# RNA and transcriptome

## Transcriptome changes caused by toxic RNA in myotonic dystrophy

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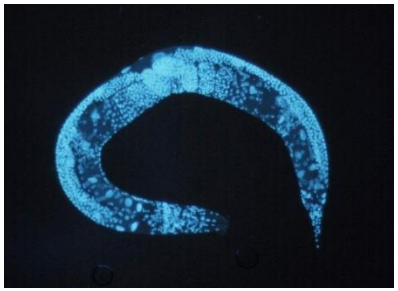
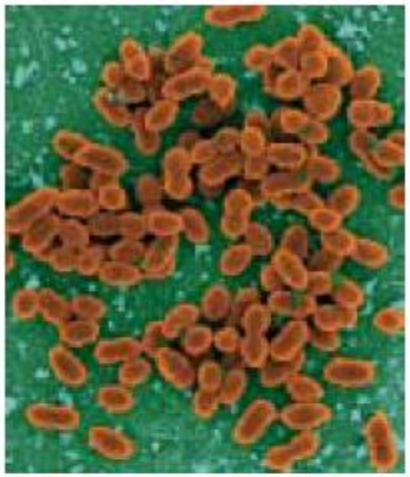
# Number of genes



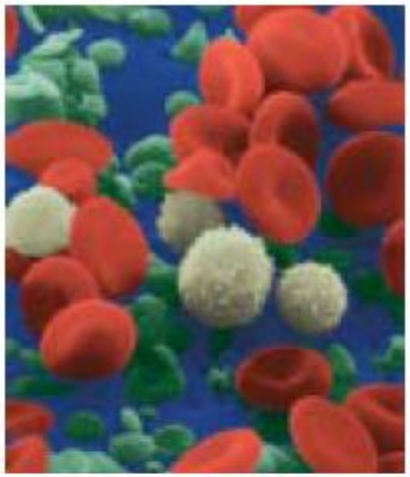
| No. cell types | Species | Genes |
|---|---|---|
| **1** | ***Mycoplasma genitalium (B)*** | **470** |
| 1 | *Haemophilus influenzae (B)* | 1 709 |
| **1** | ***Eschericia coli (B)*** | **4 288** |
| 1 | *Archaeoglobus fulgidus (A)* | 2 436 |
| 1 | *Methanococcus janaschii (A)* | 1 738 |
| 2 | *Bacillus subtilis (B)* | 4 100 |
| 2 | *Caulobacter crescentus (B)* | 4 100 |
| **3** | ***Saccharomyces cerevisiae (E)*** | **6 241** |
| ~30 | *Arabidopsis thaliana (E)* | 24 000 |
| **~50*** | ***Caenorhabditis elegans (E)*** | **18 424** |
| **~50** | ***Drosophila melanogaster (E)*** | **13 601** |
| **~120*** | ***Homo sapiens (E)*** | **20 000** |

***C. elegans* has 300 neurons, humans have 10 billion**

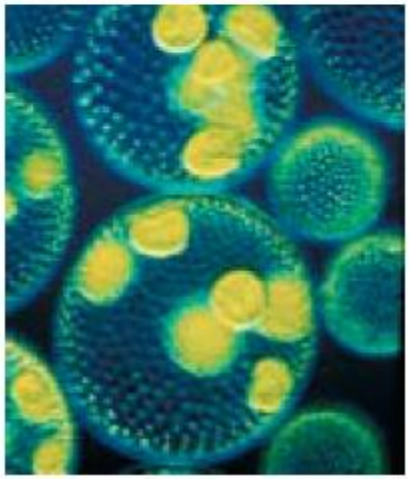
# Ekspresja różnych genów decyduje o strukturze i funkcji komórek


(a)

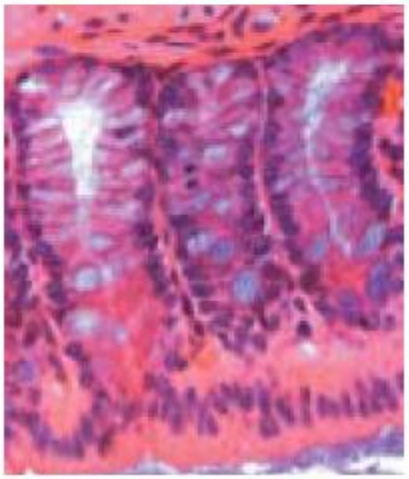

(b)


(c)


(d)

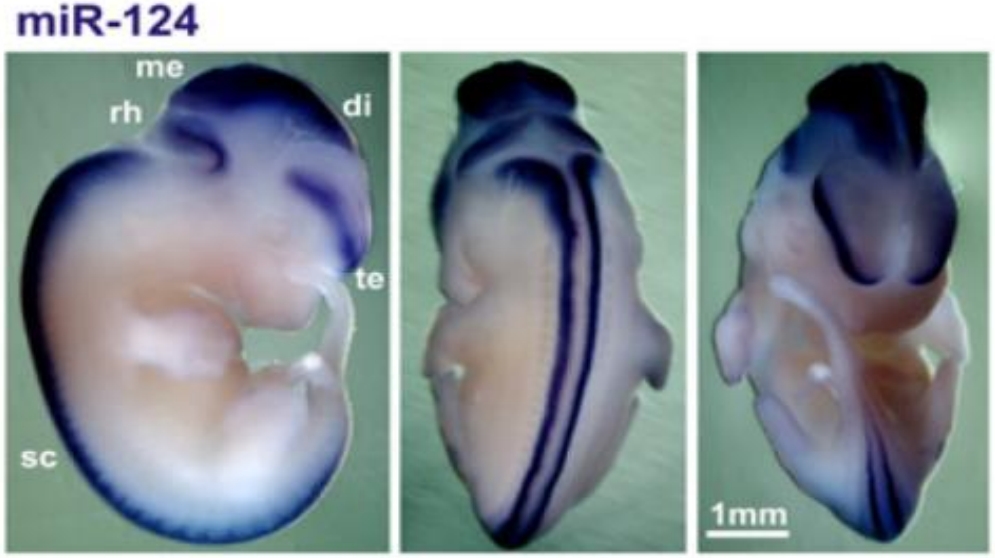

(e)


(f)


(g)


(h)

Molecular Cell Biology; Darnel et al.,5th edition


miR-124
me
rh
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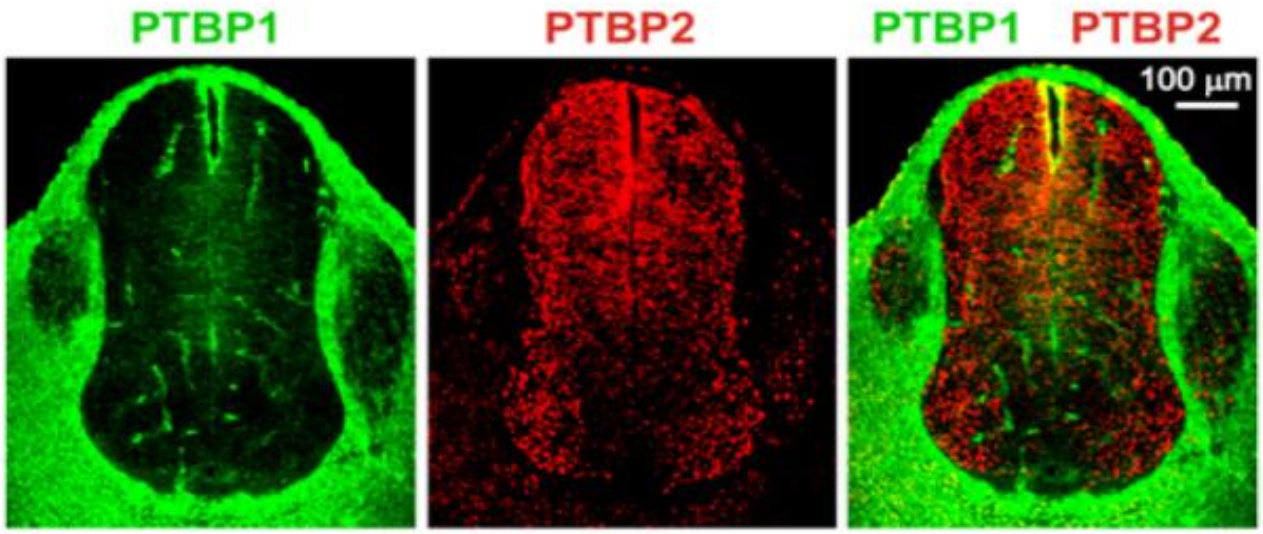

PTBP1
PTBP2
PTBP1 PTBP2
100 µm

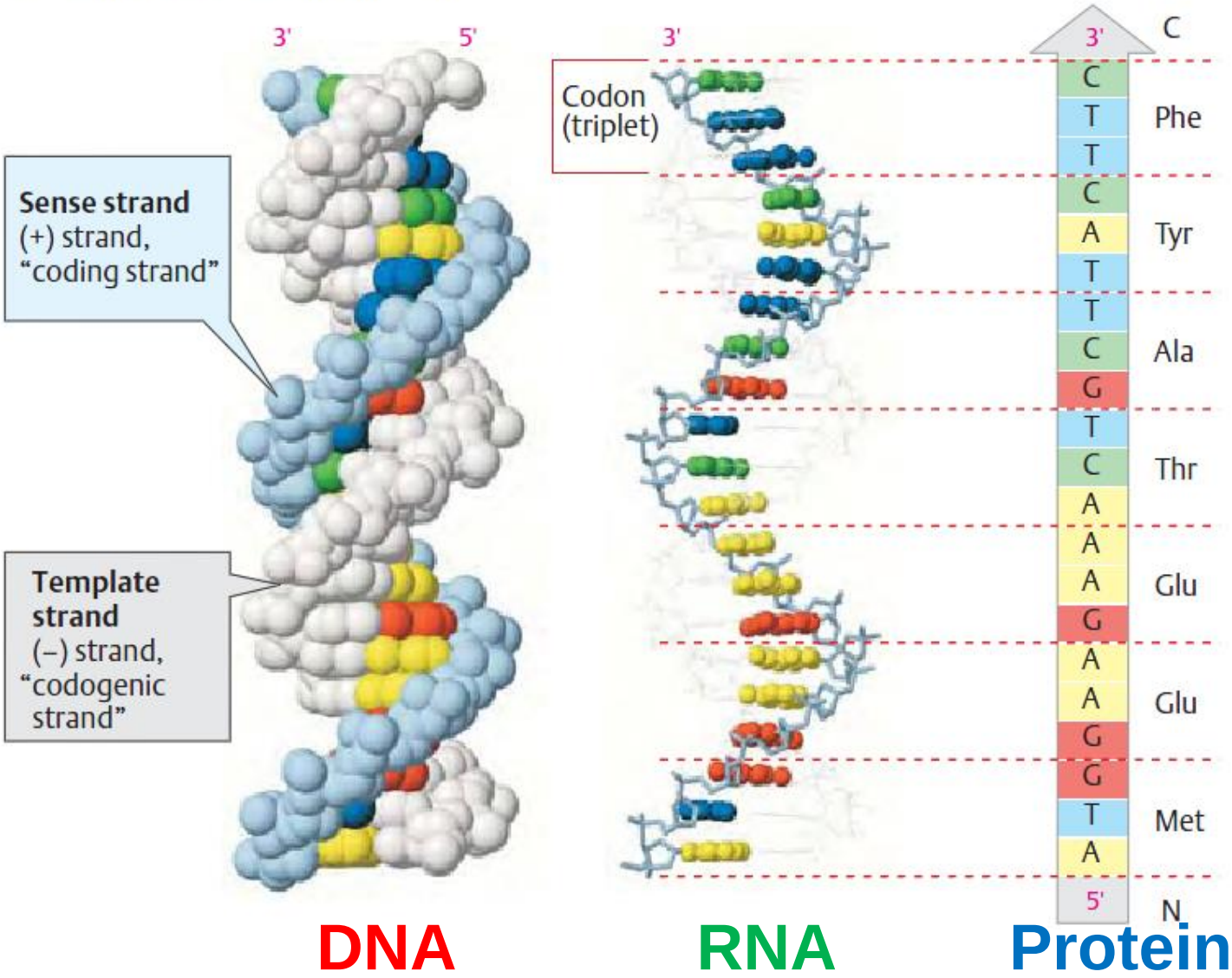
# Transcription

## DNA-Dependent Synthesis of RNA

# Genes Are Segments of DNA That Code for Polypeptide Chains and RNAs

## Transcription

## Translation



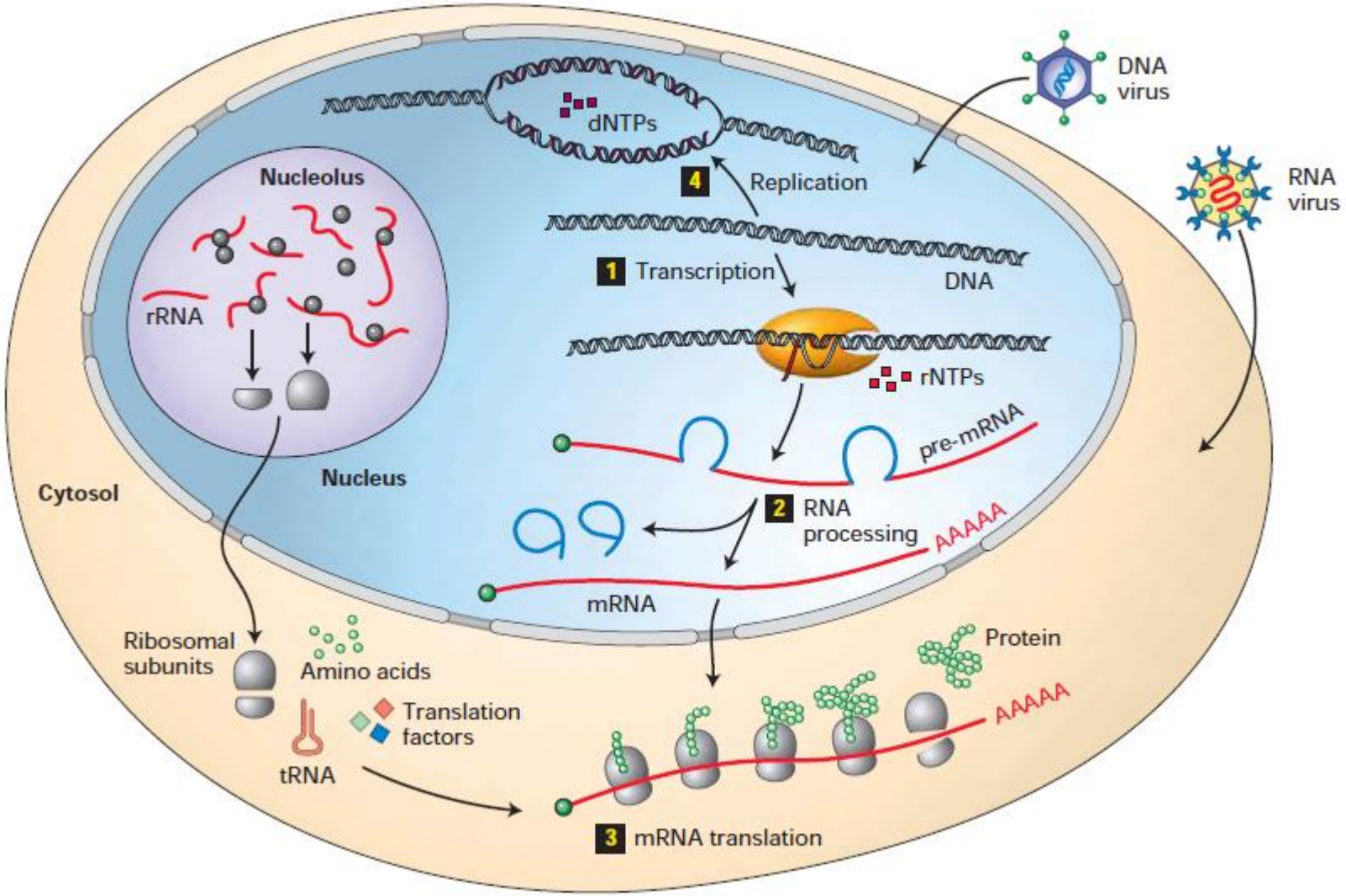

**DNA** **RNA** **Protein**

# Typy RNA

W komórkach występują różne typy RNA pełniące szereg funkcji:

- **mRNA** – (messenger) matryca dla biosyntezy białka w procesie translacji,
- **tRNA** – (transfer) cząsteczka adaptorowa – przenośnik aminokwasów,
- **rRNA** – (ribosomal) kilka typów RNA warunkujących strukturę i funkcję rybosomów,
- **snRNA** – (smal nucleolar) uczestniczą w wycinaniu intronów z pre-mRNA,
- **7SL RNA** – składnik SRP (signal recognition particle),
- **miRNA & siRNA** – (microRNA & short interfering) biorą udział w potranskrypcyjnym wyciszaniu ekspresji genów

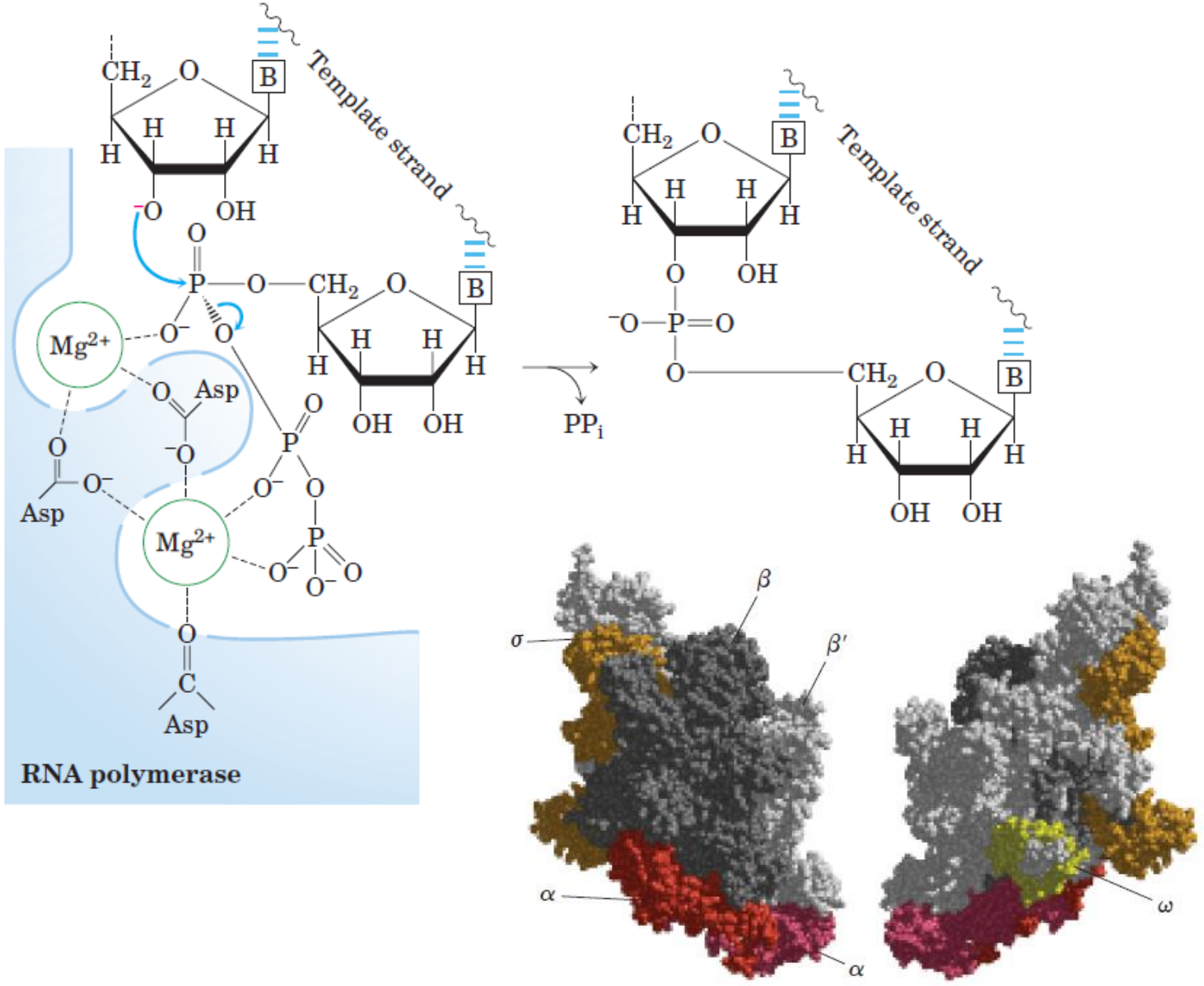
# Basic Molecular Genetic Mechanisms




**Gene** is a locus (or region) of DNA that encodes a functional RNA or protein product, and is the molecular unit of heredity.

