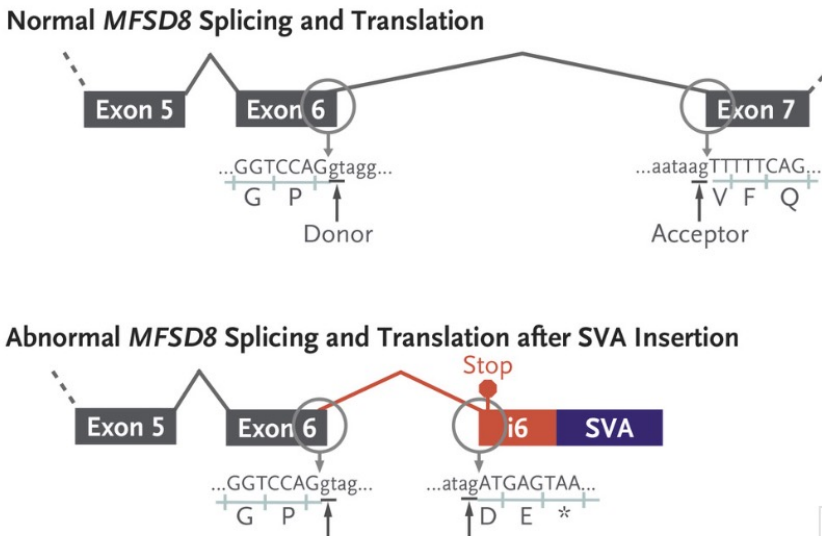
Dopo un anno di cura, scrivono gli autori, la bambina ha mostrato una diminuzione delle convulsioni di cui soffriva, anche se su altri problemi, come la cecità, non ci sono ancora miglioramenti. "La creazione di milasen in un tempo così ridotto - concludono gli autori - è uno straordinario precedente che puó rivoluzionare come le malattie genetiche vengono trattate".

