OHIO STATE UNIVERSITY - NATIONWIDE CHILDREN'S HOSPITAL

RESEARCH FOR MUSCLE BIOLOGY AND DISEASE

RNA and the Link to Motor Neuron Disease

February 26, 2015

Stephen J. Kolb, M.D., Ph.D.
Department of Neurology
Department of Molecular & Cellular Biochemistry

Wexner Medical Center

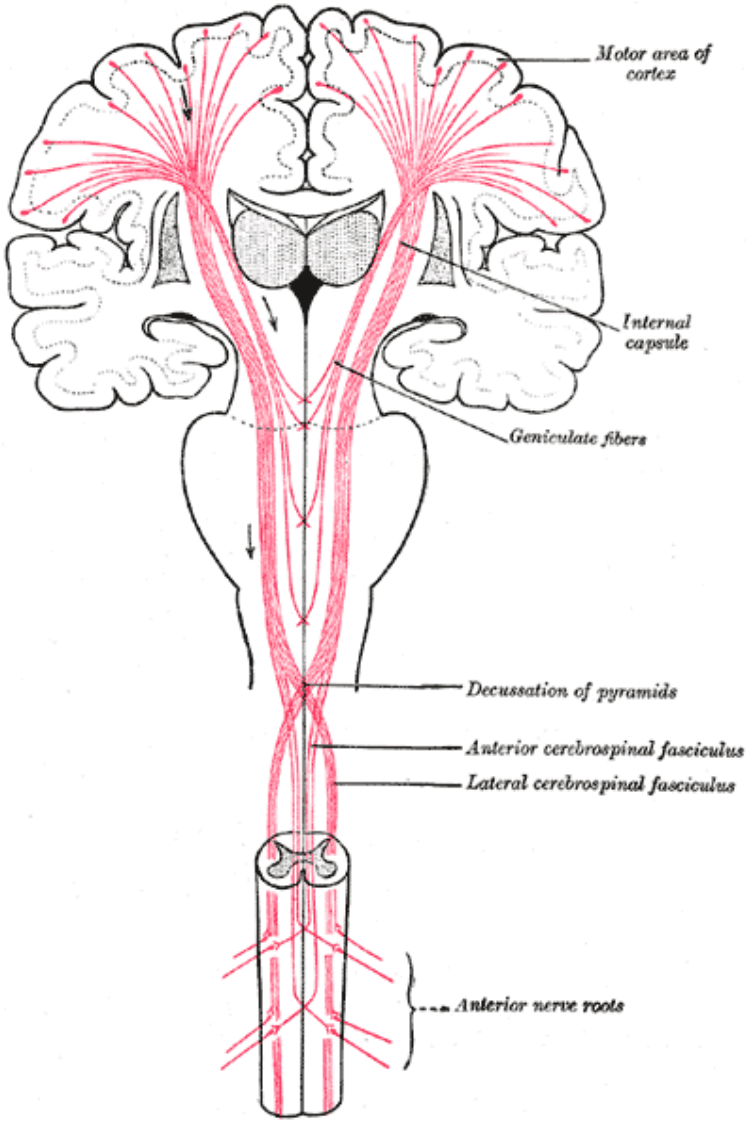
THE OHIO STATE UNIVERSITY Center for RNA Biology