Molecular Cell Biology; Darnel et al.,5th edition

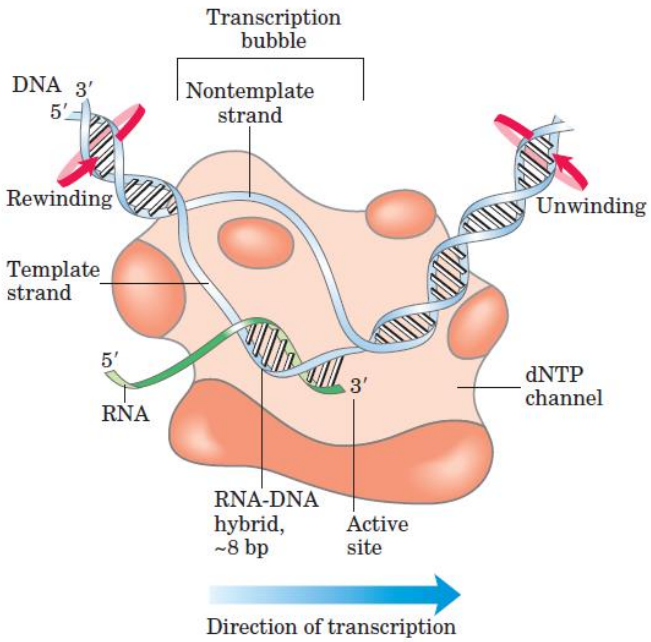
# Synthesis of RNA by RNA Polymerase



# Transcription Phases - Procariota

Transcription occurs in three phases:

- **Initiation**
- **Elongation**
- **Termination**



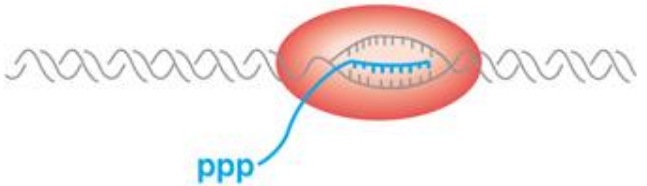



(1) Initiation:

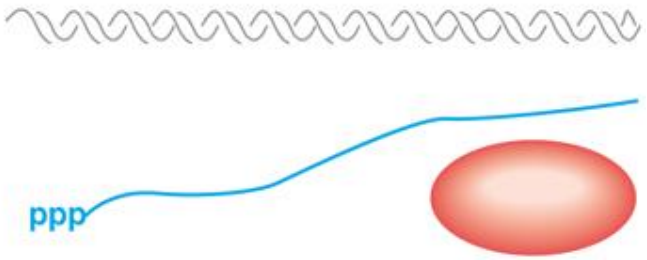
(a) RNA polymerase binds to promoter.

(b) First few phosphodiester bonds form.

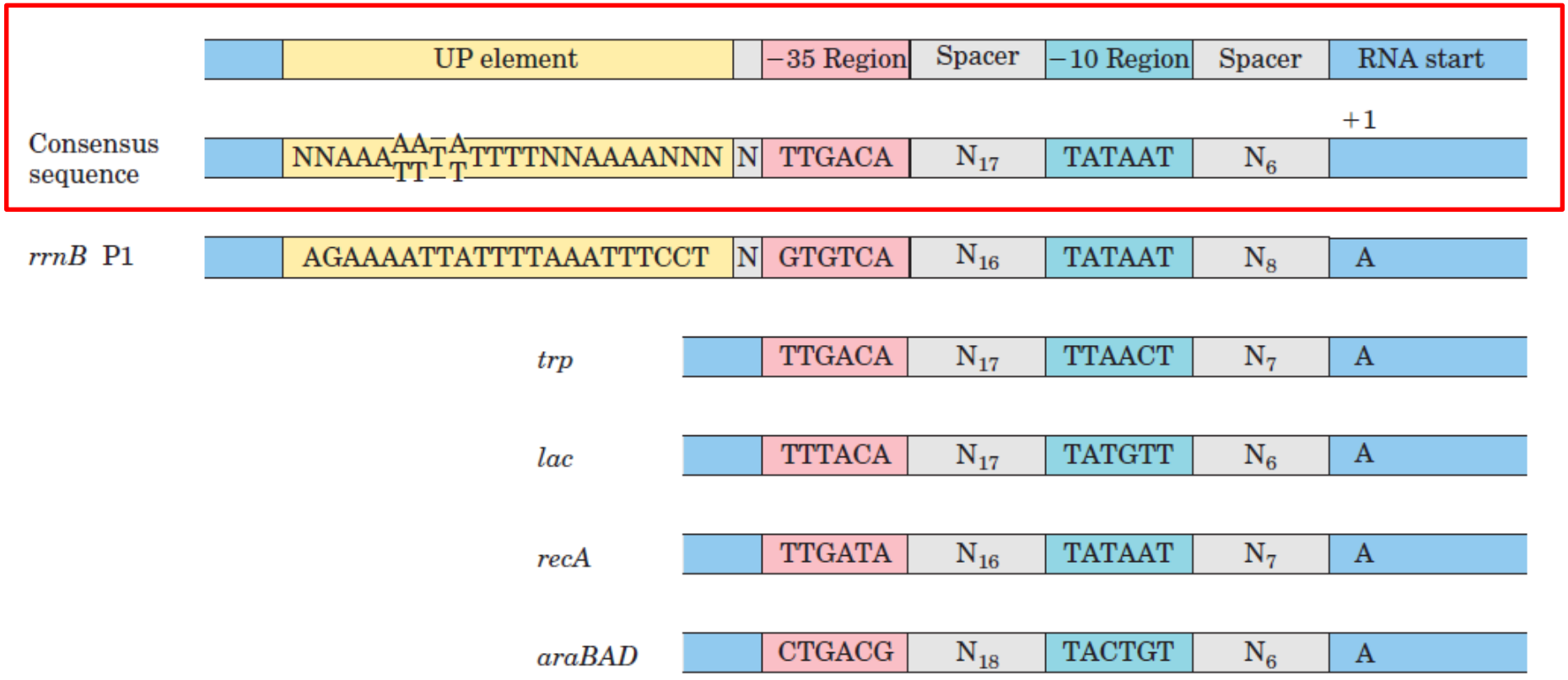

(2) Elongation.



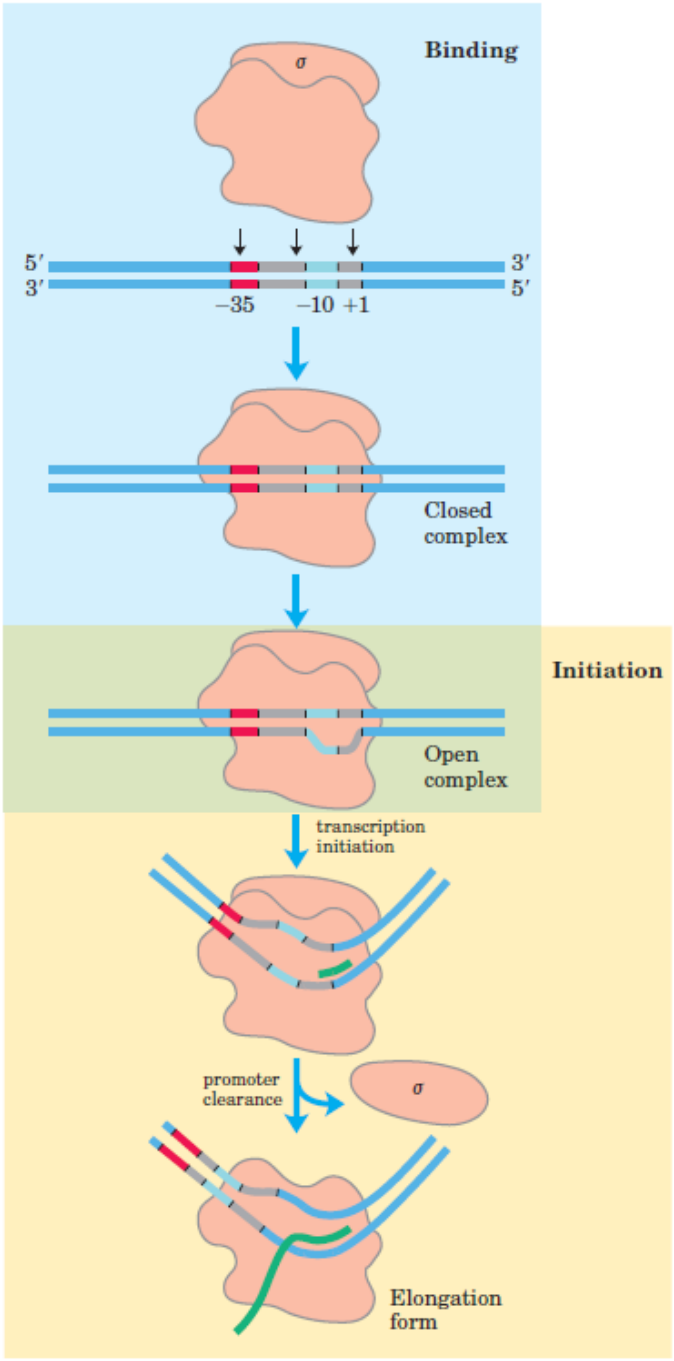

(3) Termination.

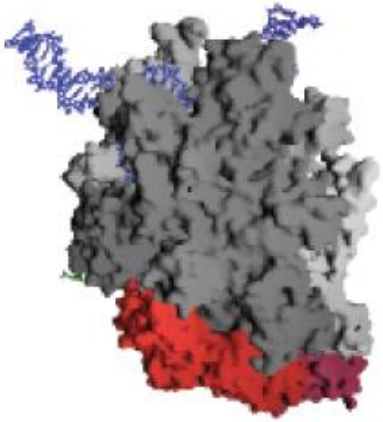


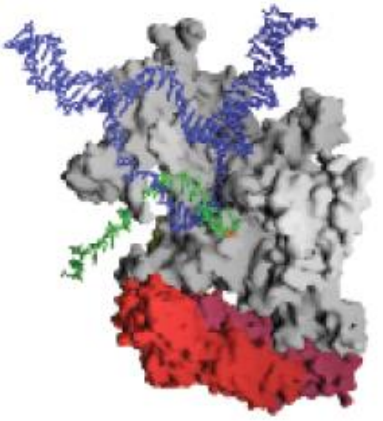
# Typical *E. coli* promoters

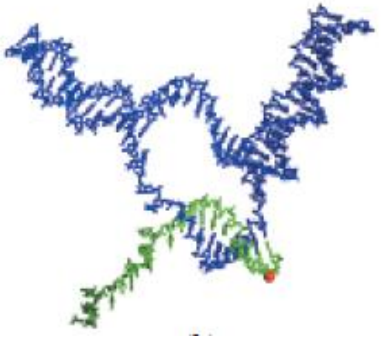


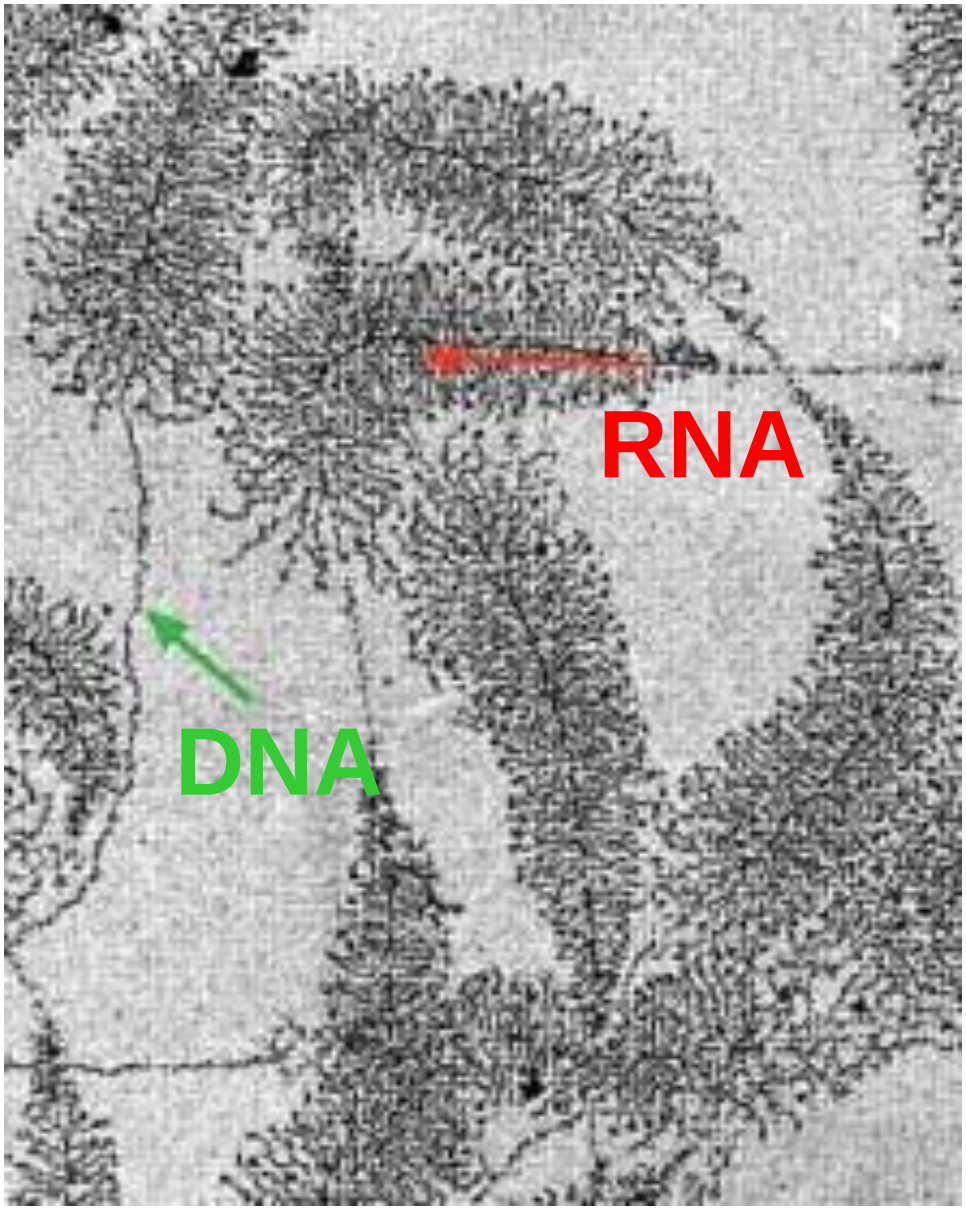
# Recognition of *E. coli* promoters by RNA Polymera