The NEW ENGLAND JOURNAL of MEDICINE

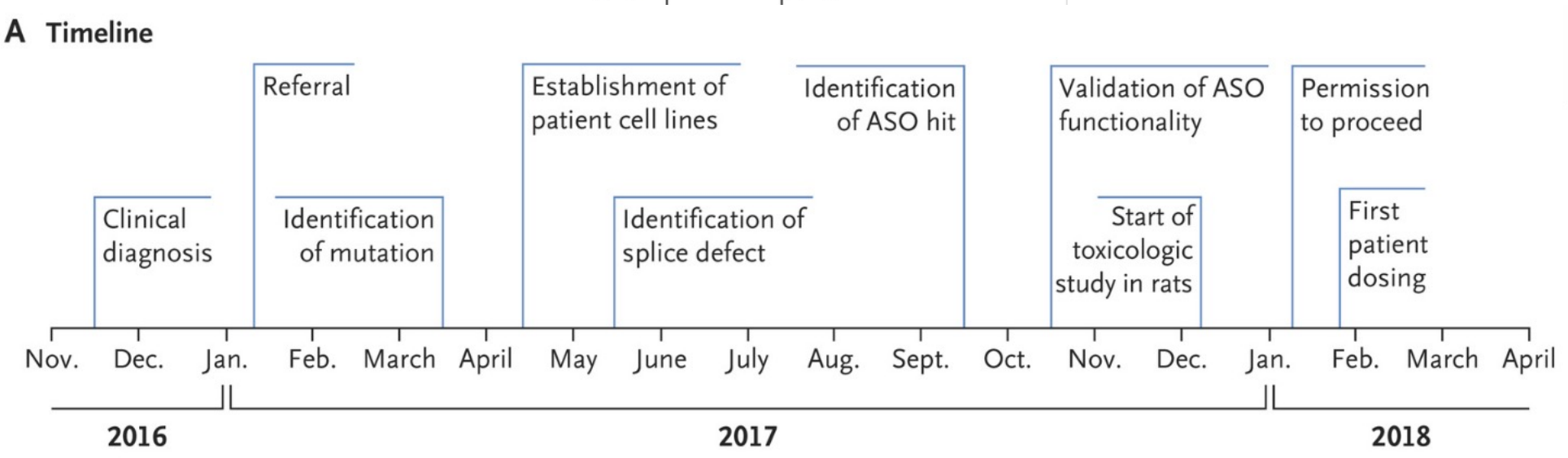
ORIGINAL ARTICLE BRIEF REPORT

Patient-Customized Oligonucleotide Therapy for a Rare Genetic Disease

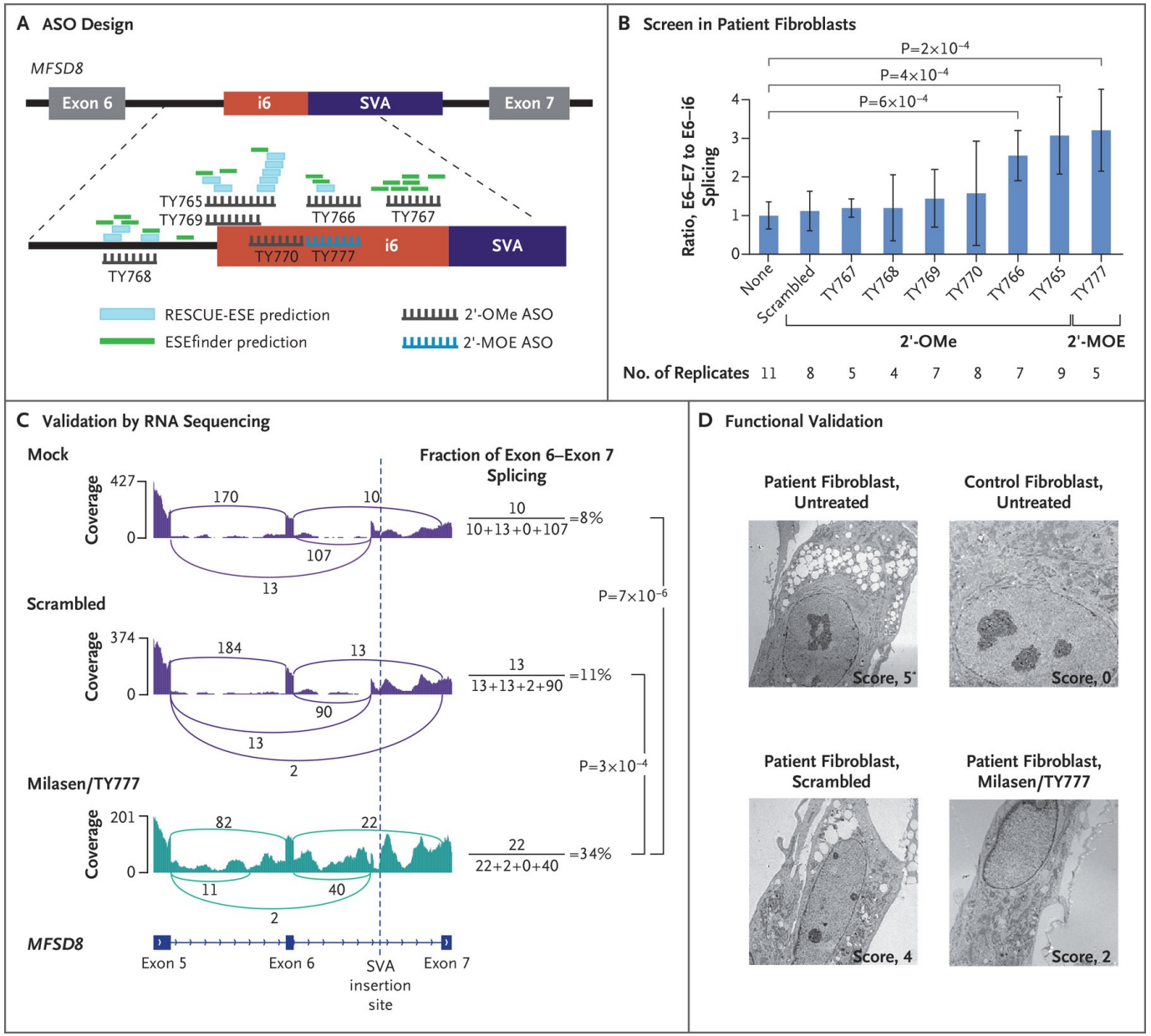
Jinkuk Kim, Ph.D., Chunguang Hu, M.D., Ph.D., Christelle Moufawad El Achkar, M.D., Lauren E. Black, Ph.D., Julie Douville, Ph.D., Austin Larson, M.D., Mary K. Pendergast, J.D., Sara F. Goldkind, M.D., Eunjung A. Lee, Ph.D., Ashley Kuniholm, B.S., Aubrie Soucy, B.A., Jai Vaze, B.A., et al.