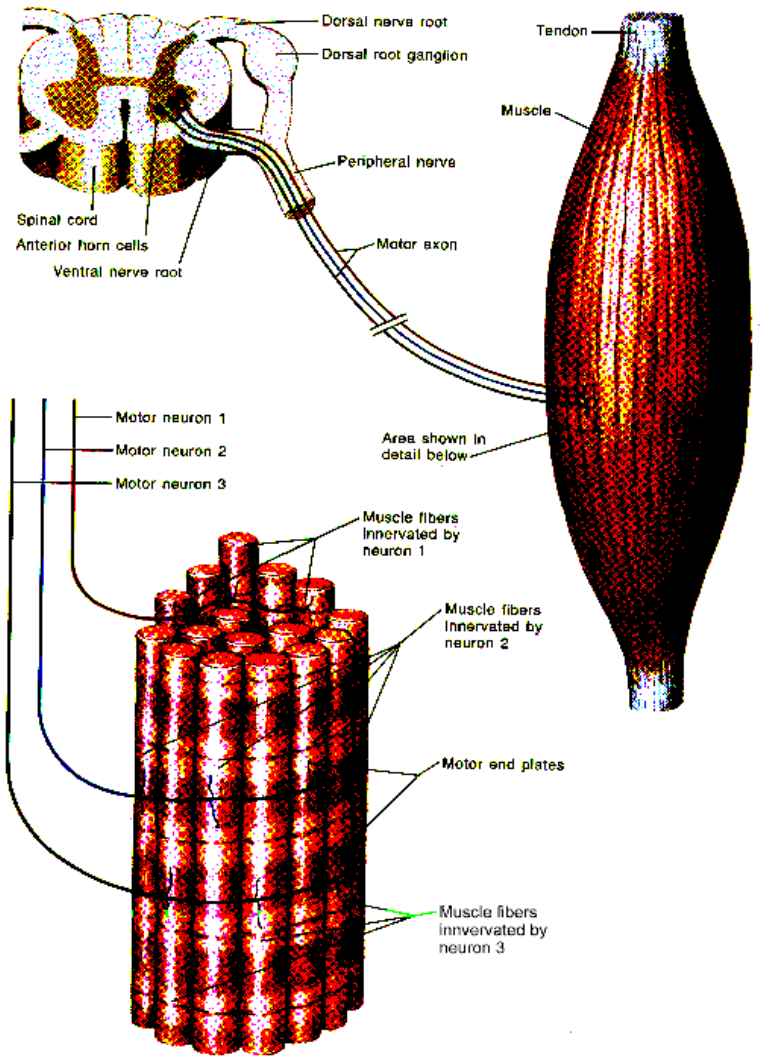
“Final Common Pathway”

“…to move things is all that mankind can do, for such the sole executant in muscle, whether in whispering a syllable or felling a forest.”