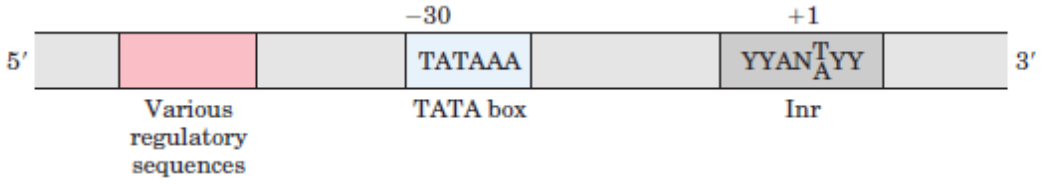



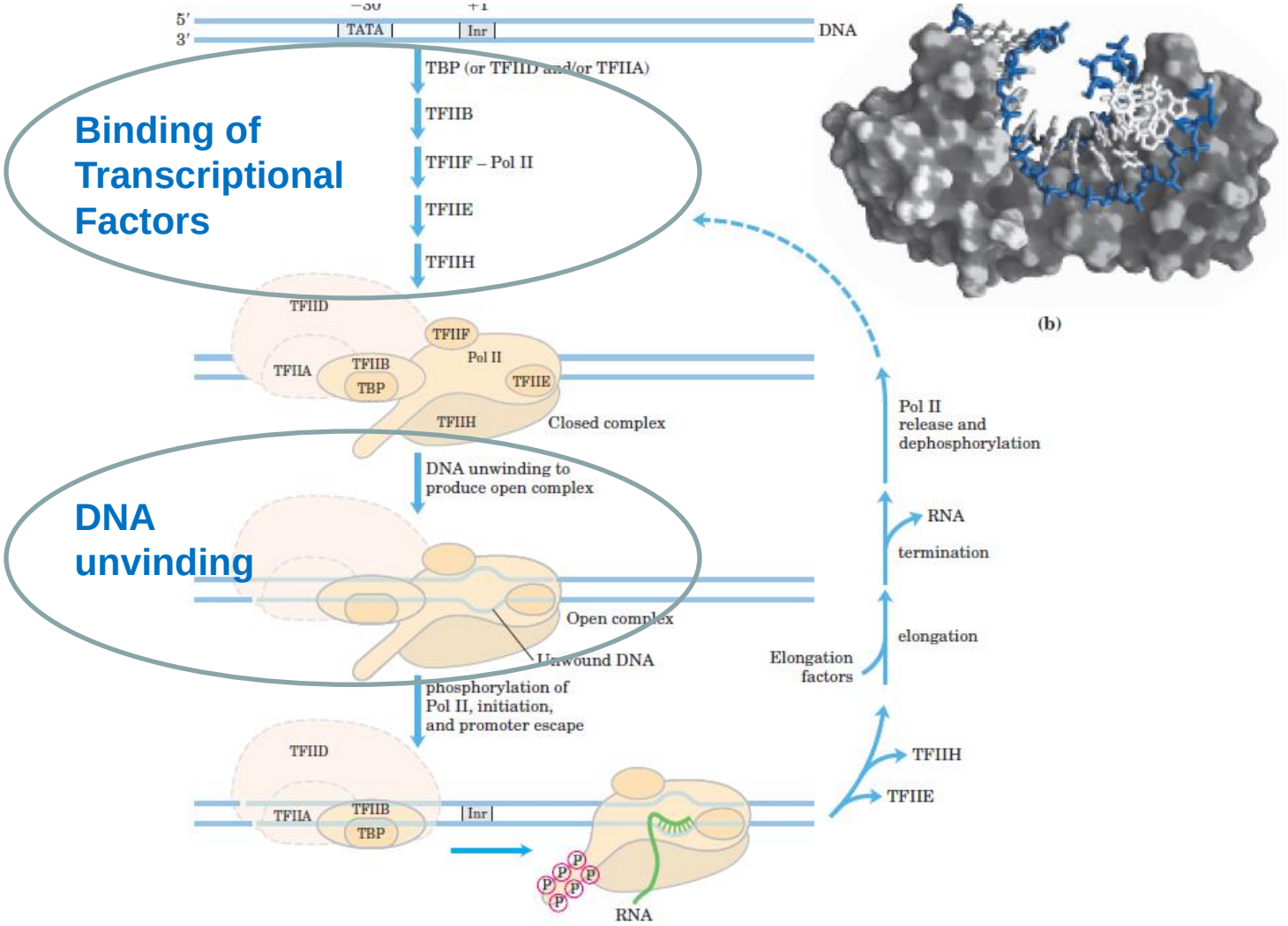




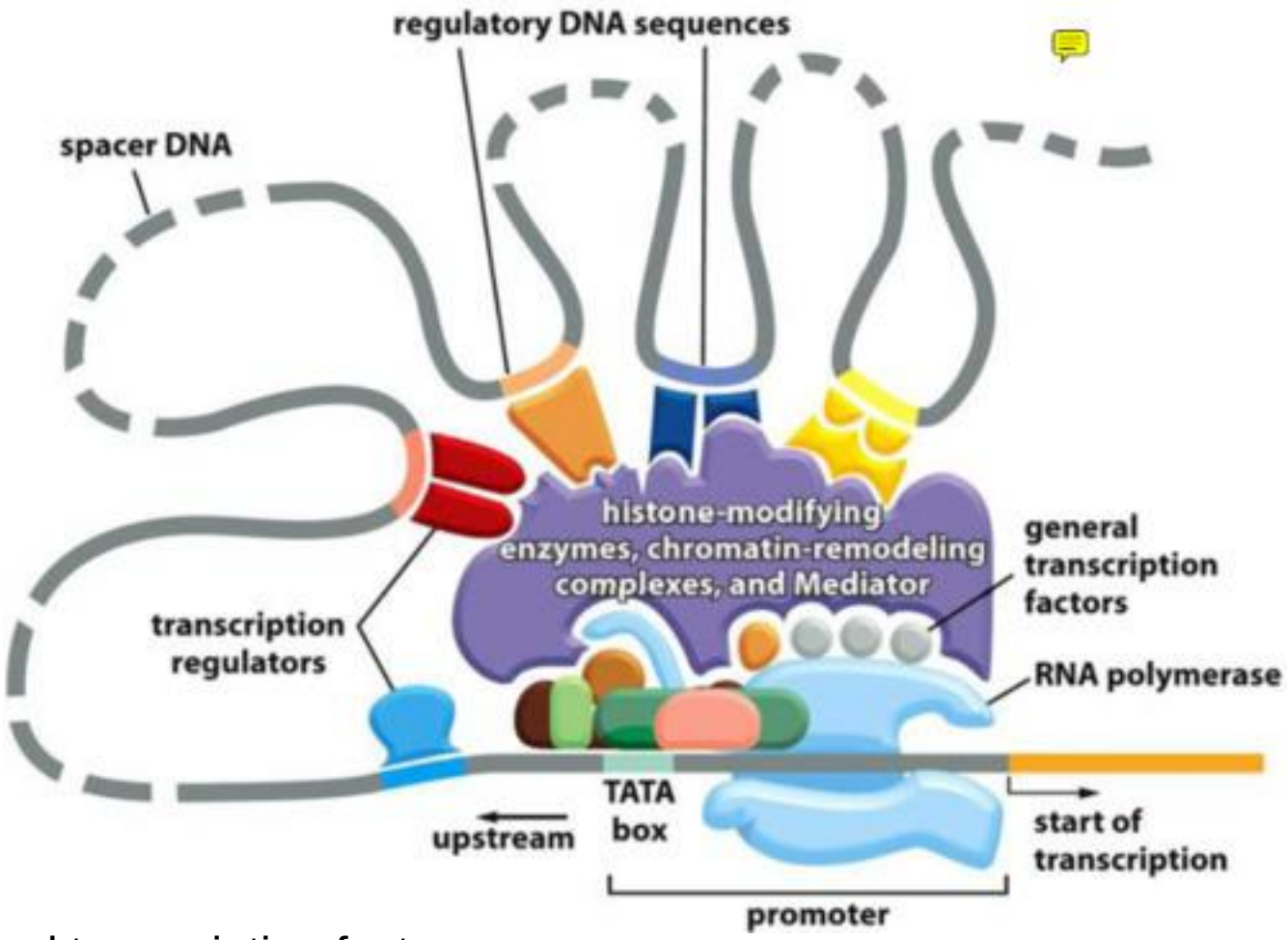

RNA
DNA

# Transcription by Eucariotic RNA polymerase II



# Many elements form the transcription initiation complex




1) general transcription factors
2) transcription regulators
3) RNA polymerase

# Eukaryotic Cells Have Three Kinds of Nuclear RNA Polymerases

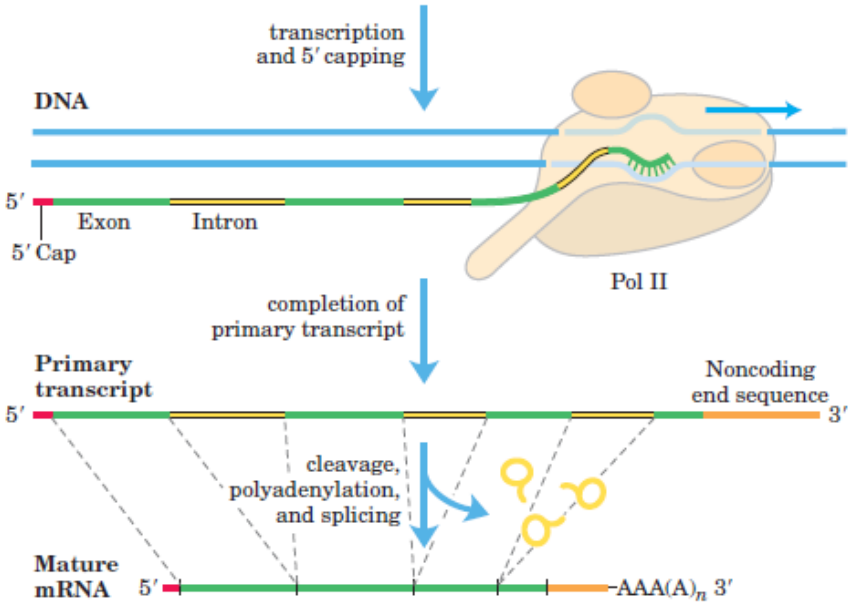
- **RNA polymerase I** (Pol I) – preribosomal RNA (**pre-rRNA**), for the 28S, 18S and 5.8S rRNAs
- **RNA polymerase II** (Pol II) – pre-mesenger RNA (**pre-mRNA**) and some specialized RNAs
- **RNA polymerase III** (Pol III) makes **tRNAs**, the 5S rRNA, and some other specialized RNAs

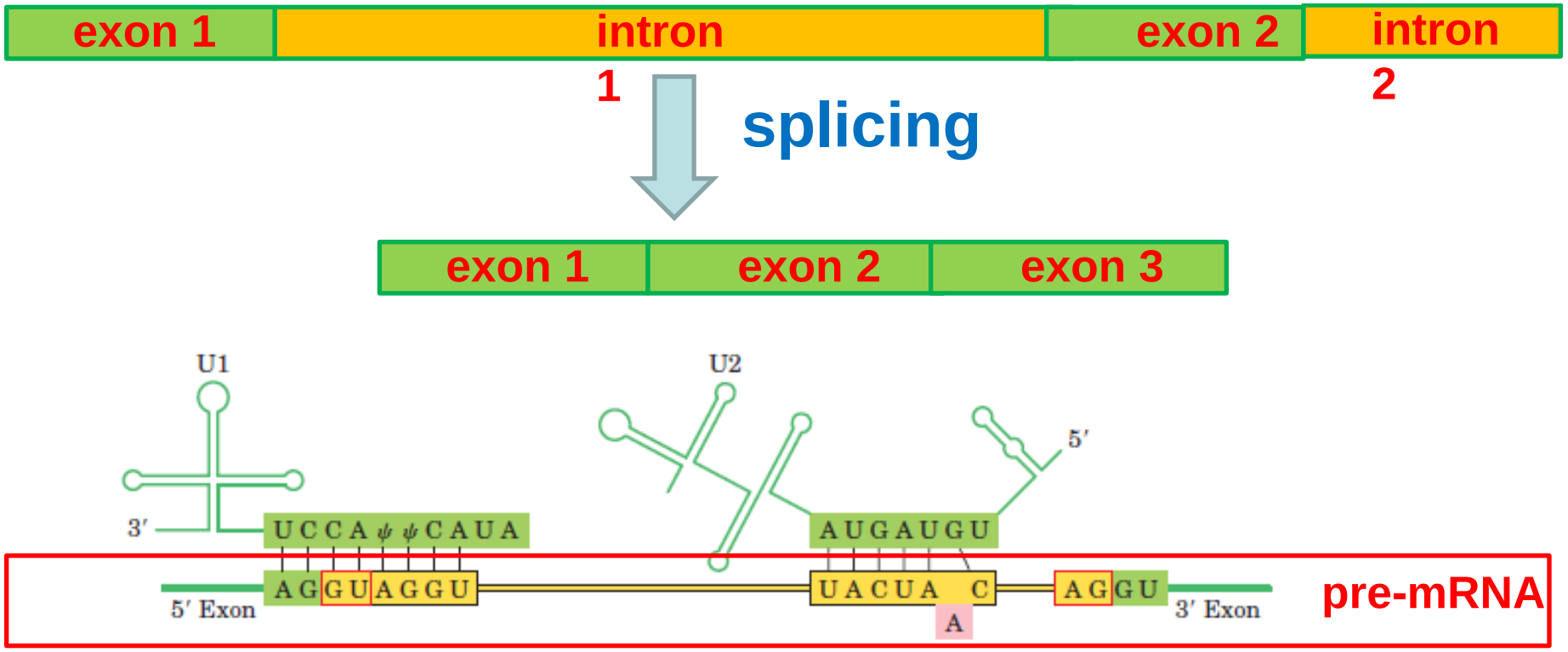
# Posttranscriptional modifications of Eucariotic pre-mRNA

1. Adding of **5’-end cap** to growing pre-mRNA
2. **Splicing** = removing of introns from pre-mRNA
3. **Polyadenylation of 3’-end** of mRNA

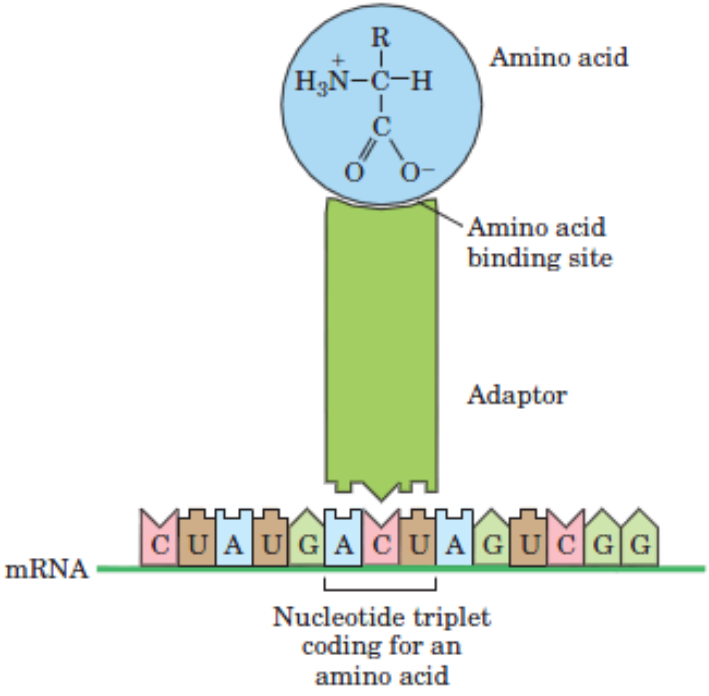
**All these proceses are co-transcriptional**

# Majority of Eucariotic pre-mRNA undergo splicing



# An initiation codon marks the start of an mRNA message




Start – initiation codon

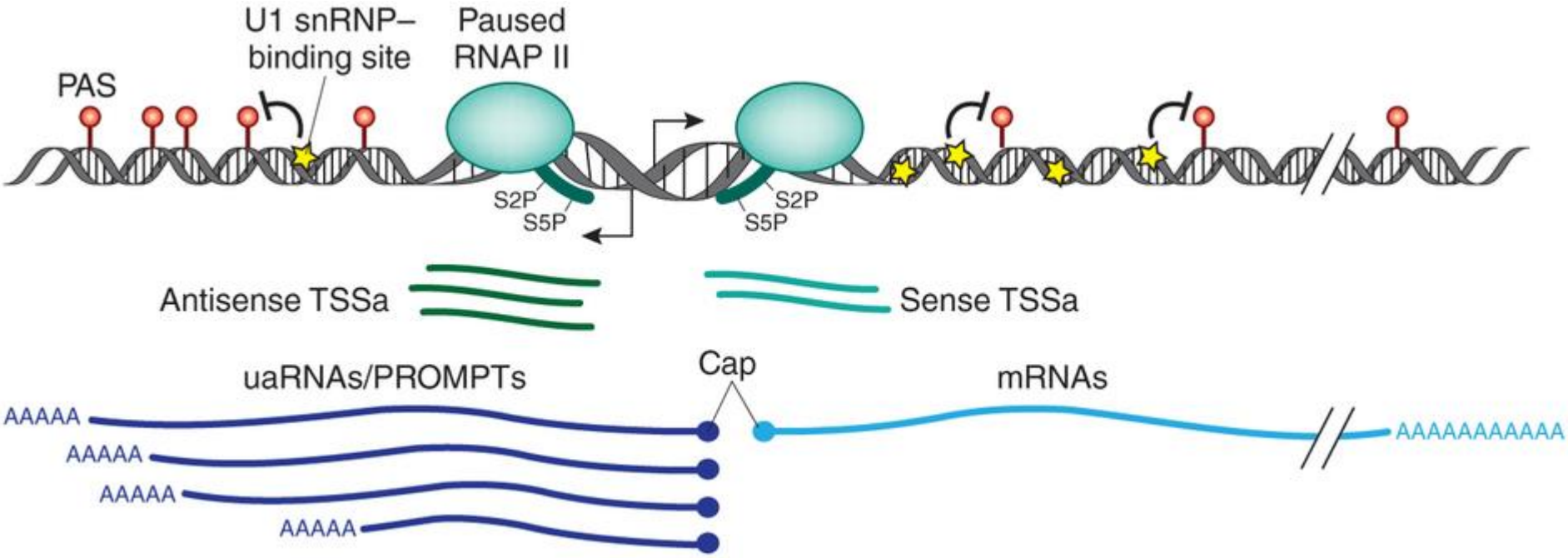
Open Reading Frame (ORF)

End

5'UTR

UTR – untranslated region

3'UTR


U1 snRNP–
binding site
Paused
RNAP II
PAS
S2P
S5P
S2P
S5P
Antisense TSSa
Sense TSSa
uaRNAs/PROMPTs
Cap
mRNAs
AAAAA
AAAAA
AAAAA
AAAAA
AAAAAAAAAAA

**RNA would be either**

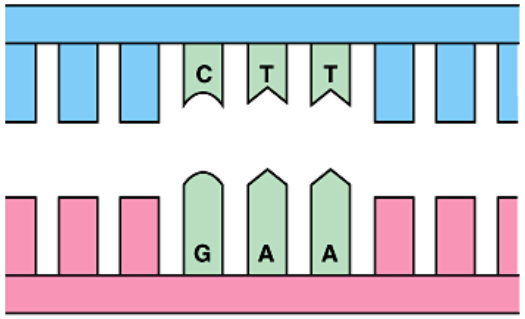
**carrier of mutation between DNA and protein**