SVA (SINE–VNTR–*Alu*) retrotransposon



Individualized genomic medicine- Milasen



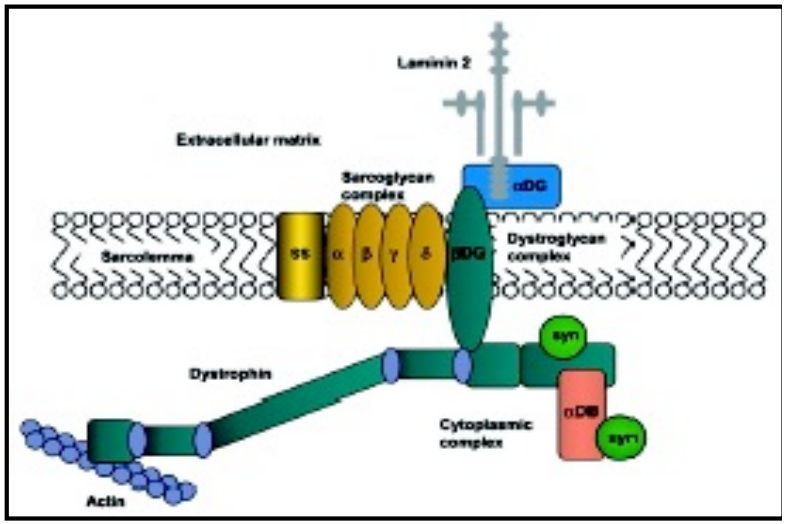
Individualized genomic medicine- Milasen

- There are tens of thousands of Milas all around the world.
- They are not covered by insurance, and drug companies aren't investing in them either.
- The cost to make one such drug, including lab tests and dealing with regulators, could be $3 to $5 million.
- Mila's treatment was paid for using funds from Boston Children's Hospital, research grants, and two private foundations, including Vitarello's Mila's Miracle Foundation, which has been raising funds for research a ASO treatment.

The exon skipping strategy in the therapeutic treatment of Duchenne Muscular Dystrophy

Duchenne Muscular Dystrophy (DMD)

- X-linked recessive disorder
- affects 1 in 3500 live males
- DMD muscles degenerate with activity
- leads to ***death*** by the third decade of life