- Charles Sherrington, 1924

Charles Scott Sherrington 1857-1952





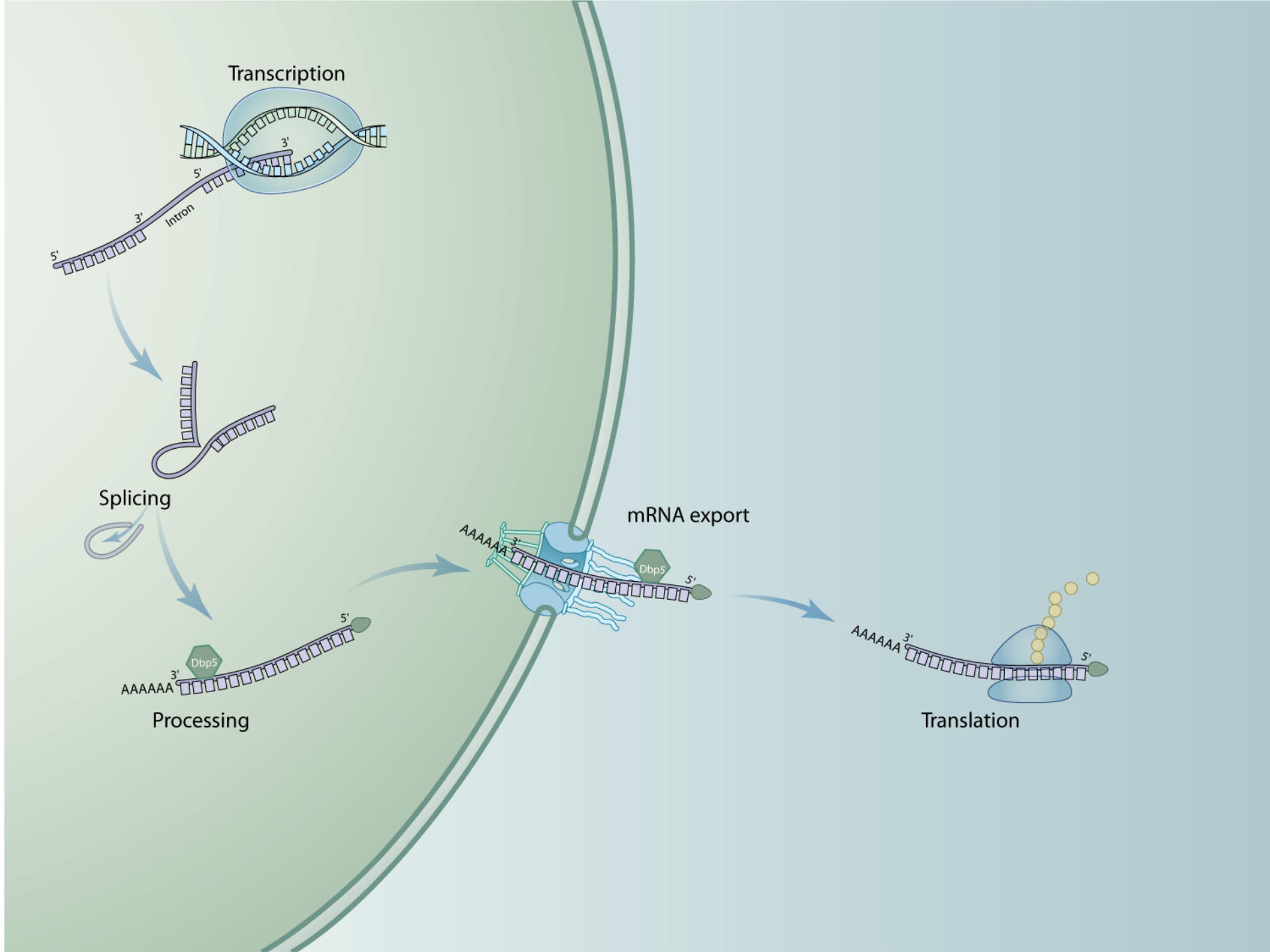
Gray's Anatomy