**or**

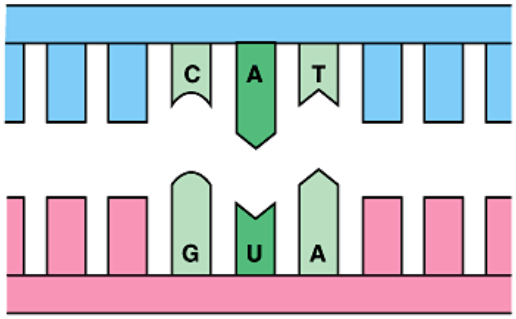
**direct cause of disease**

# Mutacje DNA mogą powodować ciężkie choroby genetyczne - *anemia sierpowata* (ang. sickle cell anemia)

**Normalny DNA hemoglobiny**

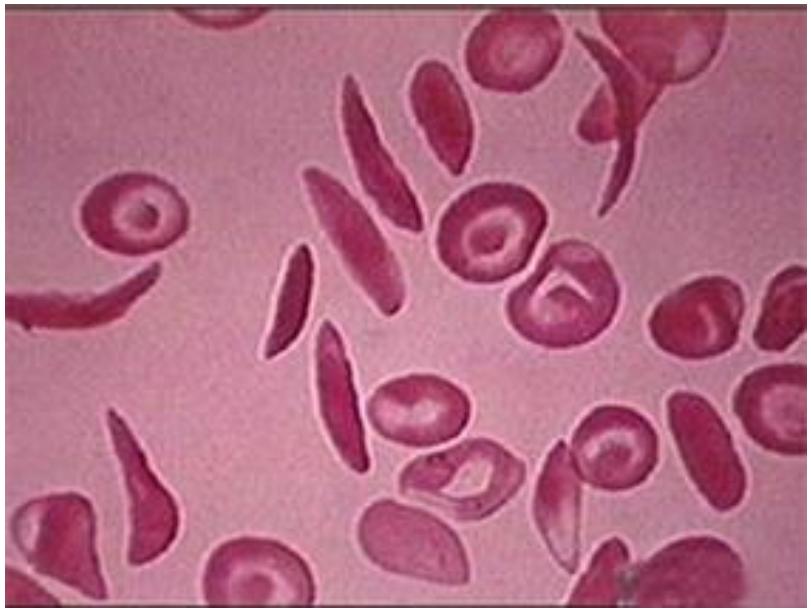



**Zmutowany DNA hemoglobiny**

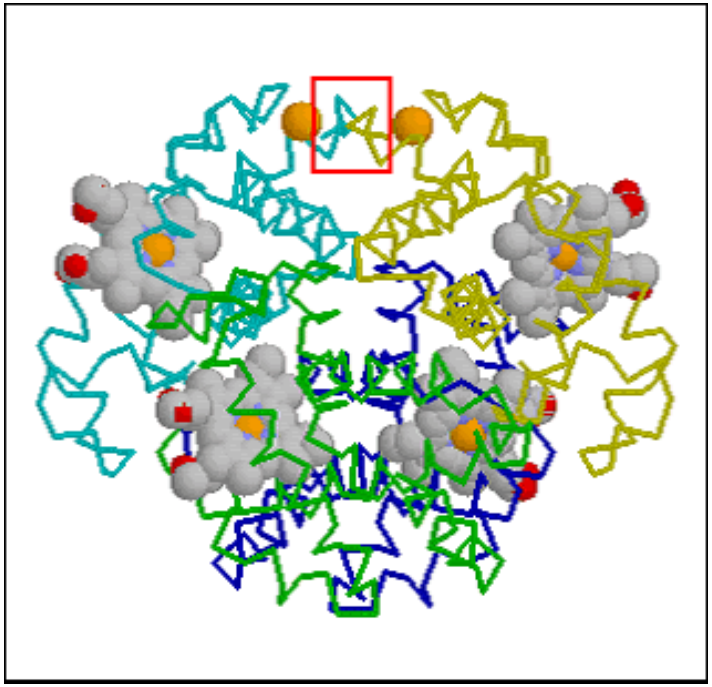



**Normalna hemoglobina**

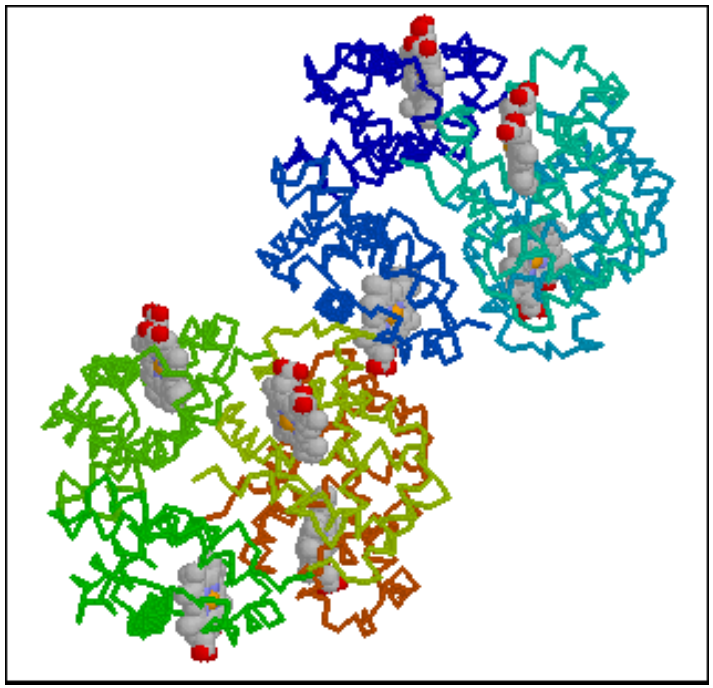

**Hemoglobina w anemii sierpowatej**

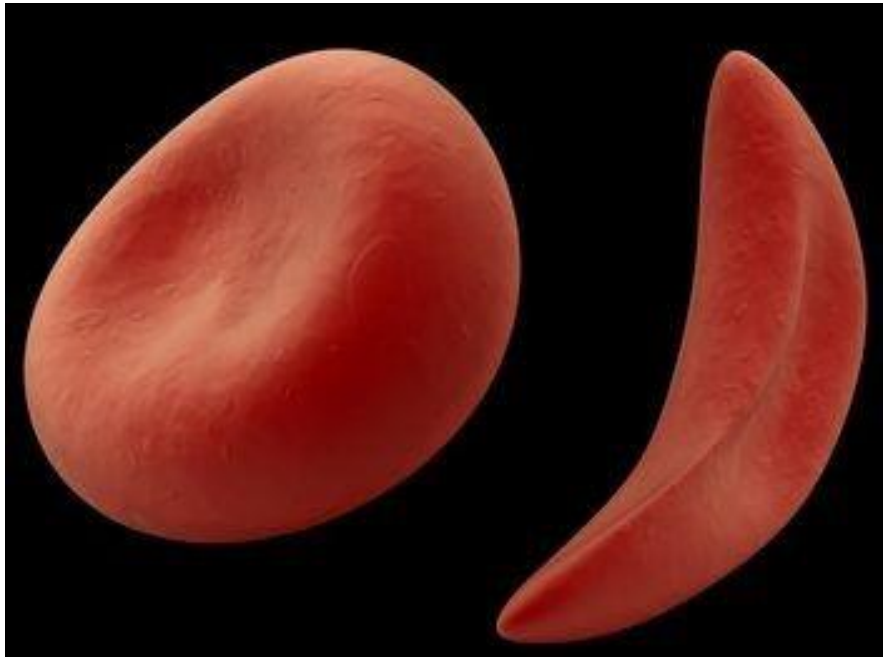



**normalna hemoglobina**



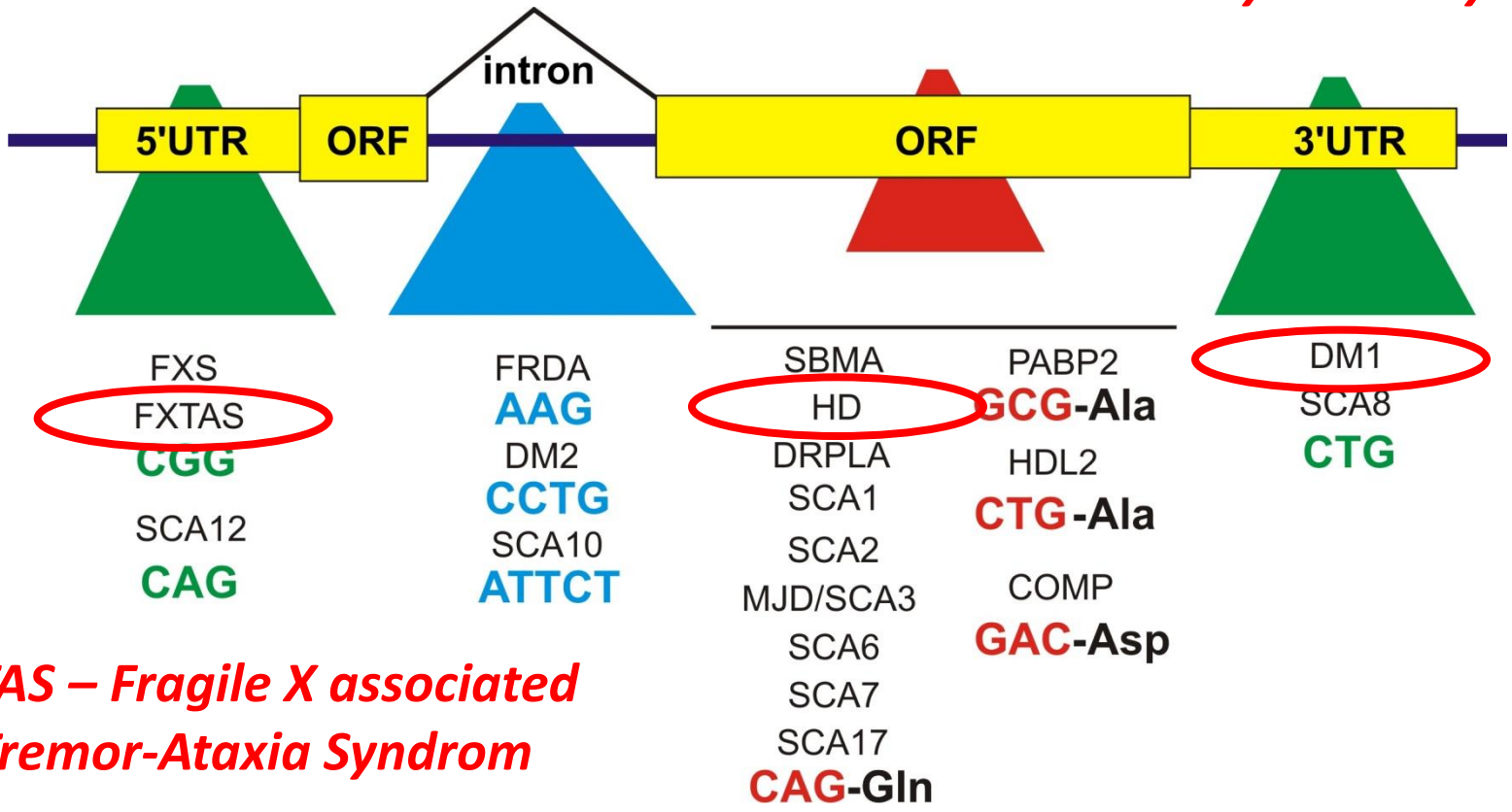
**zmutowana hemoglobina**





# Triplet Repeat Expansion Diseases (TREDs)

***DM1 – Myotonic Dystrophy***



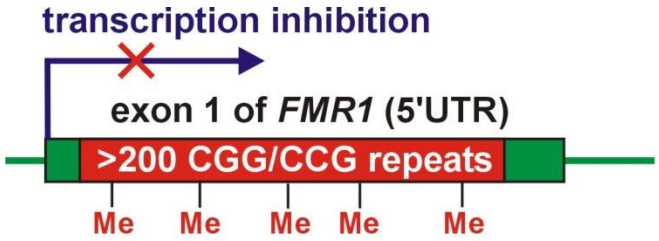

***FXTAS – Fragile X associated Tremor-Ataxia Syndrom***

***HD – Huntington Disease***

Sobczak *et al. Nucleic Acids Research 2012*

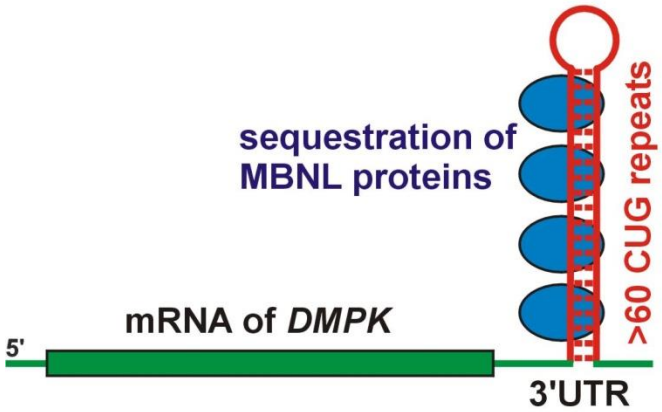
# Three main pathomechanisms of TREDs

1.




**FXS** (recessive disease)
***loss of gene function***

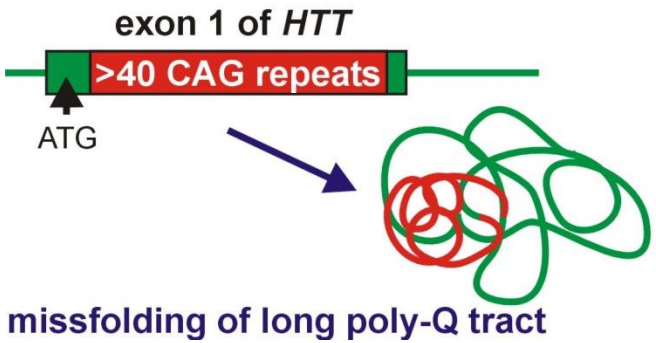
2.




**DM1** (dominant disease)
also SCA8, DM2, HDL2, FXTAS
***gain of RNA function***

***DM1 & FXTAS***

3.

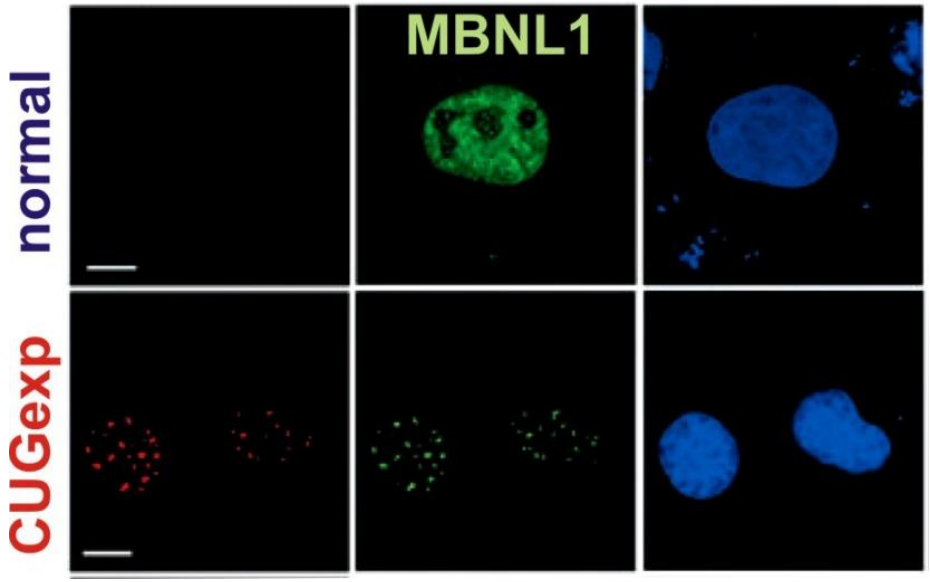



**HD** (dominant disease)
also SCAs, DRPLA, SBMA
***gain of protein function***

Kozlowski, Sobczak & Krzyzosiak, *Med. Genet*. 2

# *Myotonic dystrophy (DM)*

Curshmann-Steinert disease




**DM is an autosomal dominant** disease

**Expansion of CTG** repeats in 3'UTR of ***DMPK*** (*chrom. 19*)
**Expansion of CCTG** repeats in intron 1 of ***ZNF9*** (*chrom. 3*)

The most common form of muscular dystrophy in adults

Occurrence 1 per 6-10,000

# *DM1 – Myotonic Dystrophy type 1*

***Normal gene*** *… CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG …*

***Mutated gene*** *… CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG*
*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG*
*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG*
*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG …*

# *DM1 – Myotonic Dystrophy type 1 germline expansion*

***Normal gene*** *… CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG …*

***Mutated gene*** *… CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG*
*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG*
*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG*
*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG*
*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG*
*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG*
*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG*
*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG …*

# *DM1 – Myotonic Dystrophy type 1 somatic expansion*

***Normal gene*** *… CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG …*

***Mutated gene*** *…*

*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG*
*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG*
*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG*
*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG*
*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG*
*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG*
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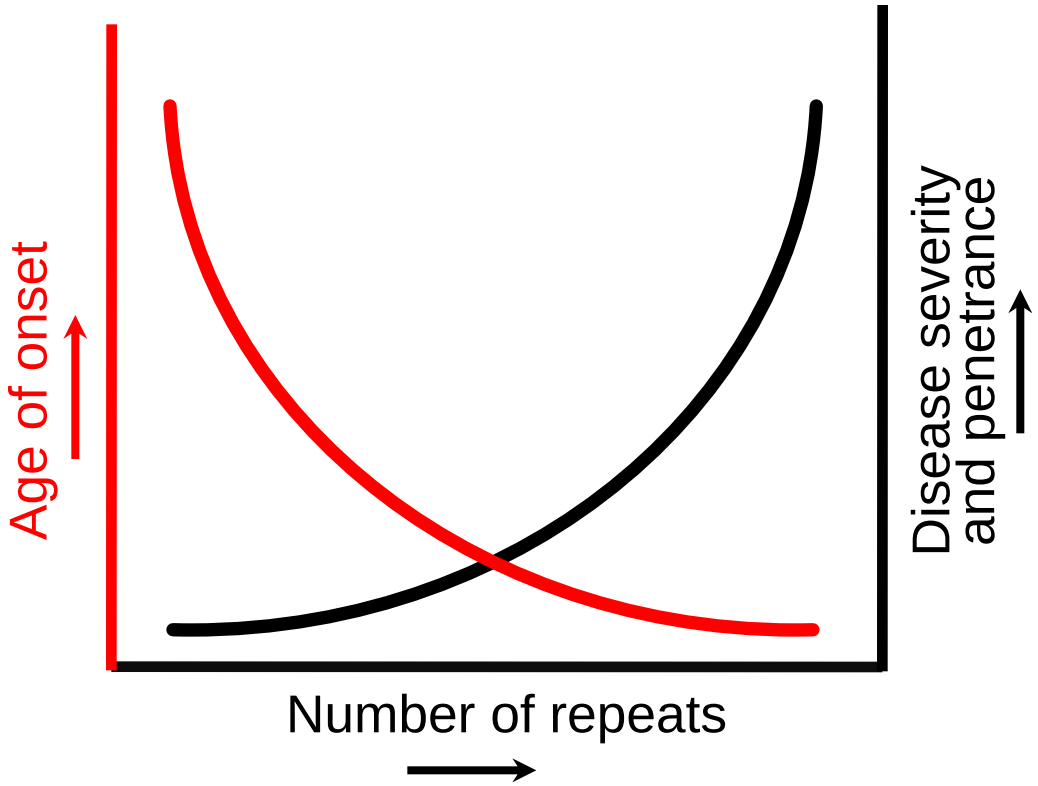
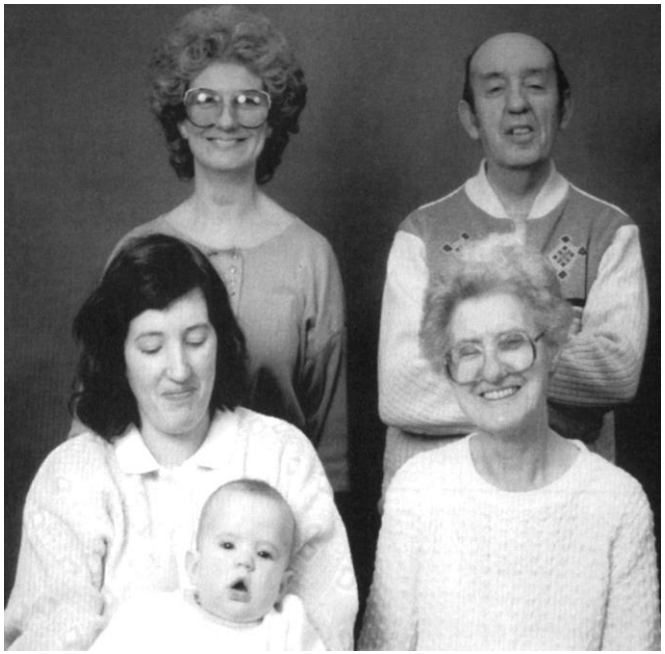
*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG*
*CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG …*