The gene is too big for a classical gene therapy intervention

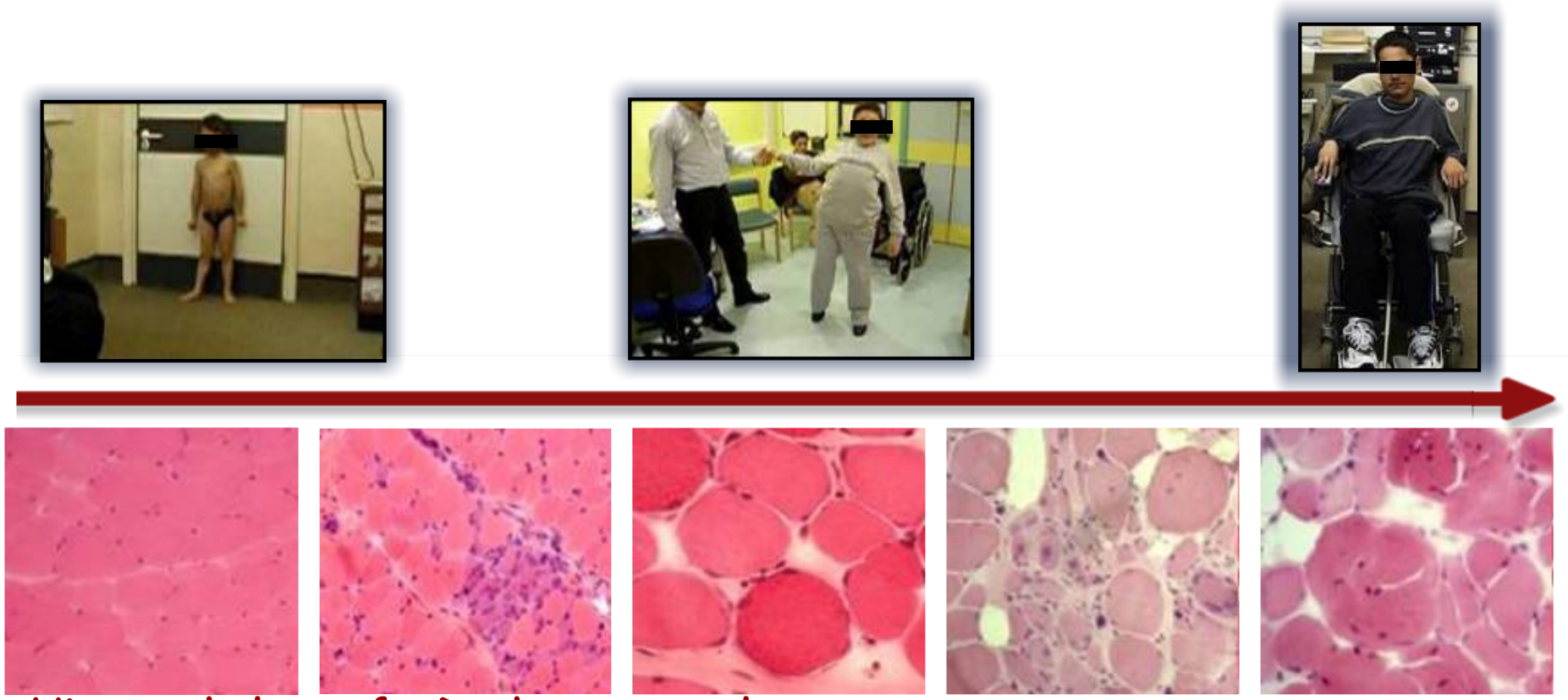
Dystrophin

- **protein= 427 KDa**
- **DNA= 2,5 Mb**
- **cDNA= 14 Kb**

Duchenne Muscular Dystrophy (DMD)

- is a severe disorder characterized by rapid progression of muscle degeneration, leading to loss of ambulation and death.