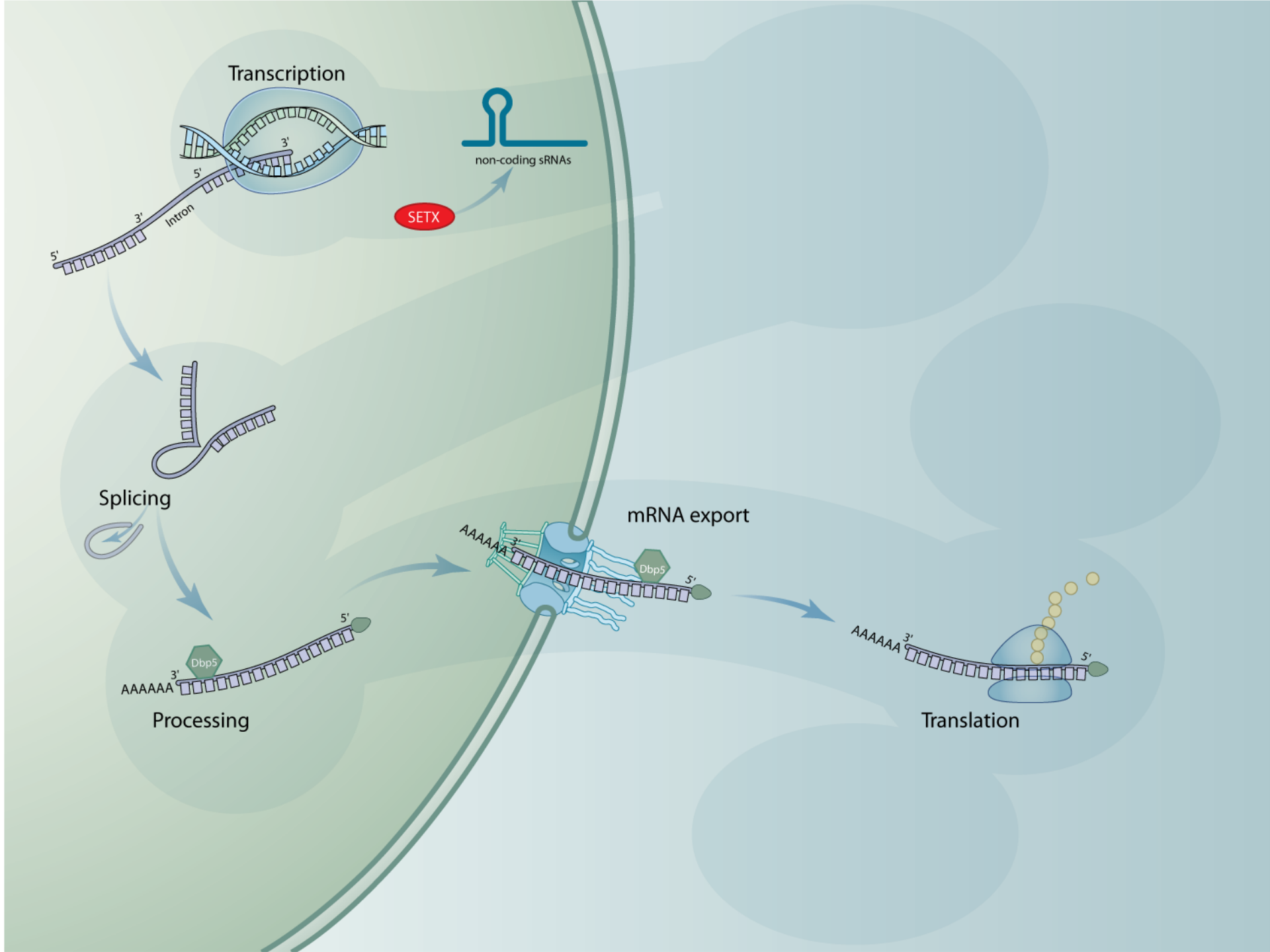
RNA processing is a Big Deal

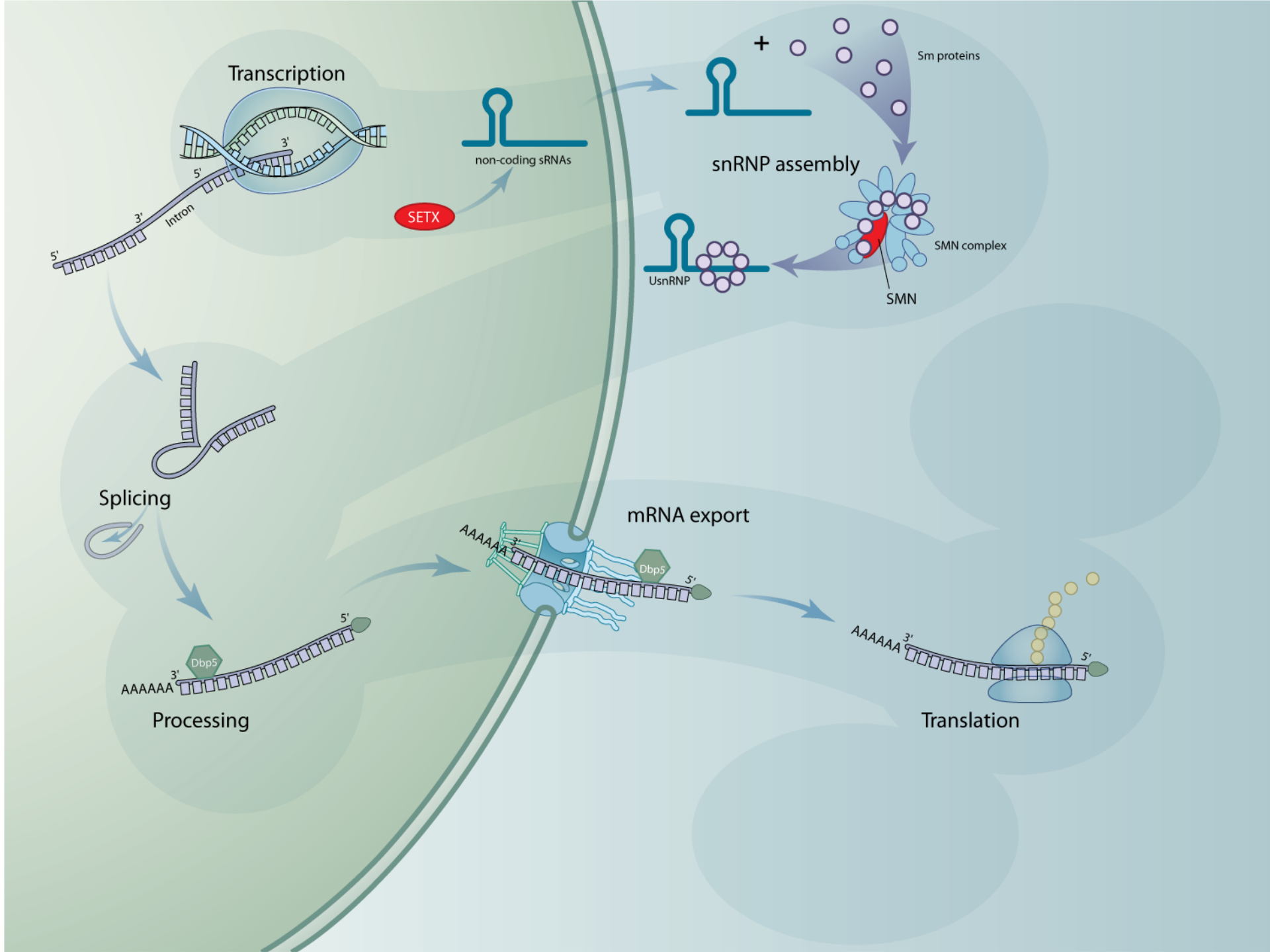
Human Genome
< 20,000 protein encoding transcripts