# Genetic anticipation in myotonic dystrophy

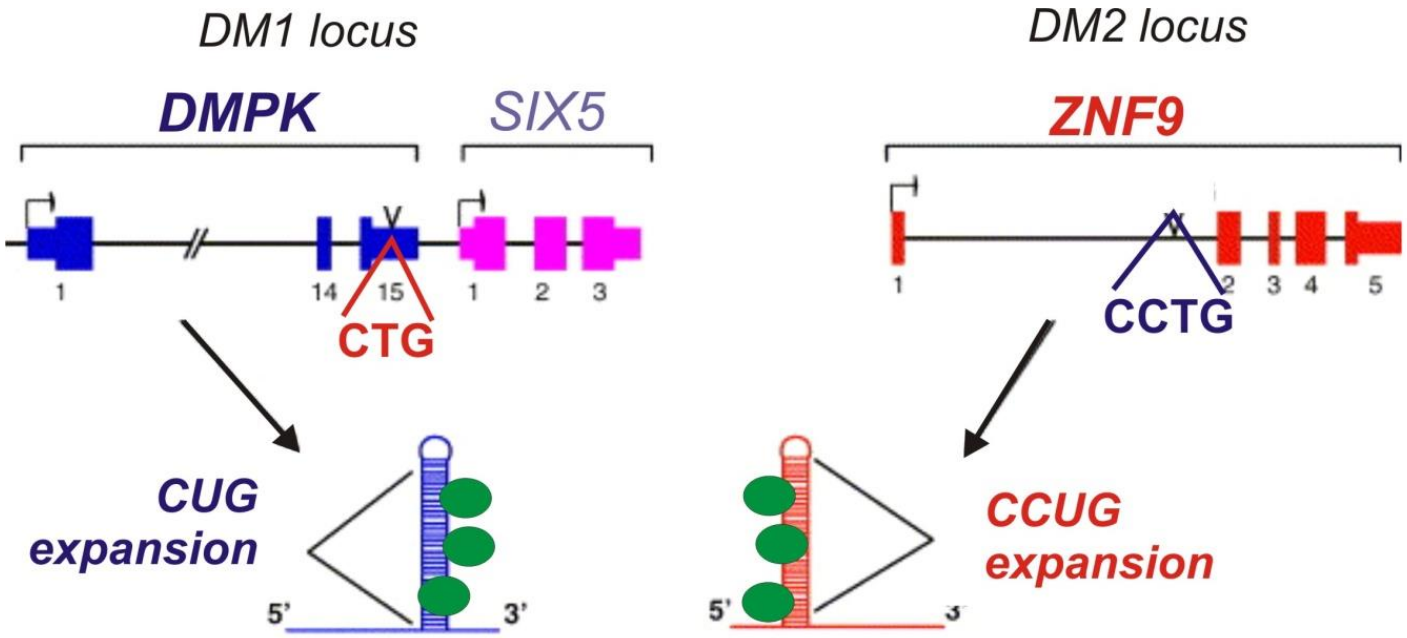


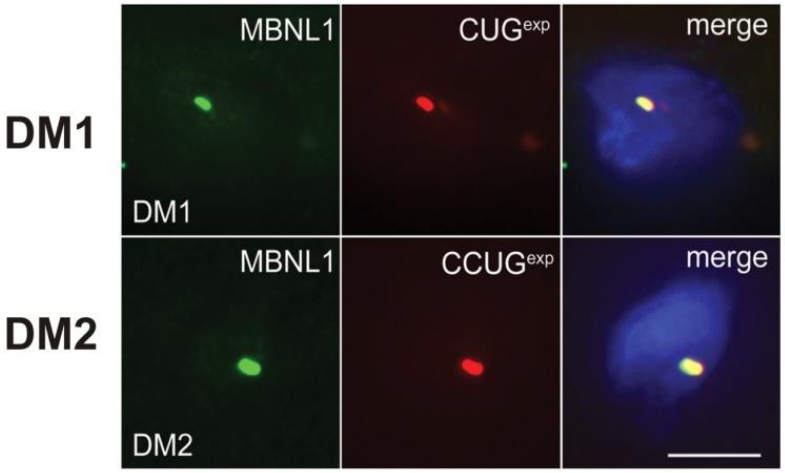

Anticipation in a four-generation family with myotonic dystrophy



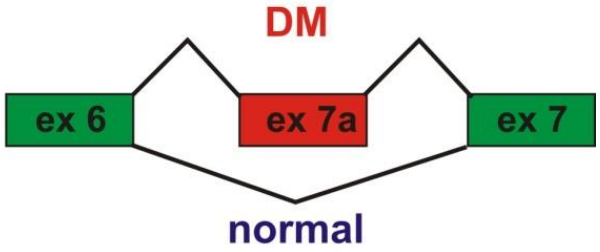
# Pathomechanism of Myotonic Dystrophy (DM)

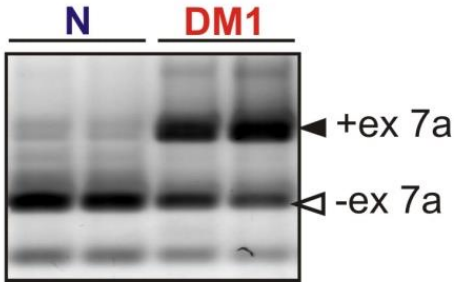
## *- dominant effect of toxic RNA*

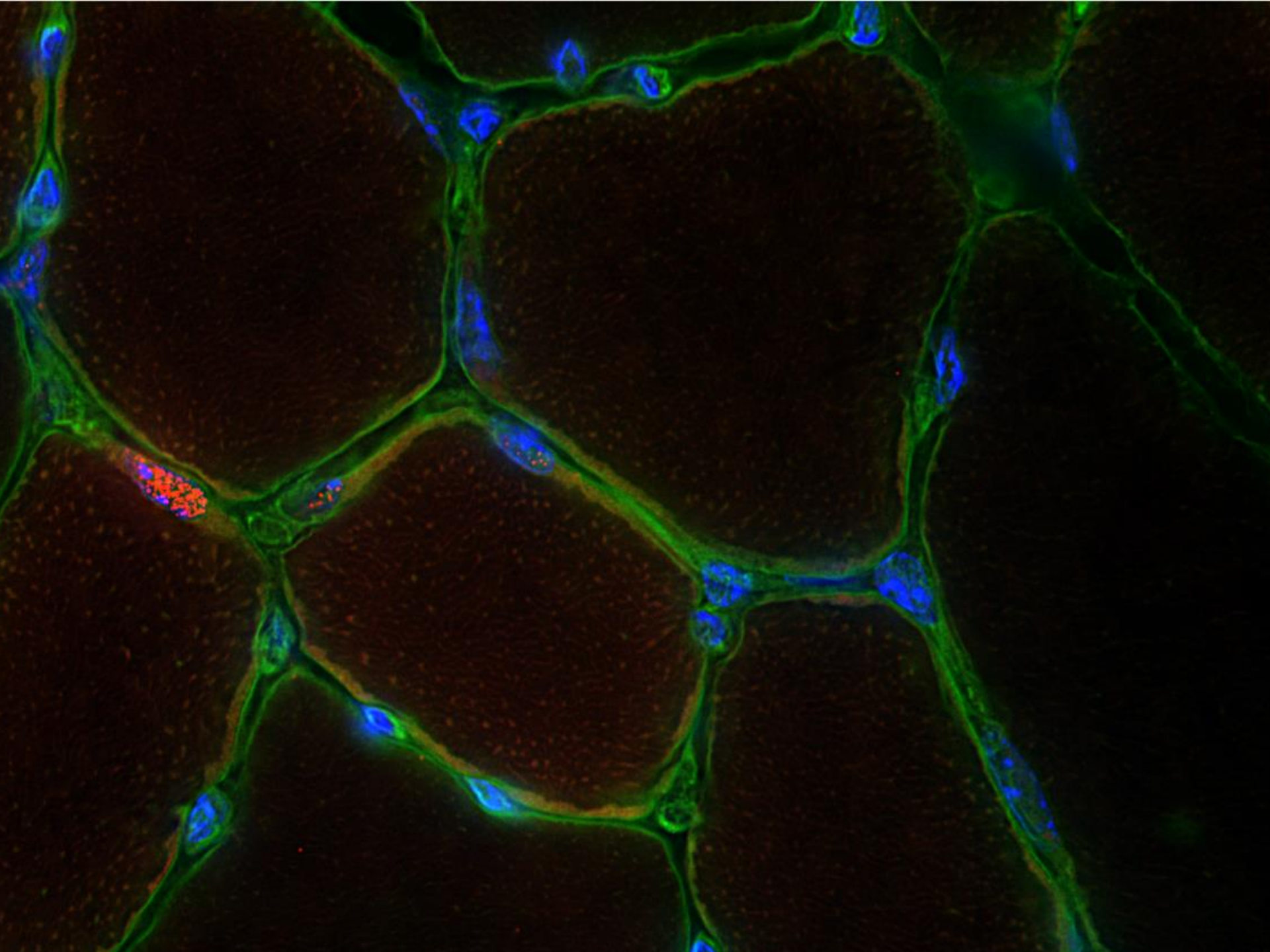



splicing changes

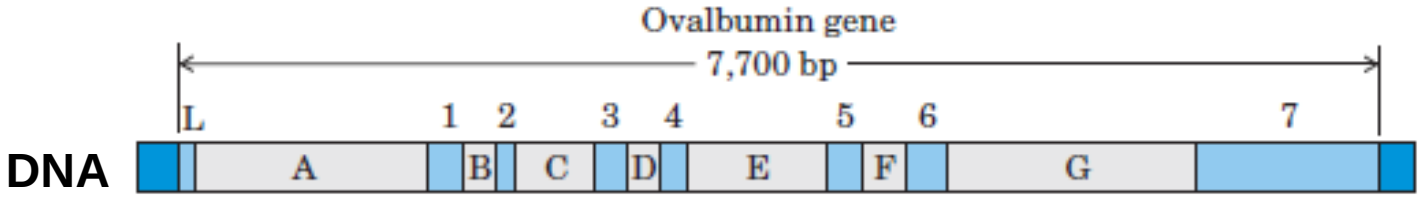




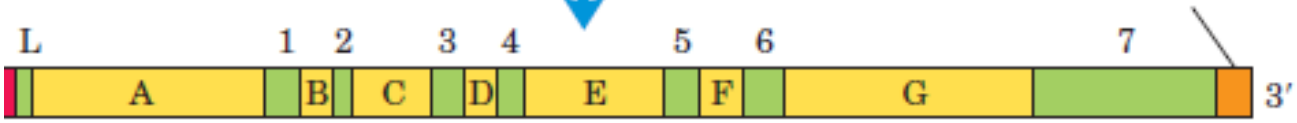
# *Looking for splicing abnormalities in DM1 and DM2*