- X-linked recessive disorder that affects 1 in 3500 live males

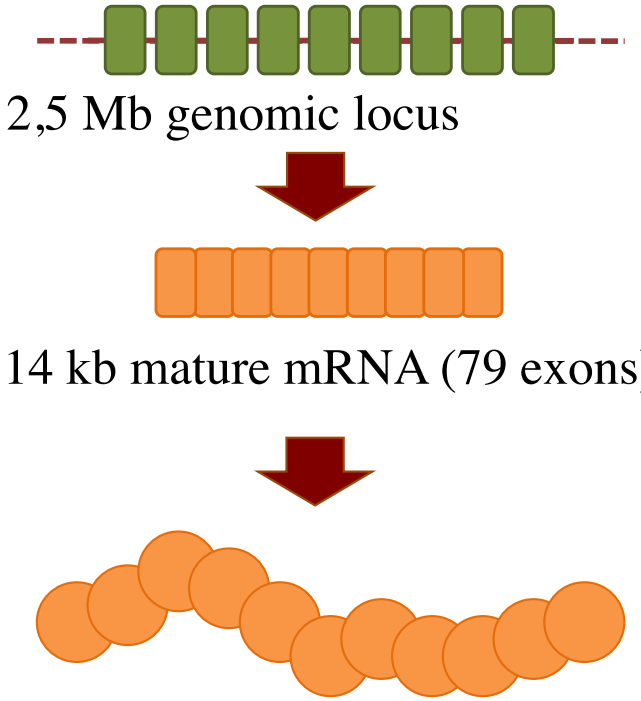


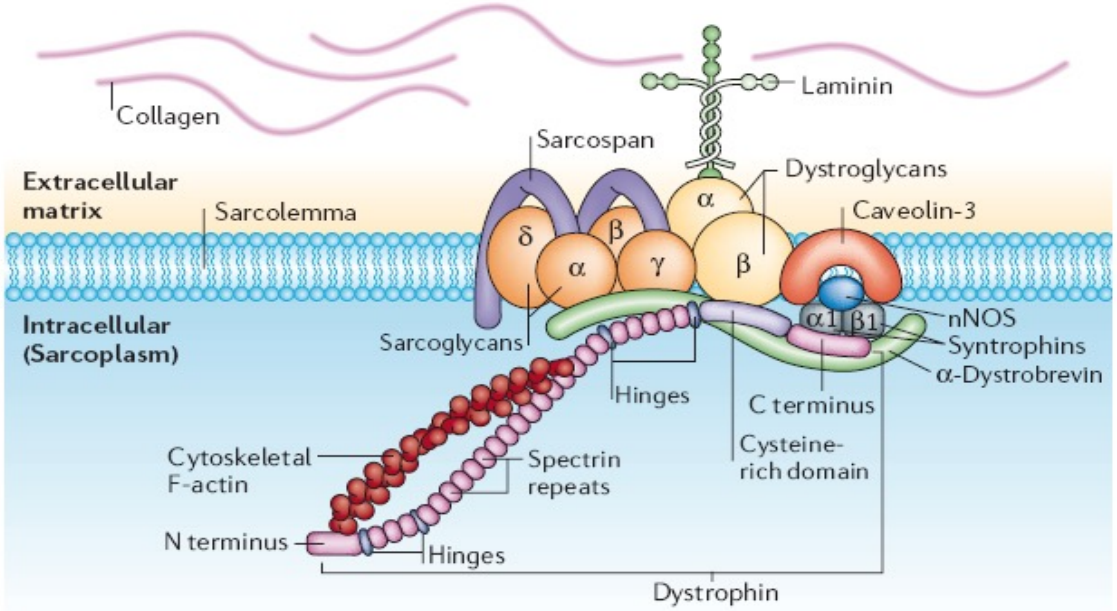
Histopathology of a Duchenne muscle

Dystrophin-The LONGEST GENE of our genome

•Patients with DMD are deficient in dystrophin, a protein that connects the cytoskeleton of a muscle fiber to the surrounding extraxellular matrix

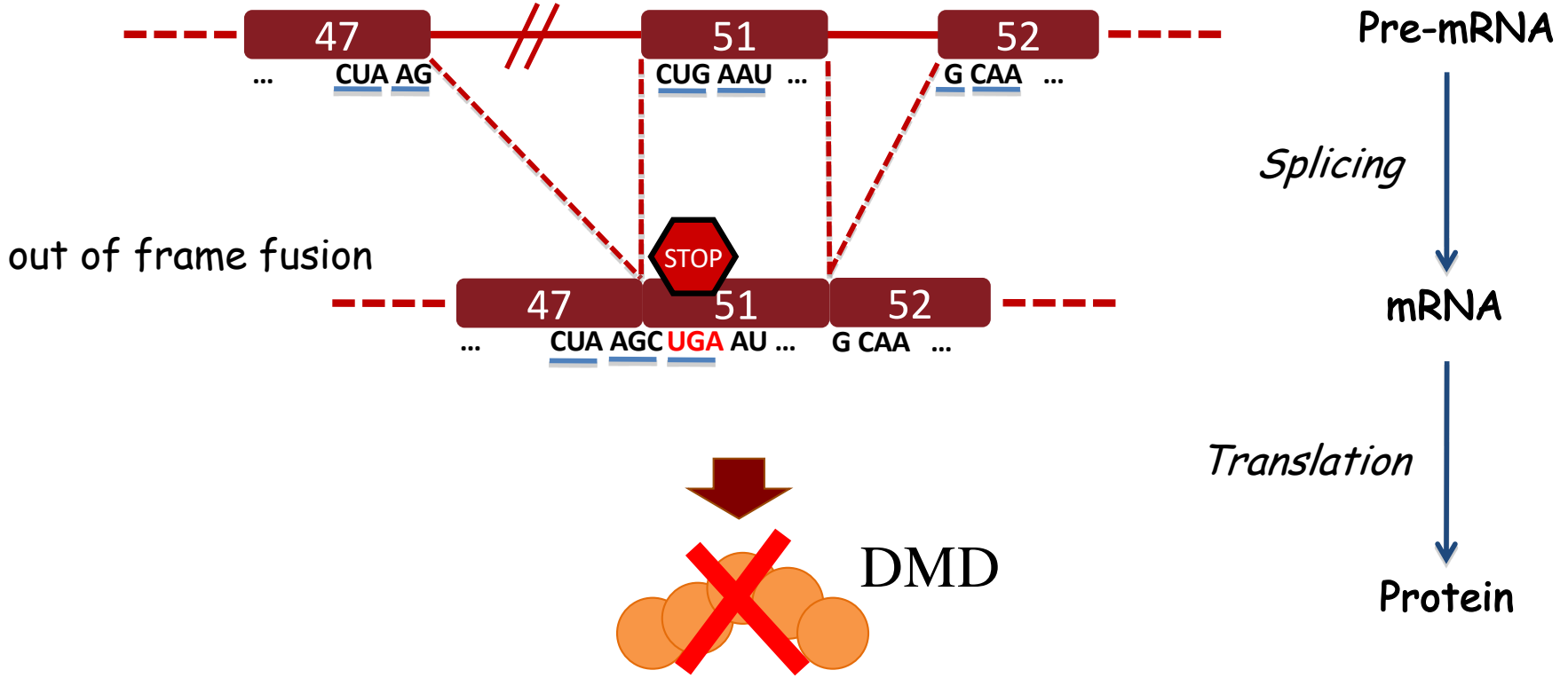
•this deficiency causes sarcolemmal instability and muscle degeneration