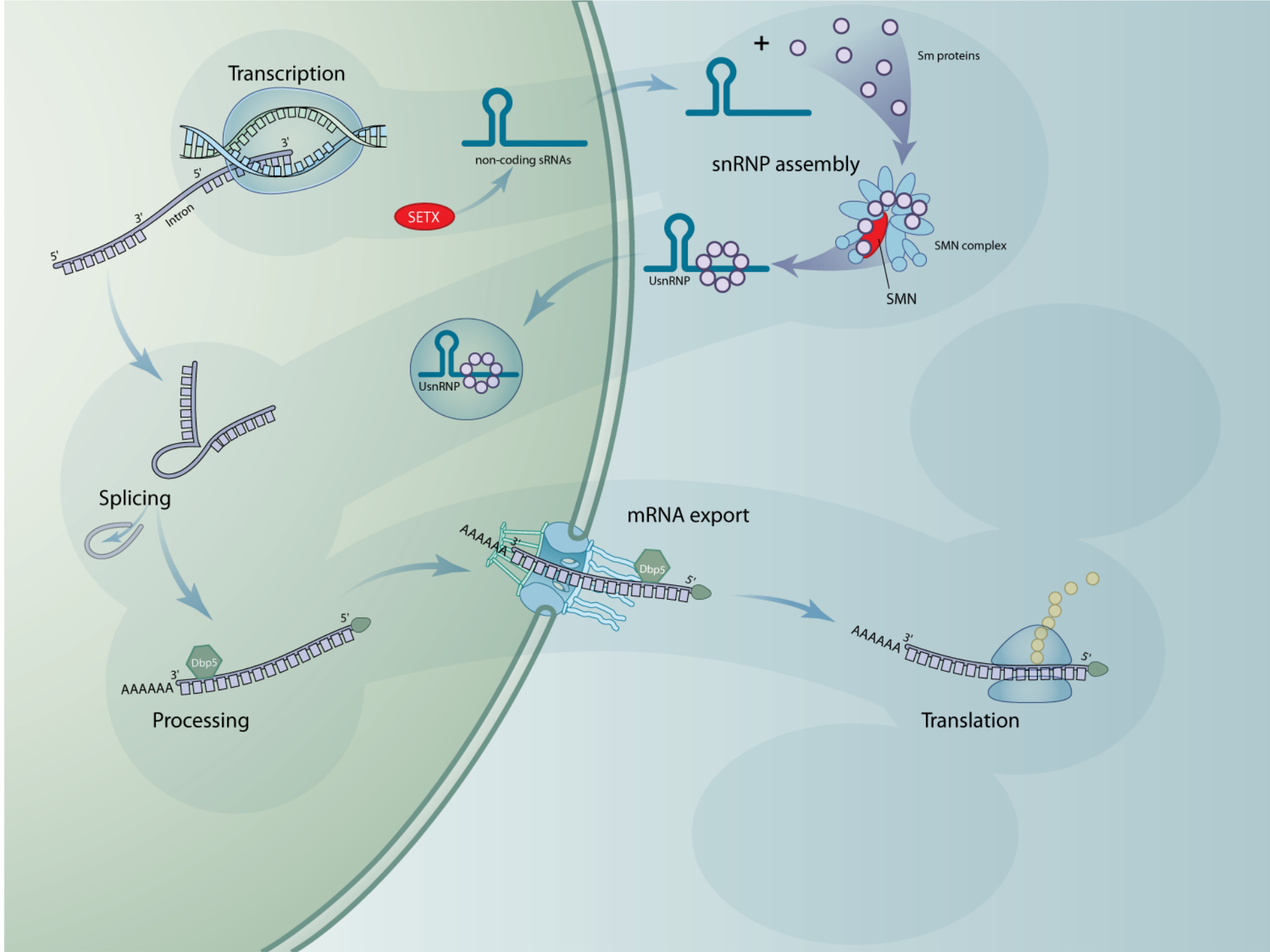
Human Transcriptome
> 80,000 protein encoding transcripts