# from DNA to protein




DNA

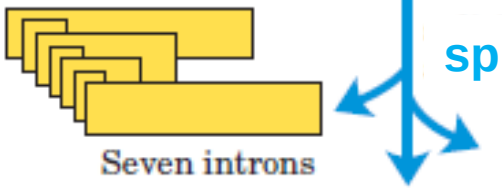
transkrypcja



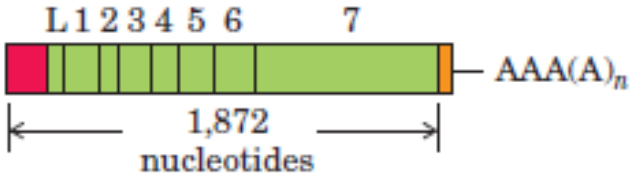

Prekursor-mRNA

introny

splicing



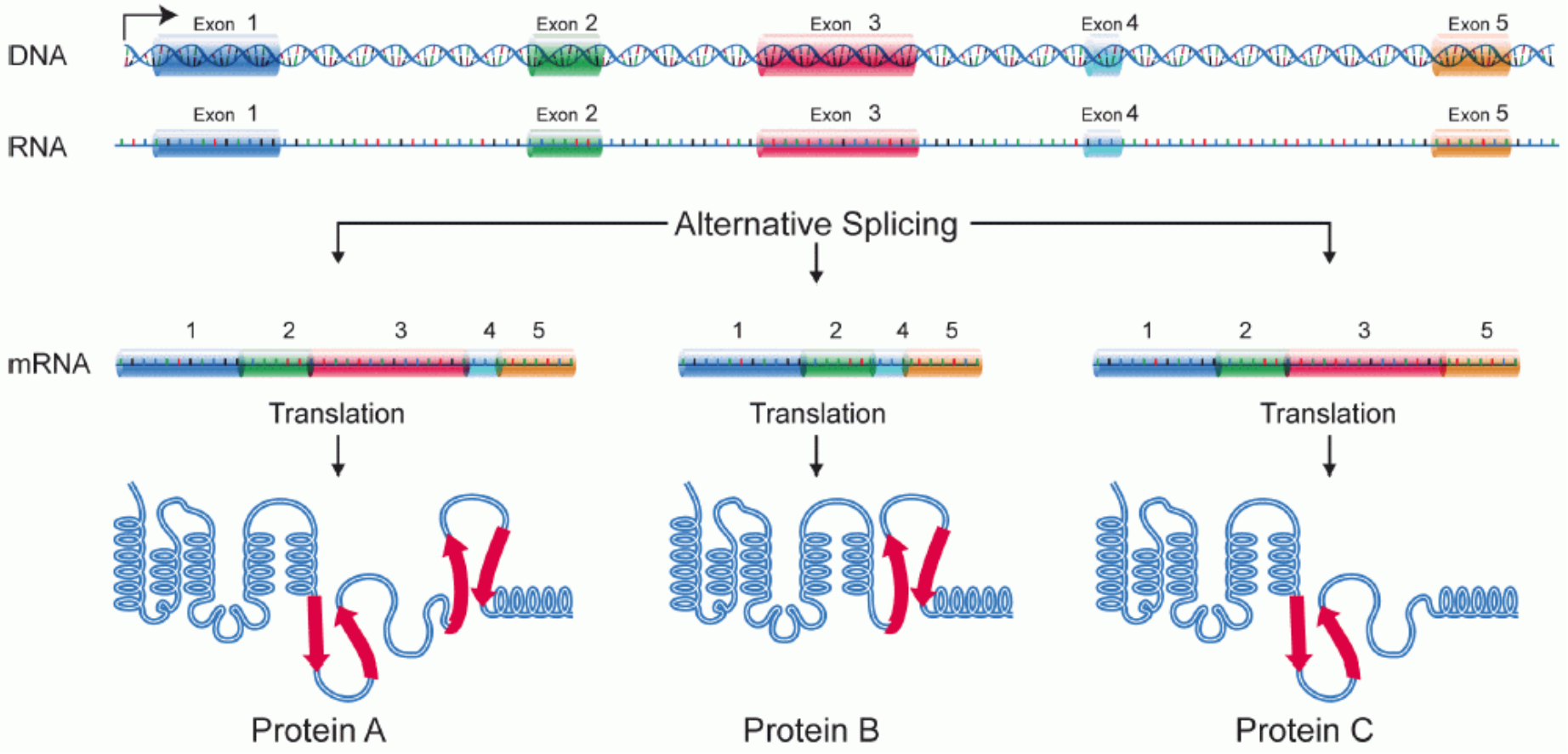

mRNA



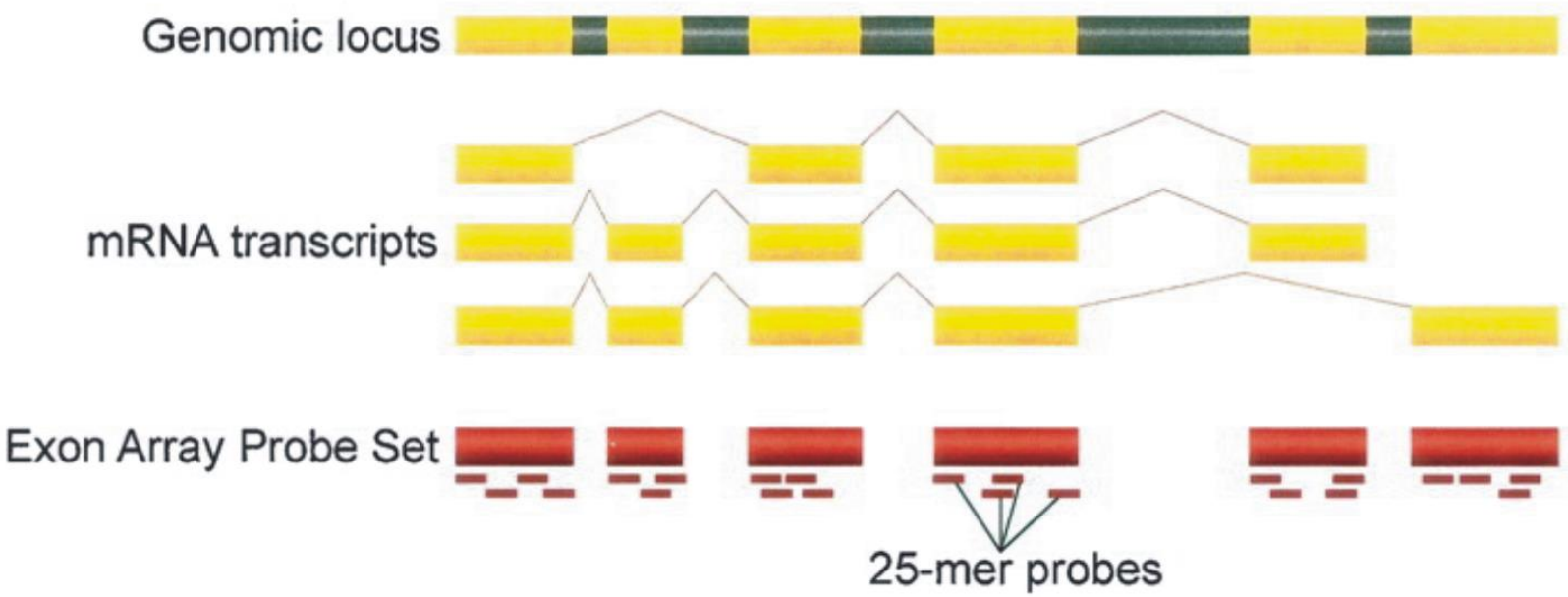

translacja

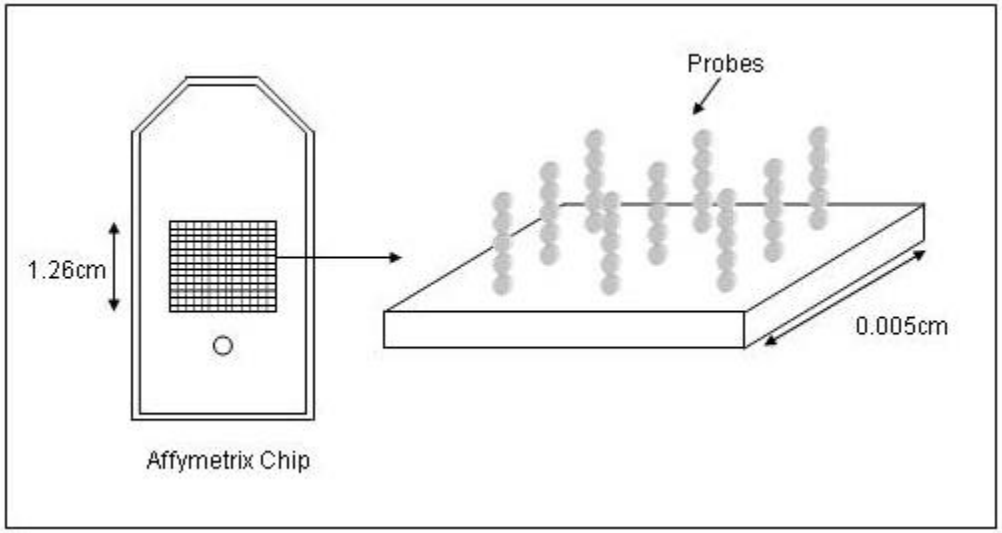
białko

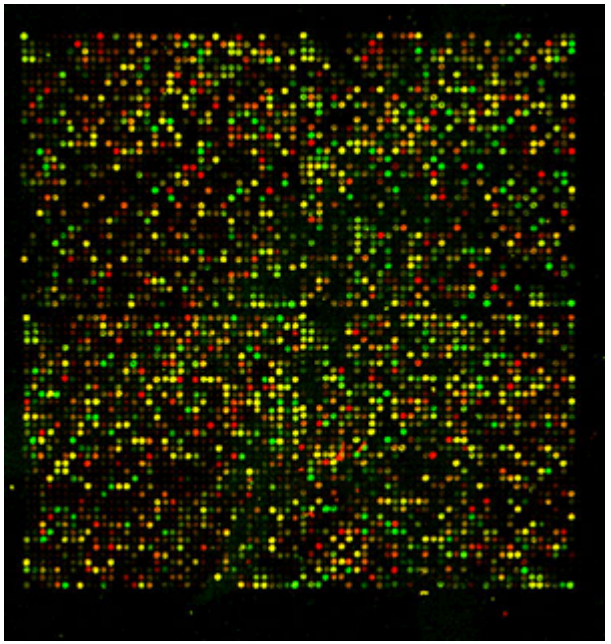
# Alternative splicing = alternative proteins



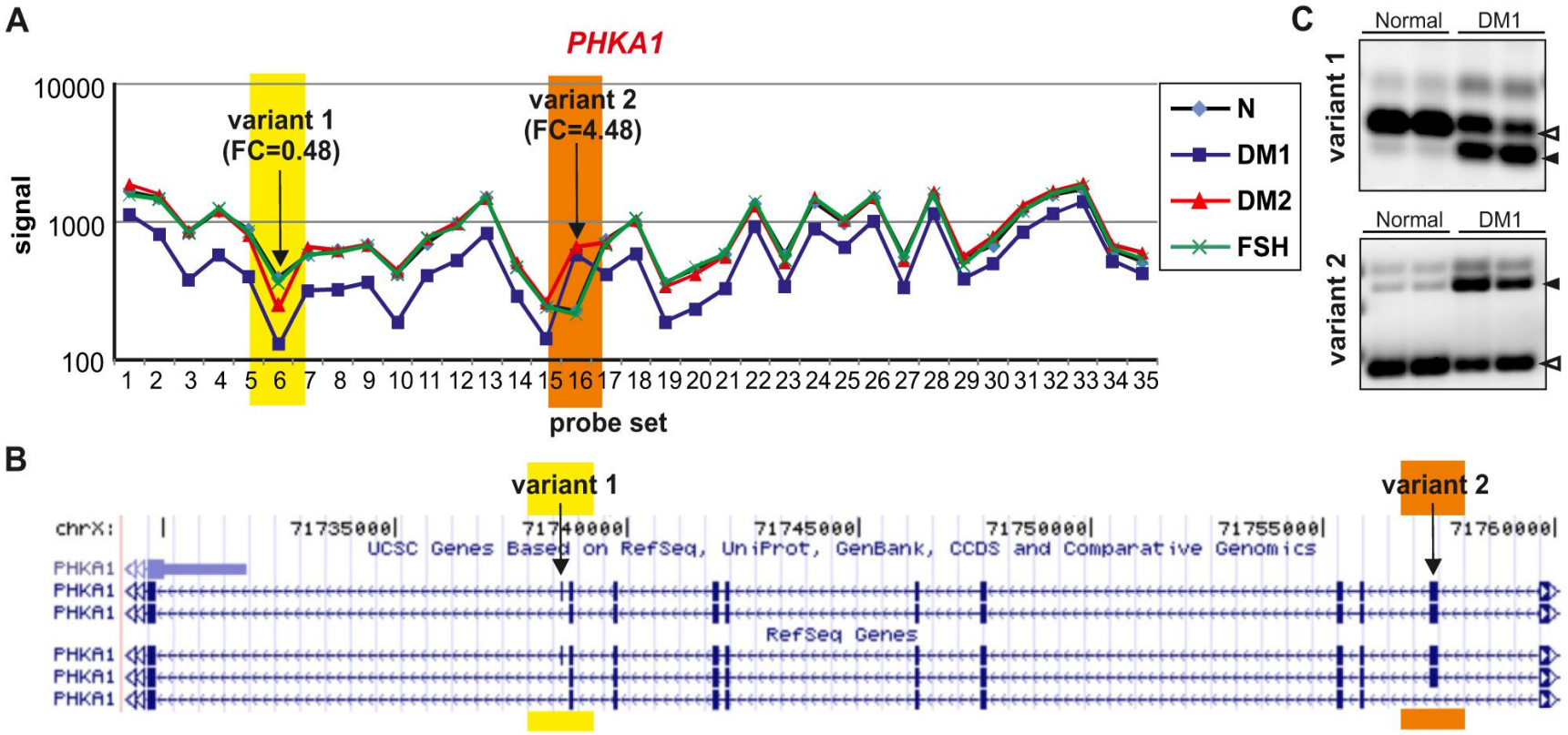
# Affymetrix GeneChip® Human Exon 1.0 ST Arrays



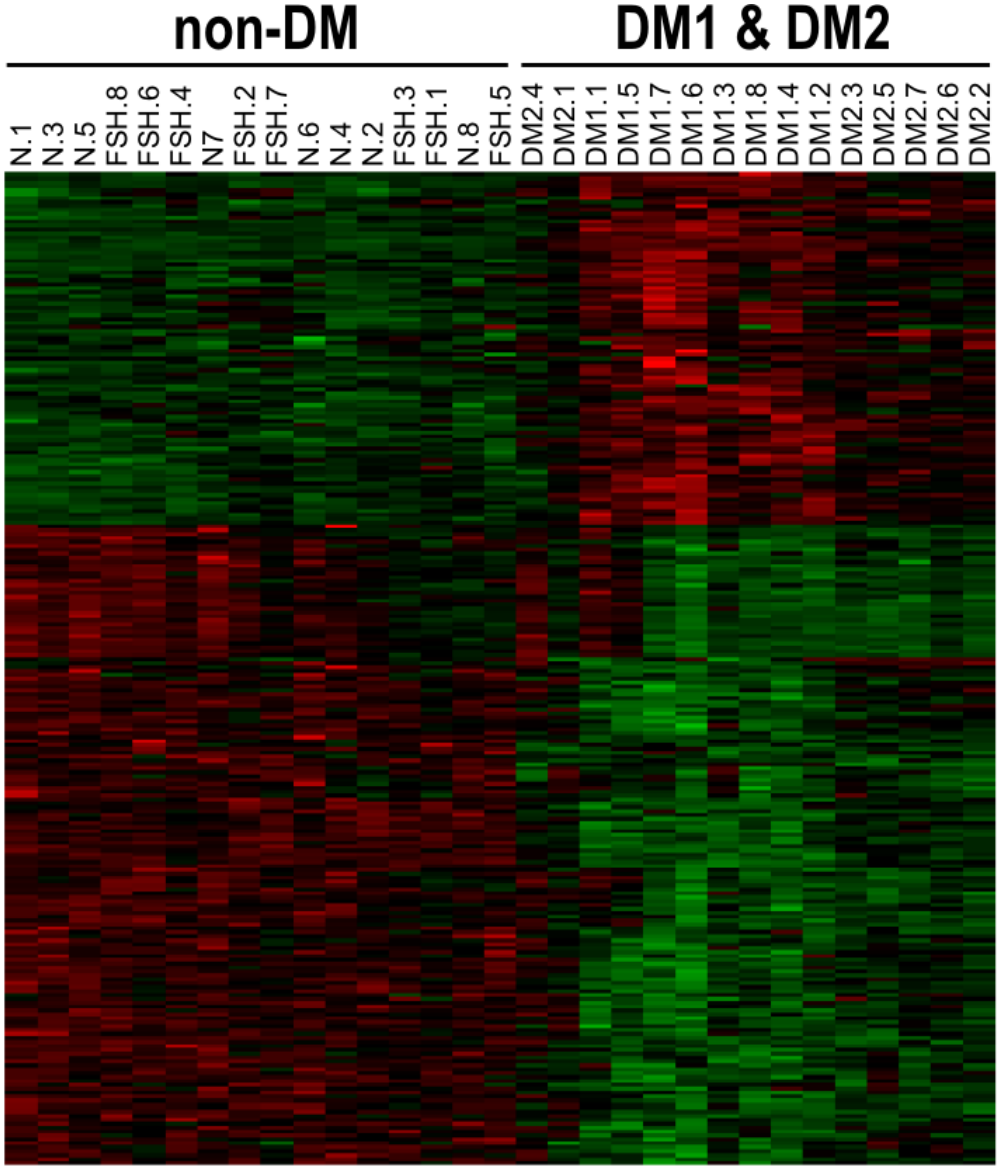

Probes
1.26cm
0.005cm
Affymetrix Chip



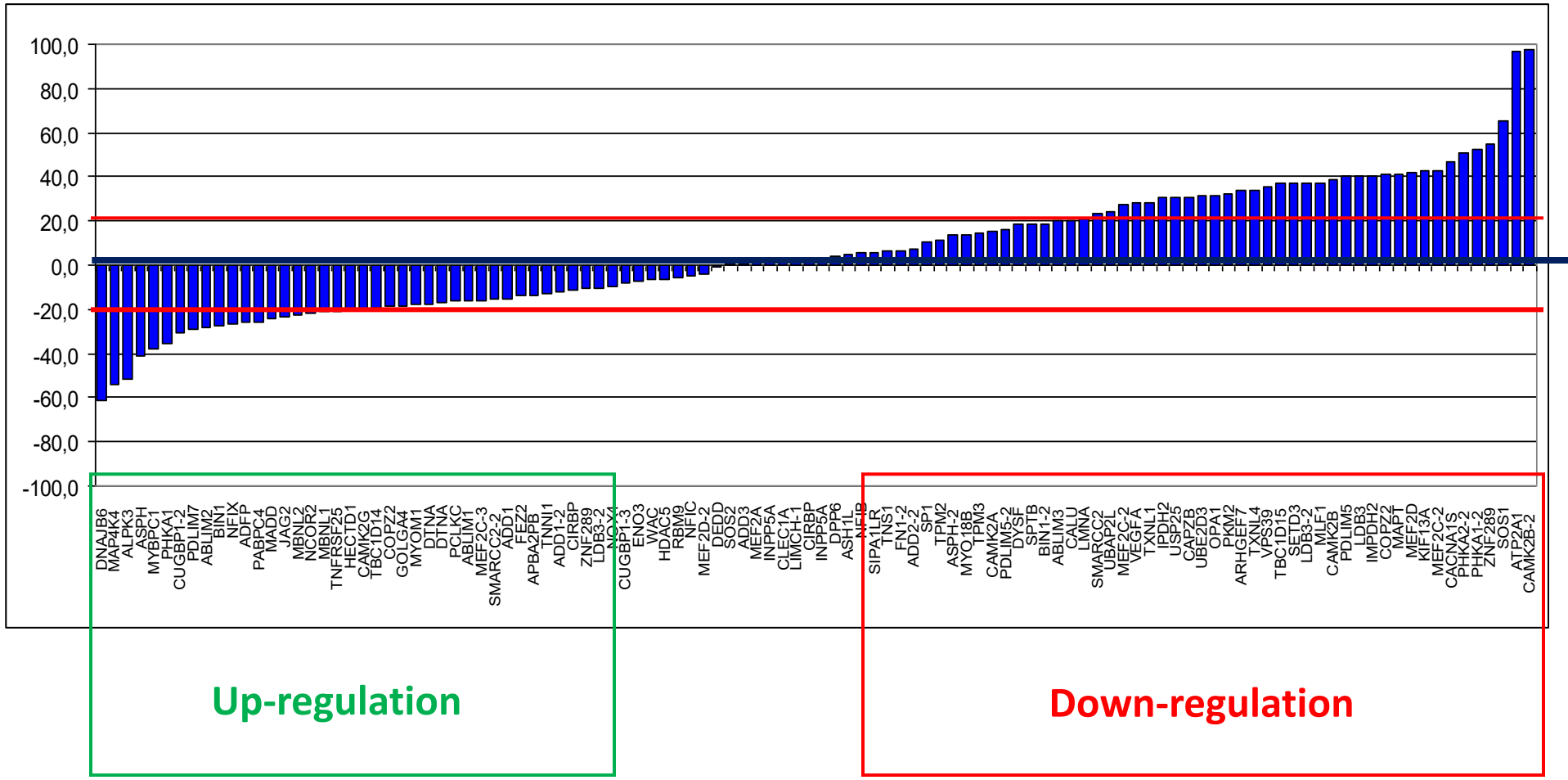
# Several genes have more than one misspliced exon in DM




Nakamori et al. *Ann. Neurology*, 2013


non-DM
DM1 & DM2
N.1
N.3
N.5
FSH.8
FSH.6
FSH.4
N7
FSH.2
FSH.7
N.6
N.4
N.2
FSH.3
FSH.1
N.8
FSH.5
DM2.4
DM2.1
DM1.1
DM1.5
DM1.7
DM1.6
DM1.3
DM1.8
DM1.4
DM1.2
DM2.3
DM2.5
DM2.7
DM2.6
DM2.2