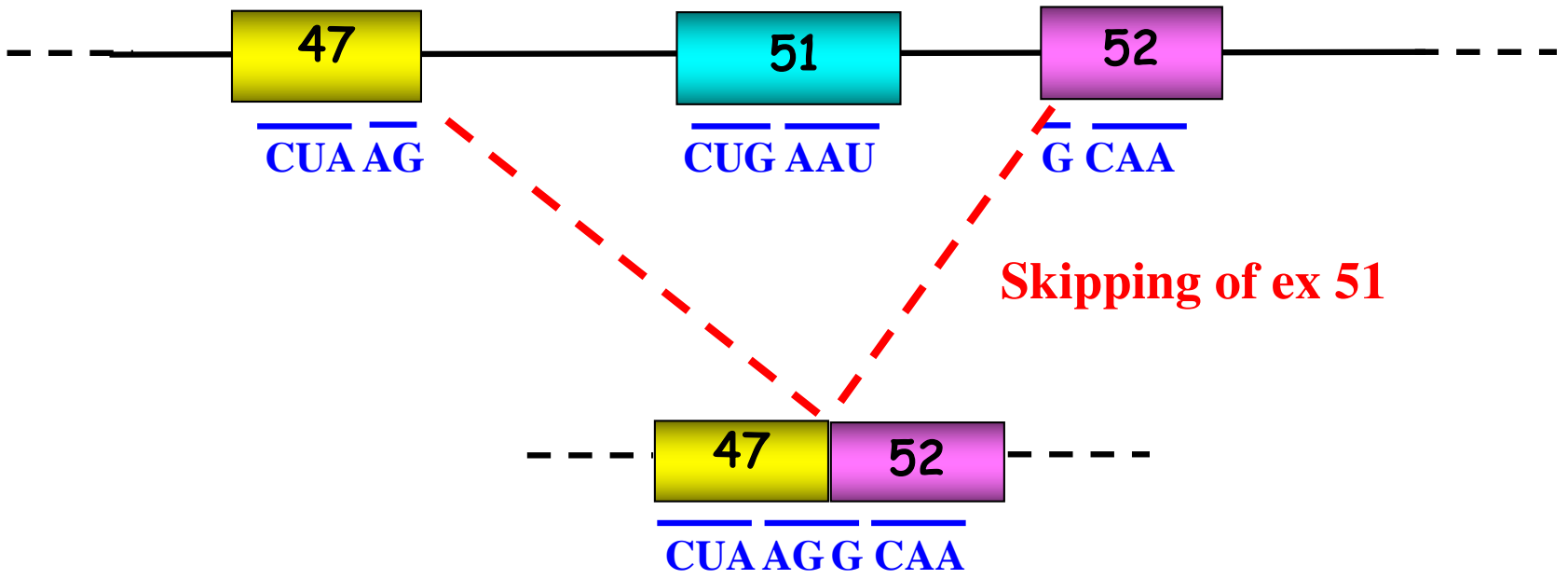


Duchenne Muscular Dystrophy
- the 48-50 deletion -

•DMD is caused by mutations in the Dystrophin gene that alter the pre-mRNA splicing and disrupt the open reading frame of the proteins, producing premature stop codons and mRNA degradation