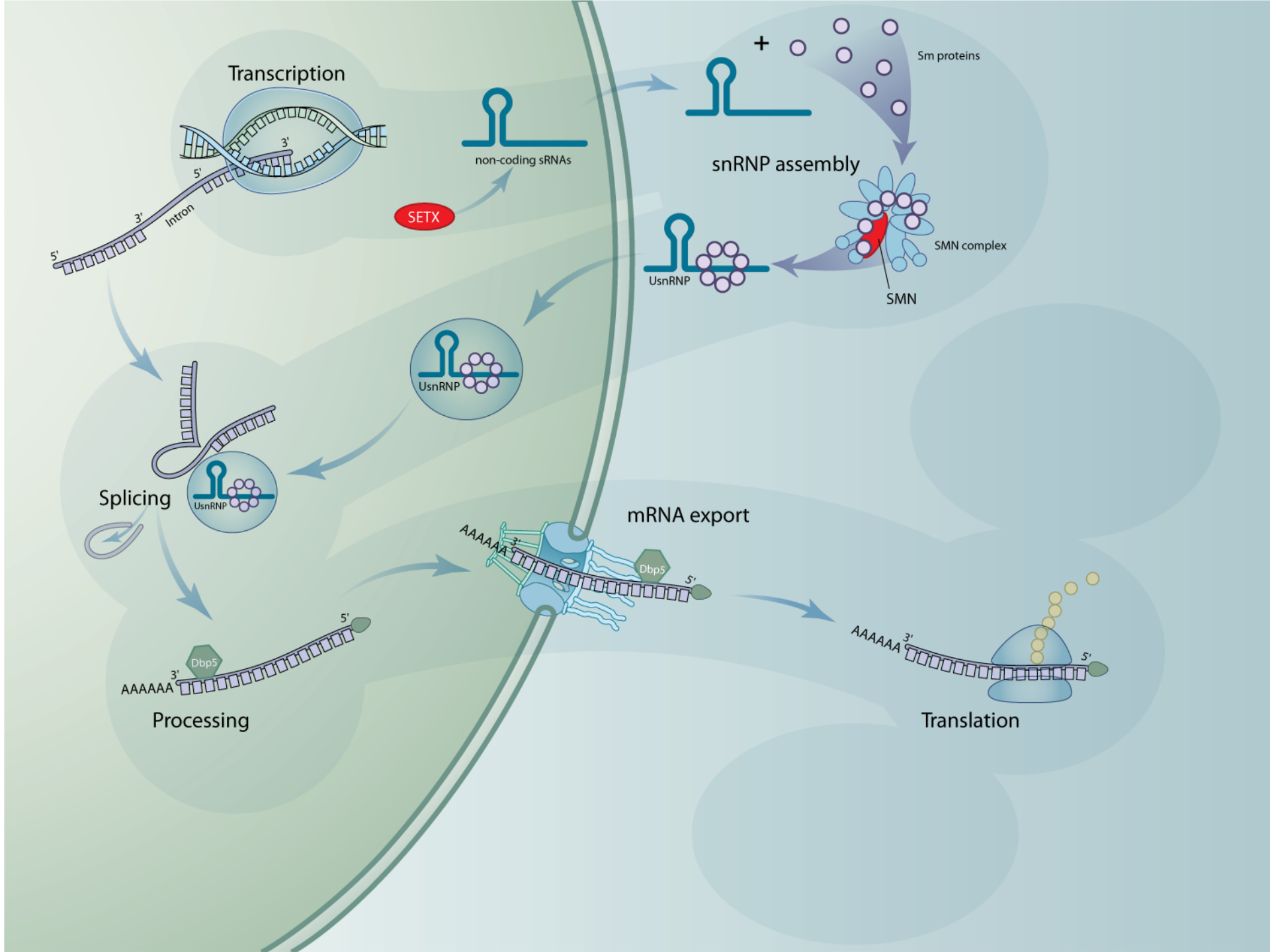
Human Proteome
250,000 – 1,000,000 distinct proteins