# There are hundreds of abnormally spliced exons in DM
# - global spliceopathy

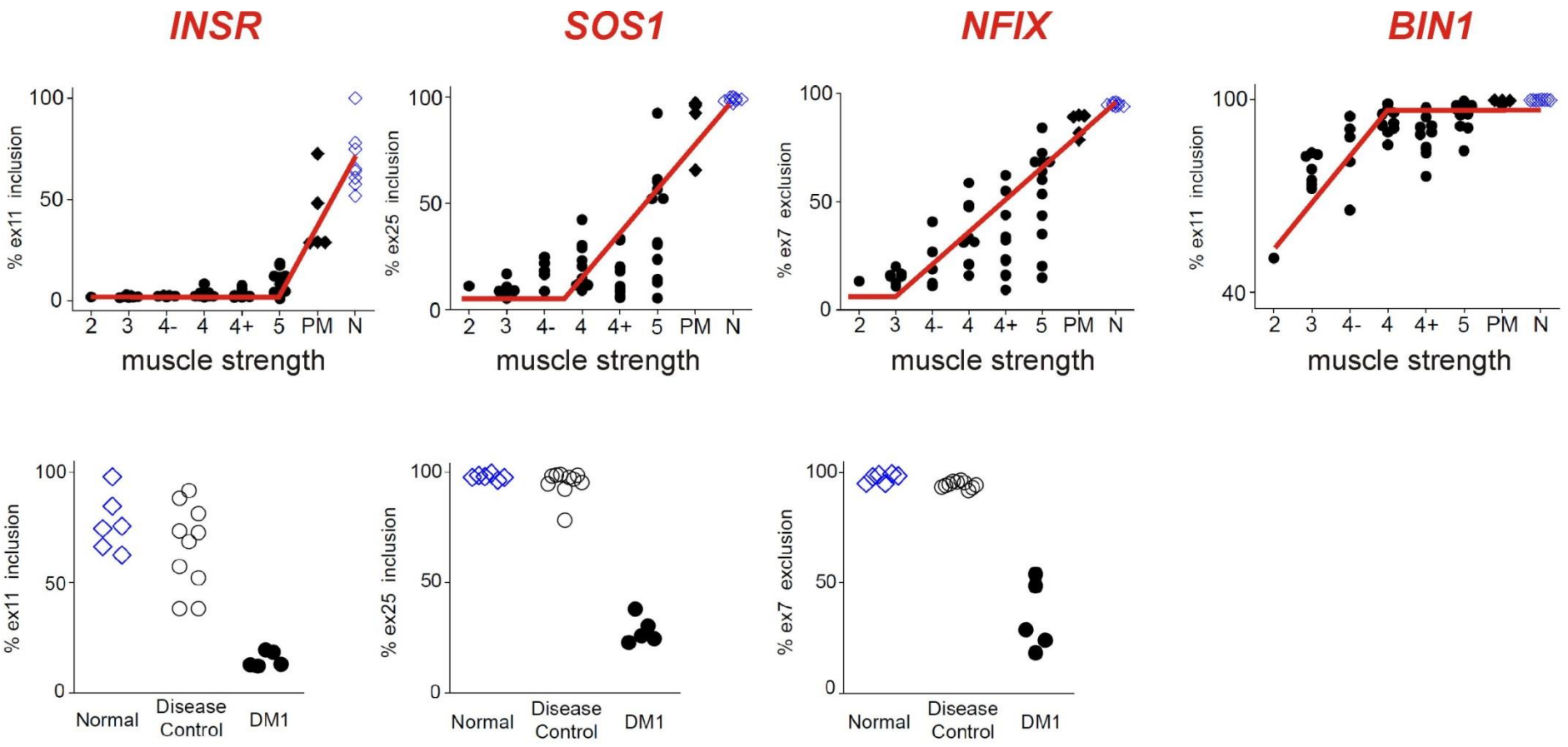



**Only about 50% of ASE shows missplicing in *Mbnl1*-KO mouse**

Nakamori et al. *Ann. Neurology*, 2013

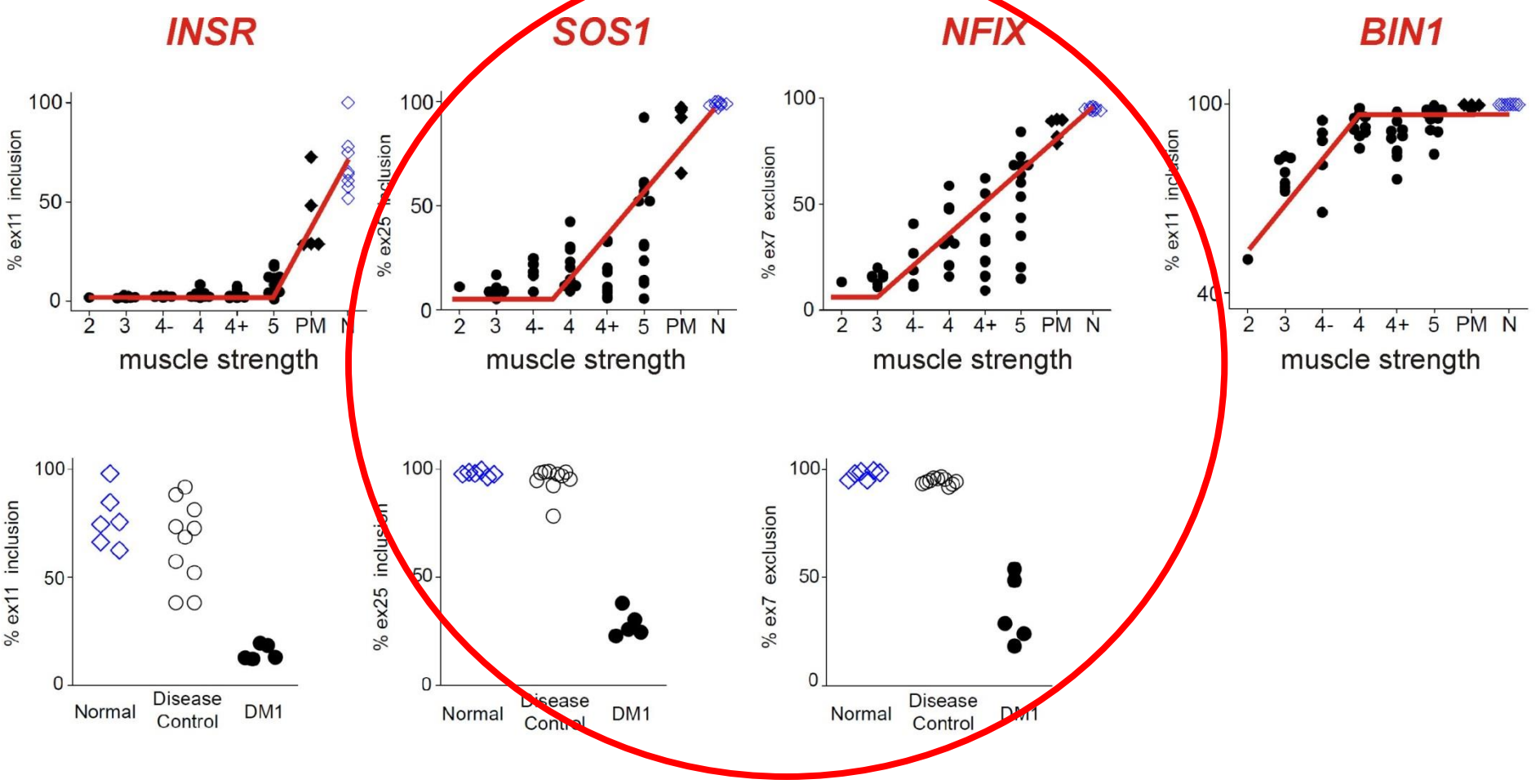
# Looking for DM1-specific biomarkers

## disease severity and therapeutic response




Nakamori et al. *Ann. Neurology*, 2013

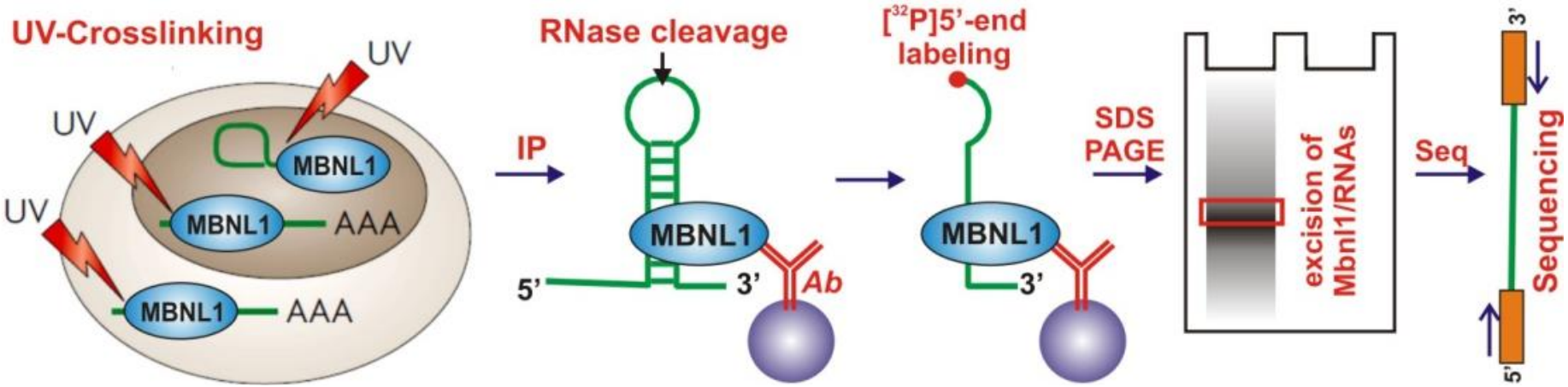
# Looking for DM1-specific biomarkers

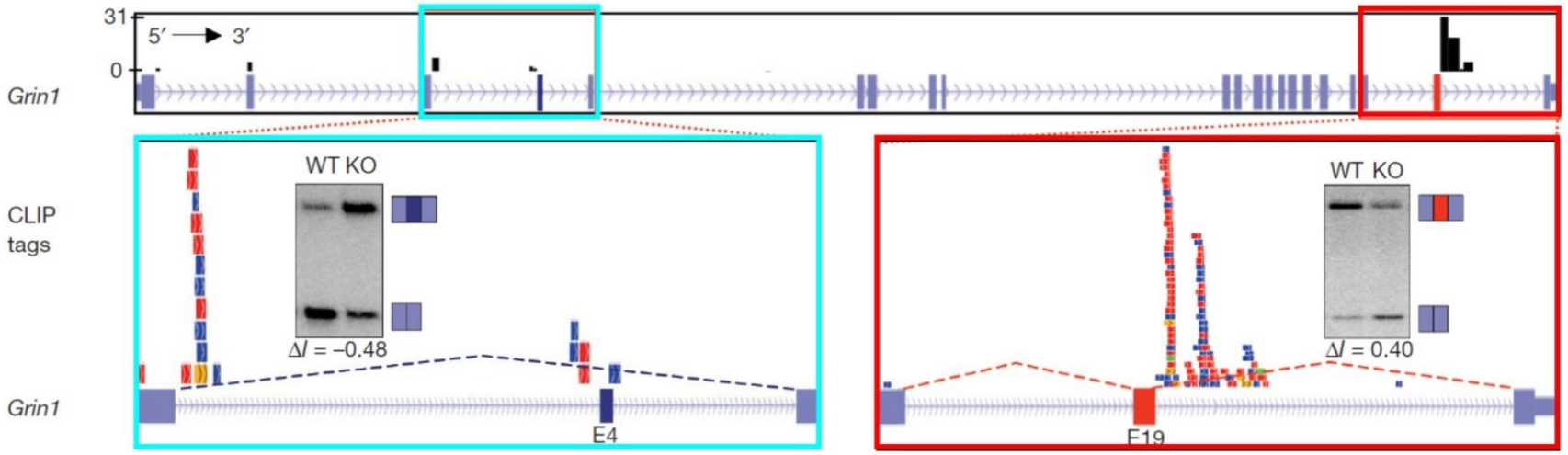



Nakamori et al. *Ann. Neurology*, 2013

# *Looking for natural RNA targets for MBNLs: Transcriptome-based approache*

# - CLIP-Seq




Licatalosi *et al. Nature* 2008

# Sequence motif enrichment

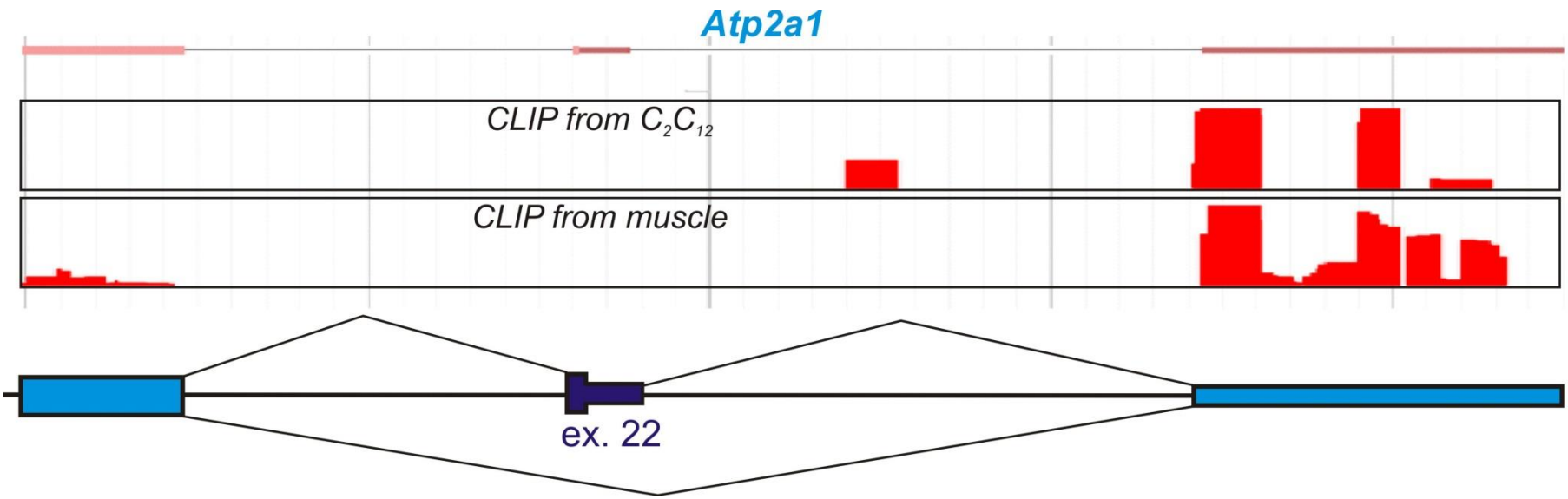
Mbnl1-CLIP

Mbnl2-CLIP

Mbnl3-CLIP

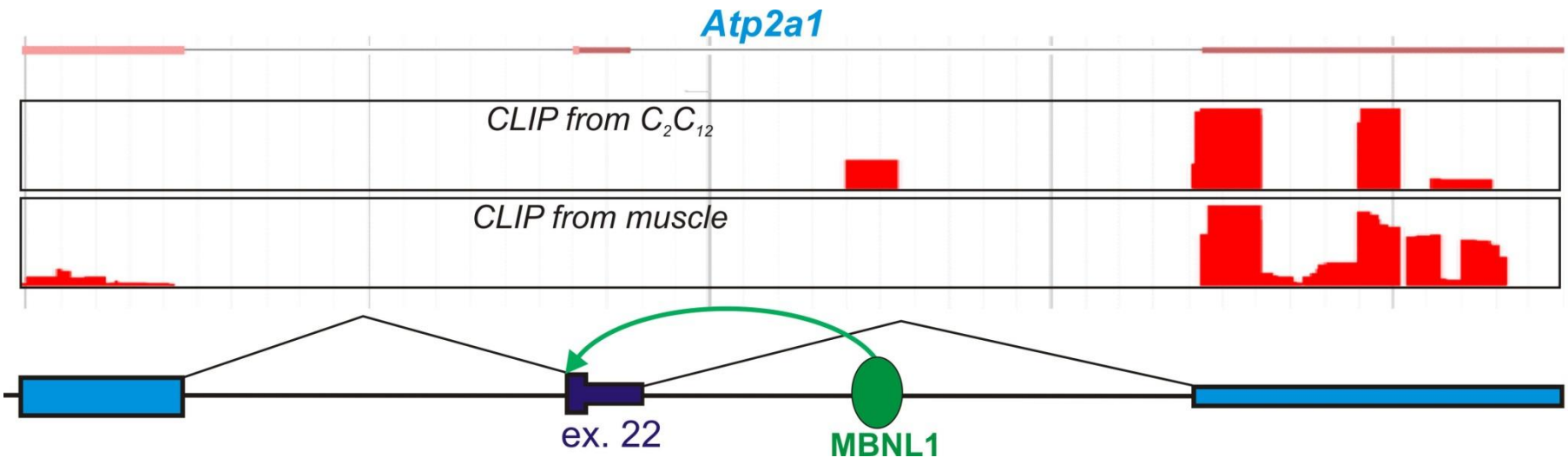
Sznajder *et al. manuscript under review*

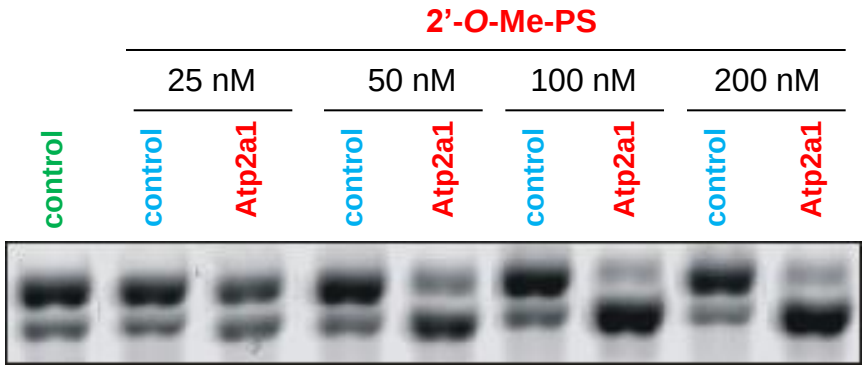
# Antisense oligos can modulate alt. splicing of a single mRNA