Exon skipping can revert the phenotype



In-frame mRNA → **translation of a shorter but still functional protein**

- Becker-type -

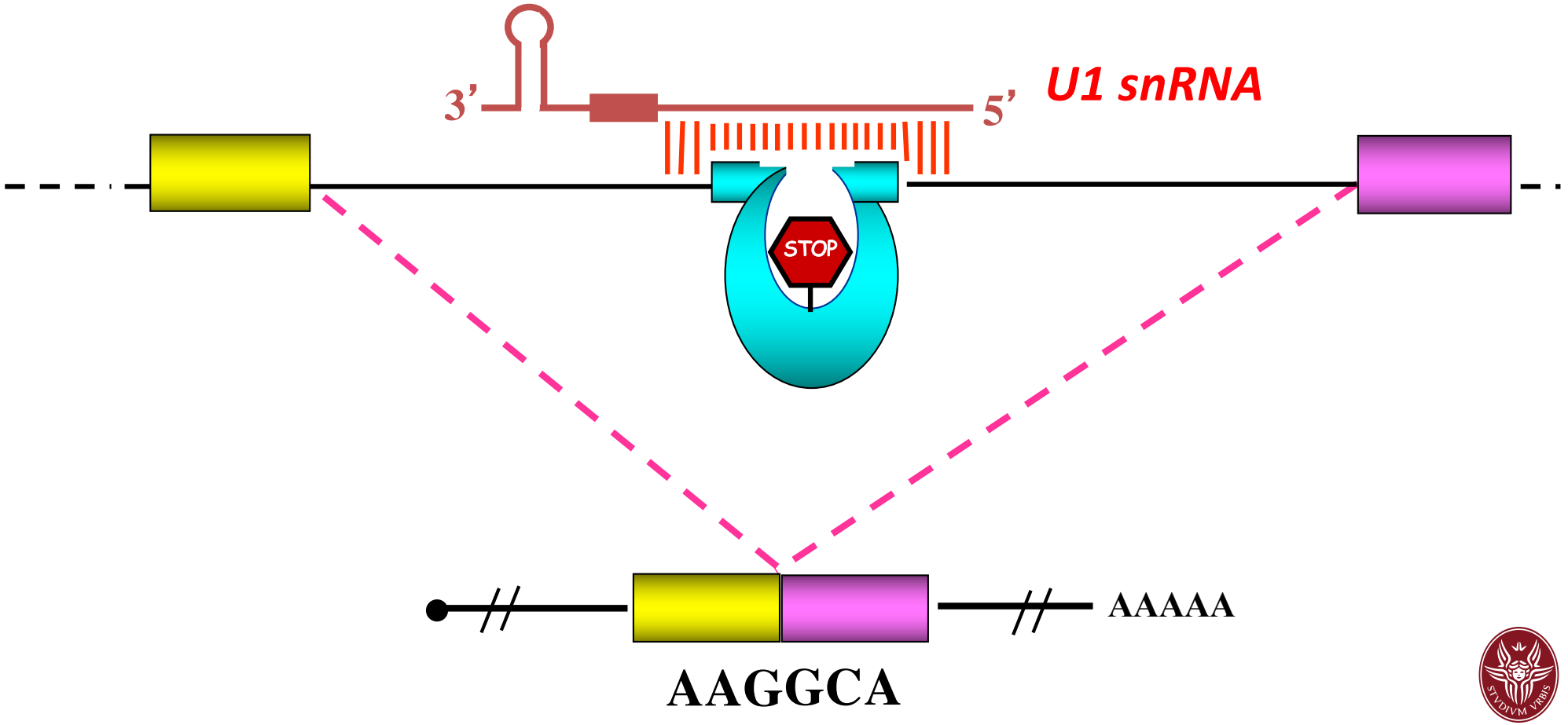
75% of all known dystrophin mutations can be cured by exon skipping

skipping of ex 51 - 18%

Antisense RNA technology applied to the correction of DMD mutations

U1 snRNA

- nuclear RNA with specific recognition for splice junctions
- is matured in the cytoplasm an then reimported in the nucleus
- few transduced nuclei in the muscle fiber can provide chimeric antisense molecules to the entire fiber



Exon skipping for the cure of DMD entered clinical trials (*Eteplirsen*)

van Deutekom, JC, *et al*. (2007).
Local dystrophin restoration with antisense oligonucleotide PRO051.
N Engl J Med **357**: 2677–2686.