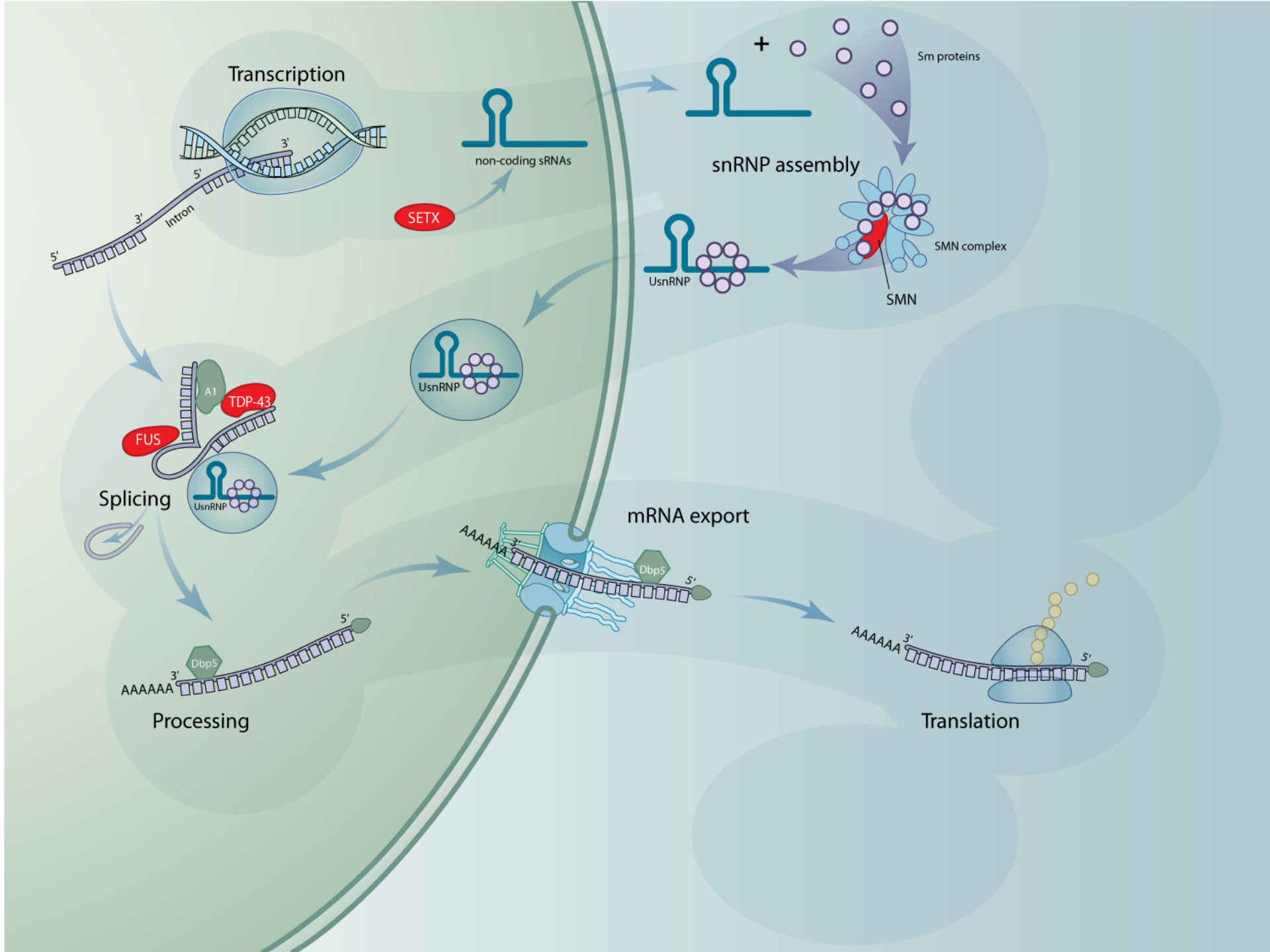

Transcription
3'
5'
3'
Intron
5'
Splicing
Dbp5
AAAAAA
3'
5'
Processing
mRNA export
AAAAAA
3'
Dbp5
5'
AAAAAA
3'
5'
Translation