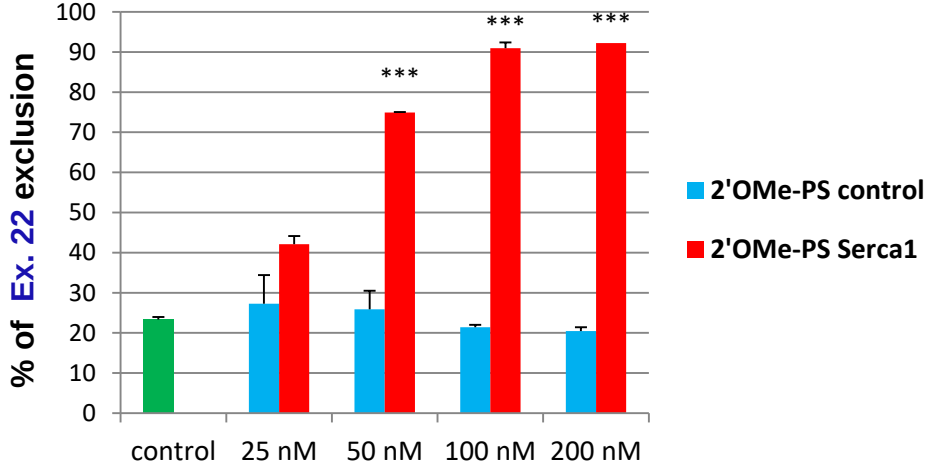



Ł. Sznajder and P. Konieczny - *Unpublished data*

# Antisense oligos can regulate alt. splicing of a single mRNA

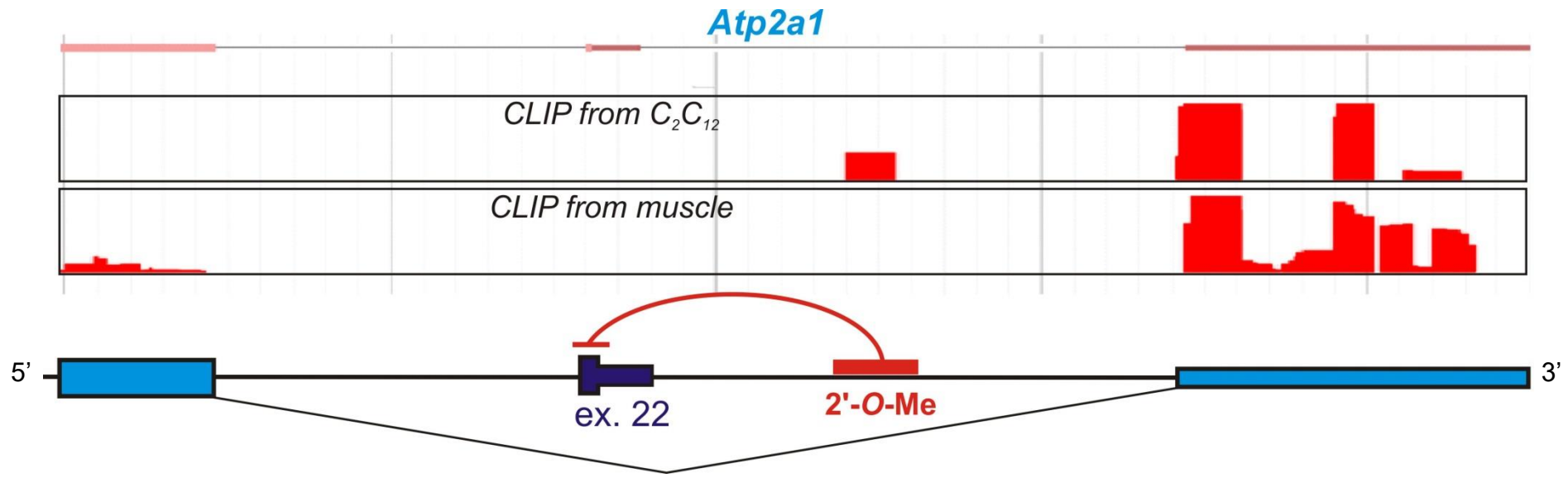


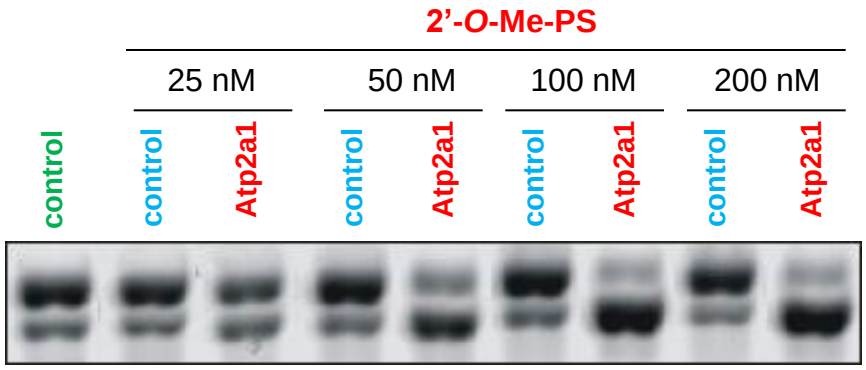



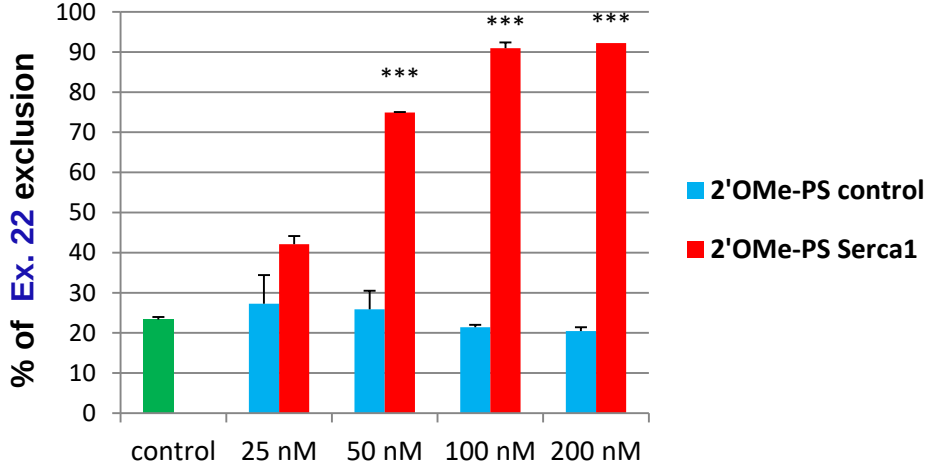




P. Cywoniuk - *Unpublished data*

# Antisense oligos can regulate alt. splicing of a single mRNA



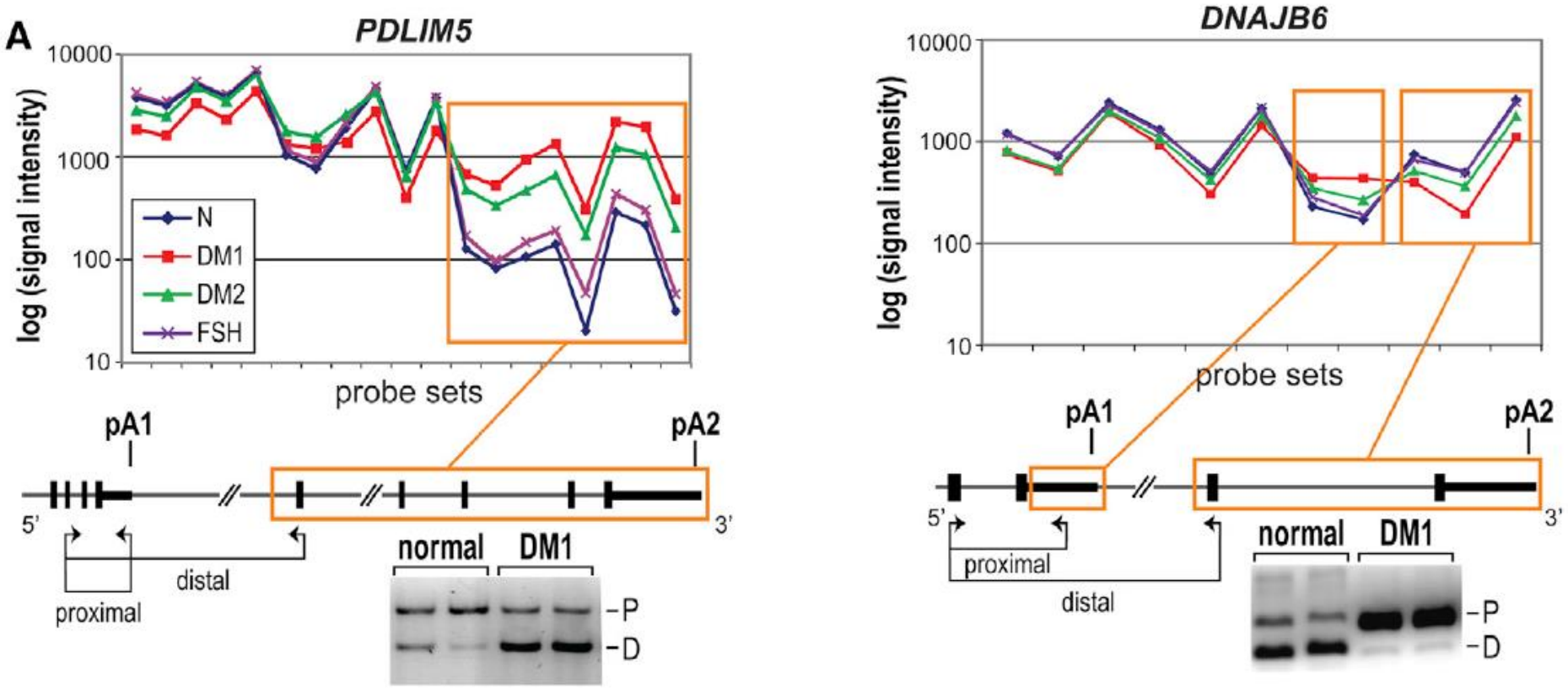




P. Cywoniuk - *unpublished data*

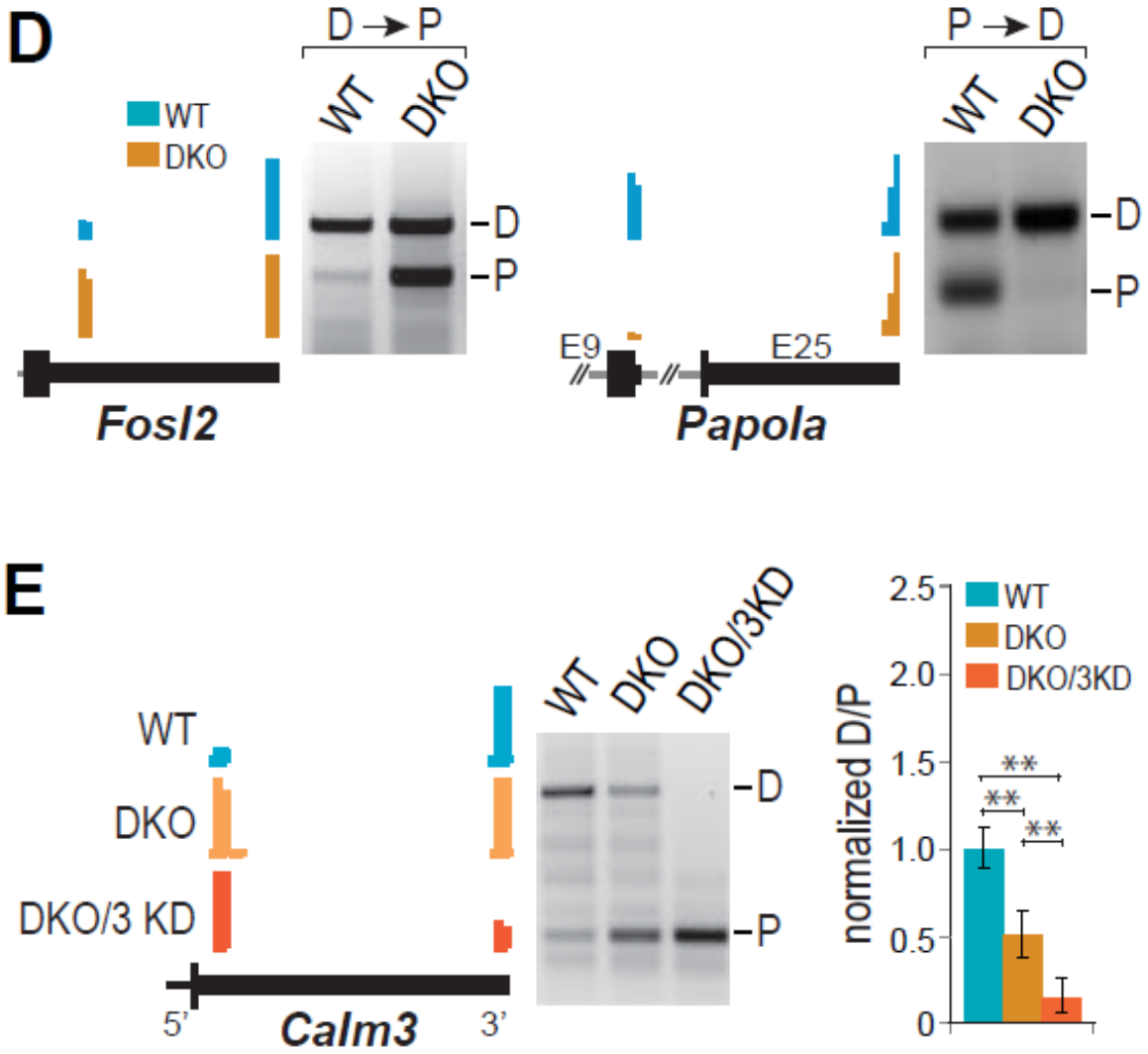
# *Looking for New Functions of MBNLs*

# Many transcripts in DM undergo alternative polyadenylation

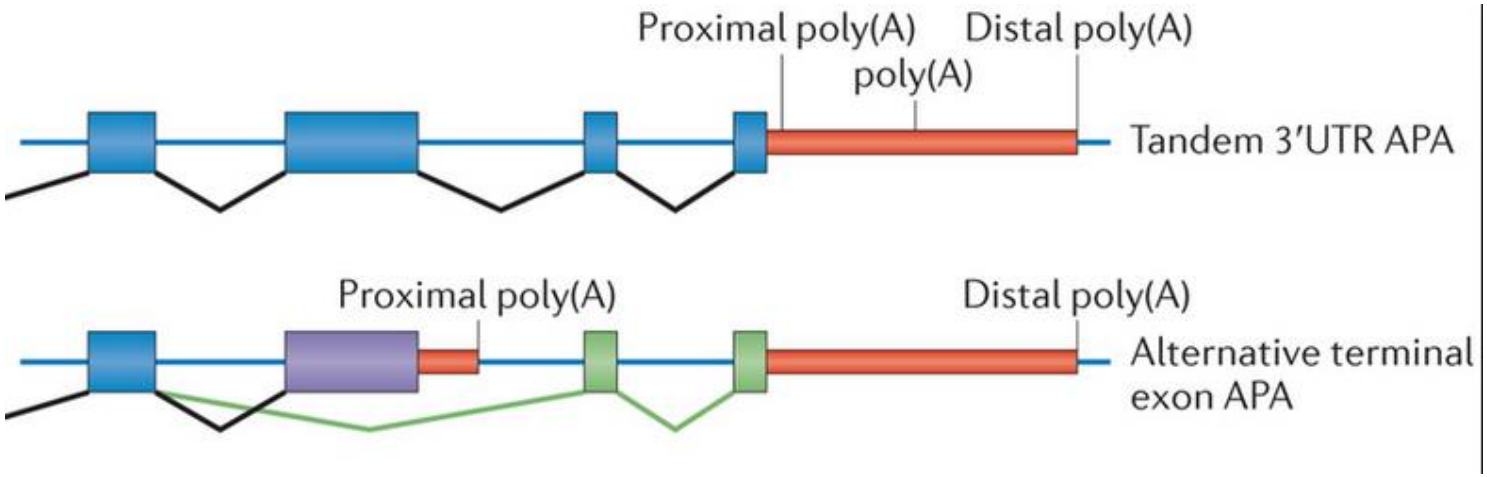


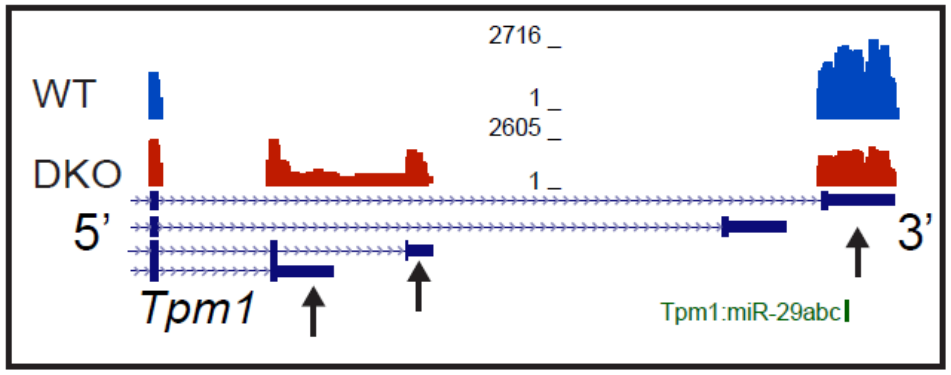

Batra et al., *Mol. Cell*, 2014

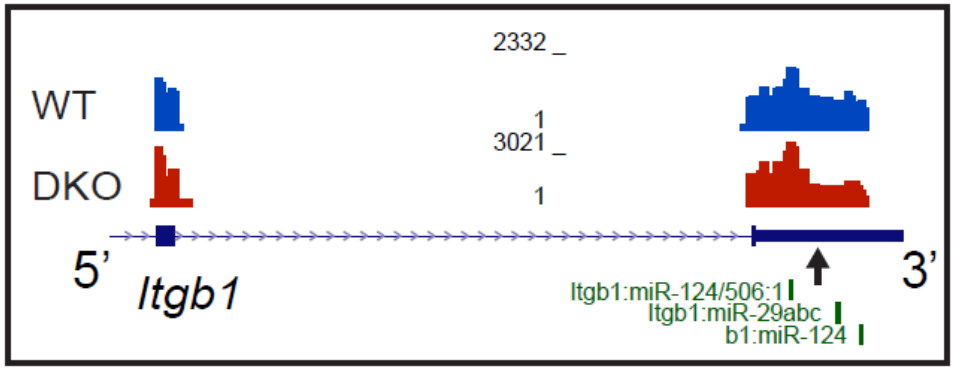
# Poly(A) Site Shifts following MBNL Depletion

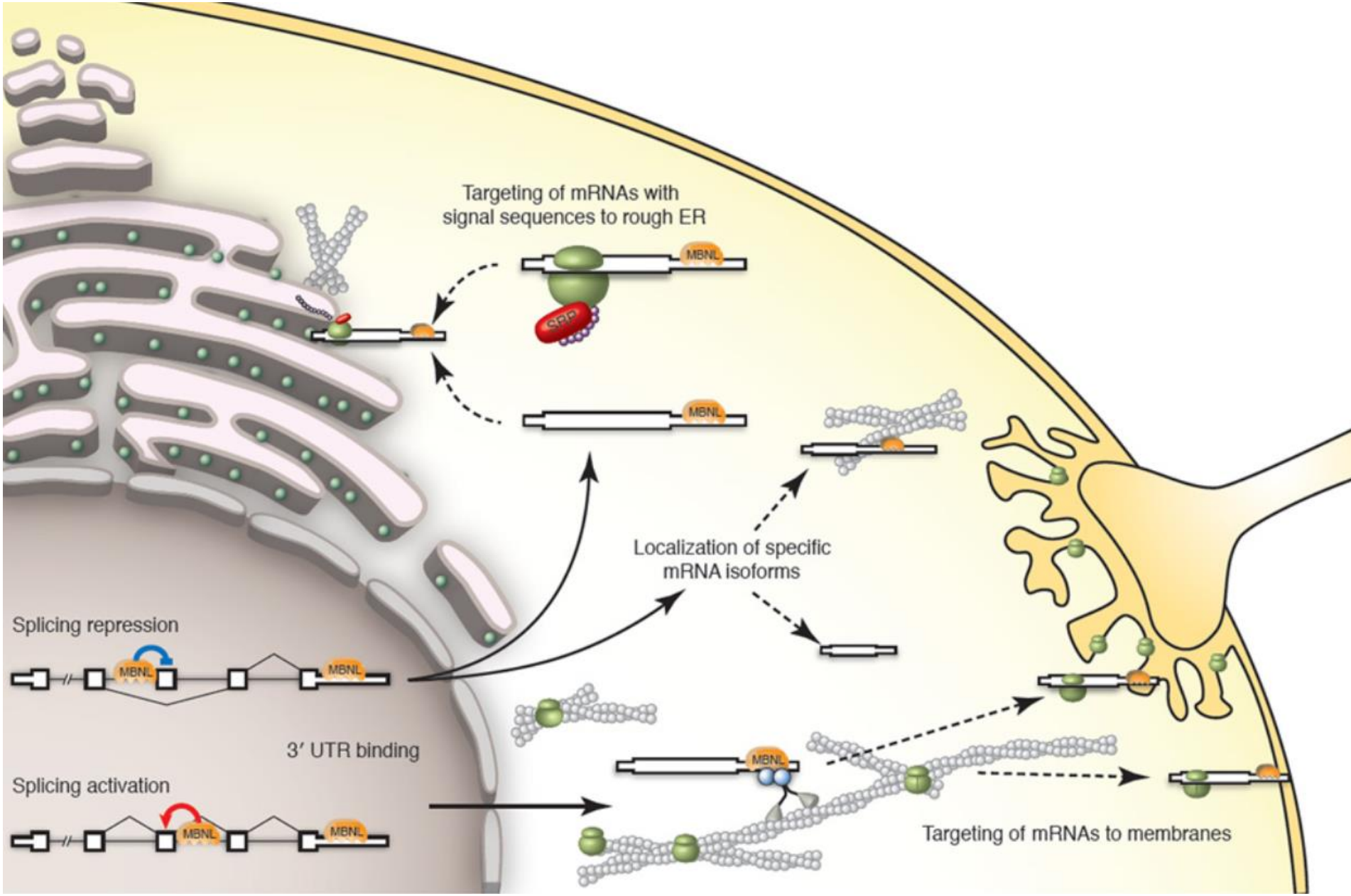



Batra et al., *Mol. Cell*, 2014


Proximal poly(A)
Distal poly(A)
poly(A)
Tandem 3′UTR APA
Proximal poly(A)
Distal poly(A)
Alternative terminal exon APA


WT
DKO
5’
3’
2716
1
2605
1
Tpm1
Tpm1:miR-29abc


WT
DKO
5’
3’
2332
1
3021
1
Itgb1
Itgb1:miR-124/506:1
Itgb1:miR-29abc
b1:miR-124




Wang et al., *Cell*, 2012