Goemans, NM, *et al*. (2011).
Systemic administration of PRO051 in Duchenne's muscular dystrophy.
N Engl J Med **364**: 1513–1522.

Cirak et al.. (2011)
Exon skipping and dystrophin restoration in patients with Duchenne muscular dystrophy after systemic phosphorodiamidate morpholino oligomer treatment: an open-label, phase 2, dose-escalation study
The Lancet **378**: 595–605

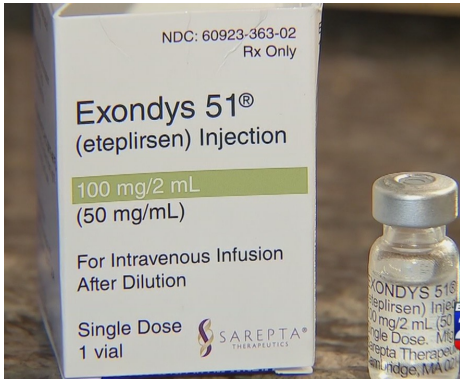
Exon skipping for the cure of DMD entered clinical trials (*Eteplirsen*)

On September 19, 2016, *eteplirsen* received accelerated conditional approval by the US FDA for boys with DMD deletion amenable to exon 51 skipping with limited data on only 12 cases and from just dystrophin protein as a surrogate marker in muscle biopsies, without proof of clinical improvement.

Eteplirsen (*ExonDys 51*) is reported to cost in the order of $300,000-400,000/year per patient for life.

Five FDA-approved splice-switching ASOs since 2016

SMA; Biogen
Approved 2016



DMD Exon 51; Sarepta
Approved 2016

DMD Exon 53; Sarepta
Approved 2019

DMD Exon 53; NS Pharma
Approved 2020

DMD Exon 45; Sarepta
Approved 2021

Exon skipping in the CRISPR/CAs9 Era

In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy

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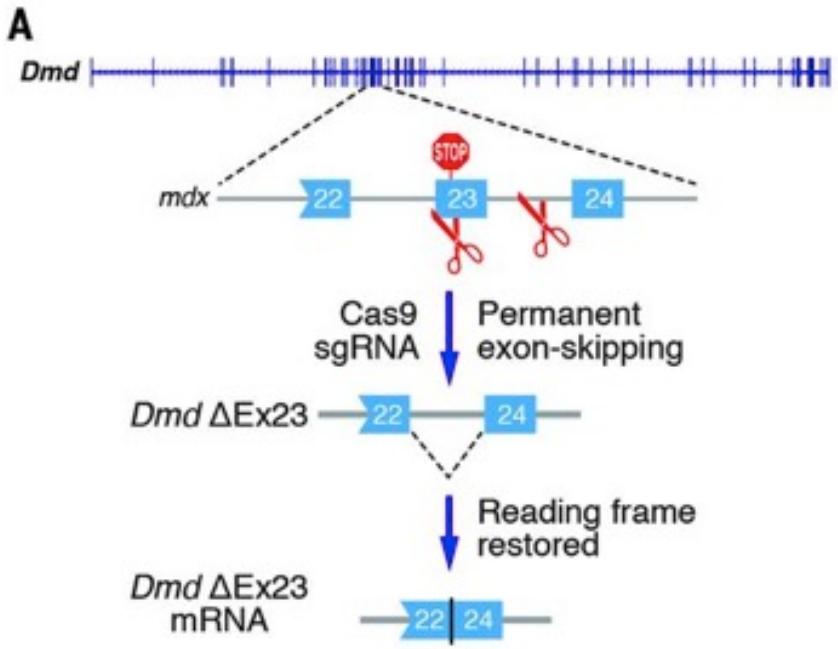

Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy

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In vivo gene editing in dystrophic mouse muscle and muscle stem cells

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Science 2016