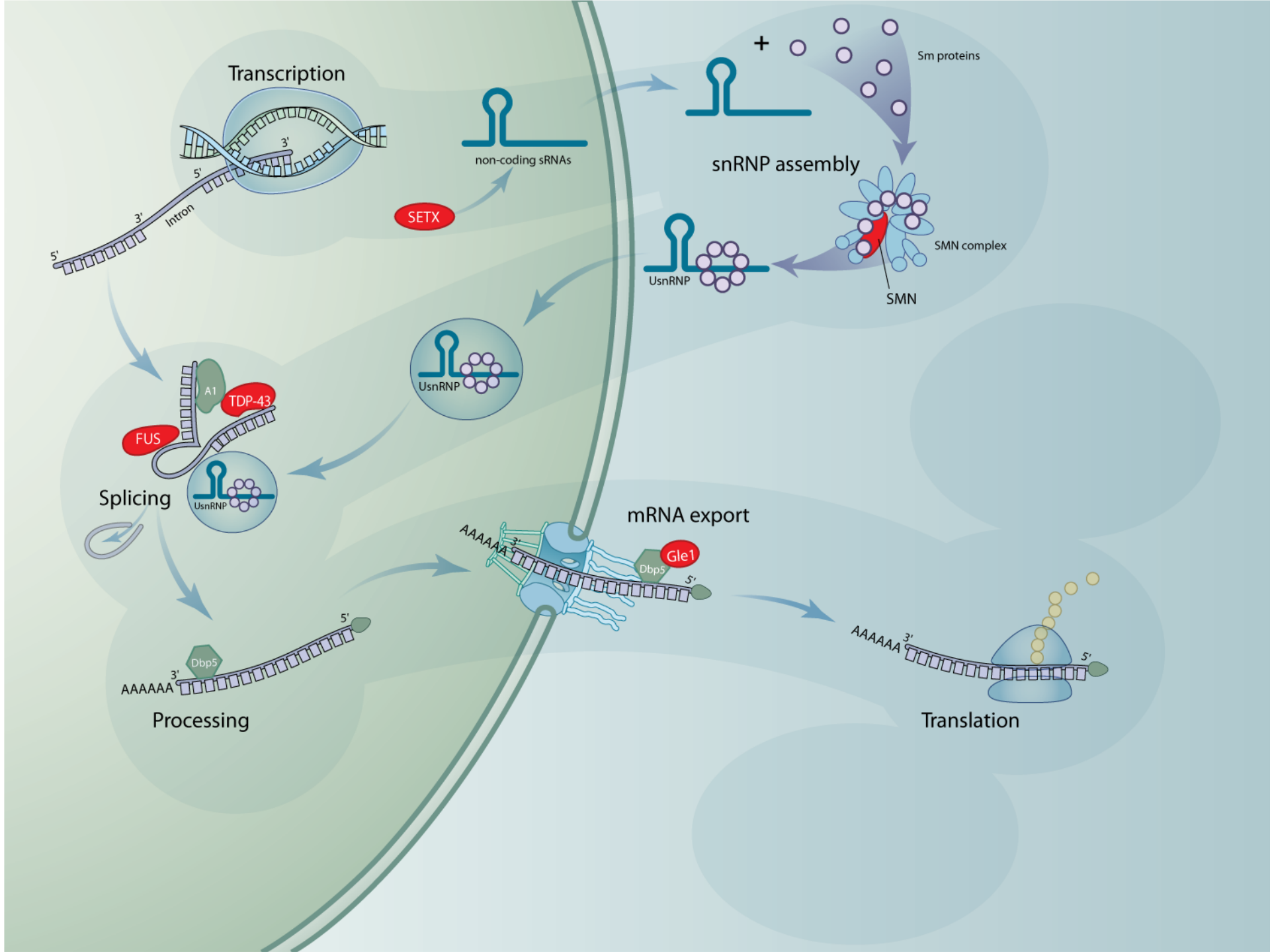

Transcription
3'
5'
3'
Intron
5'
non-coding sRNAs
SETX
Splicing
Dbp5
5'
3'
AAAAAA
Processing
mRNA export
AAAAAA
3'
Dbp5
5'
AAAAAA
3'
5'
Translation

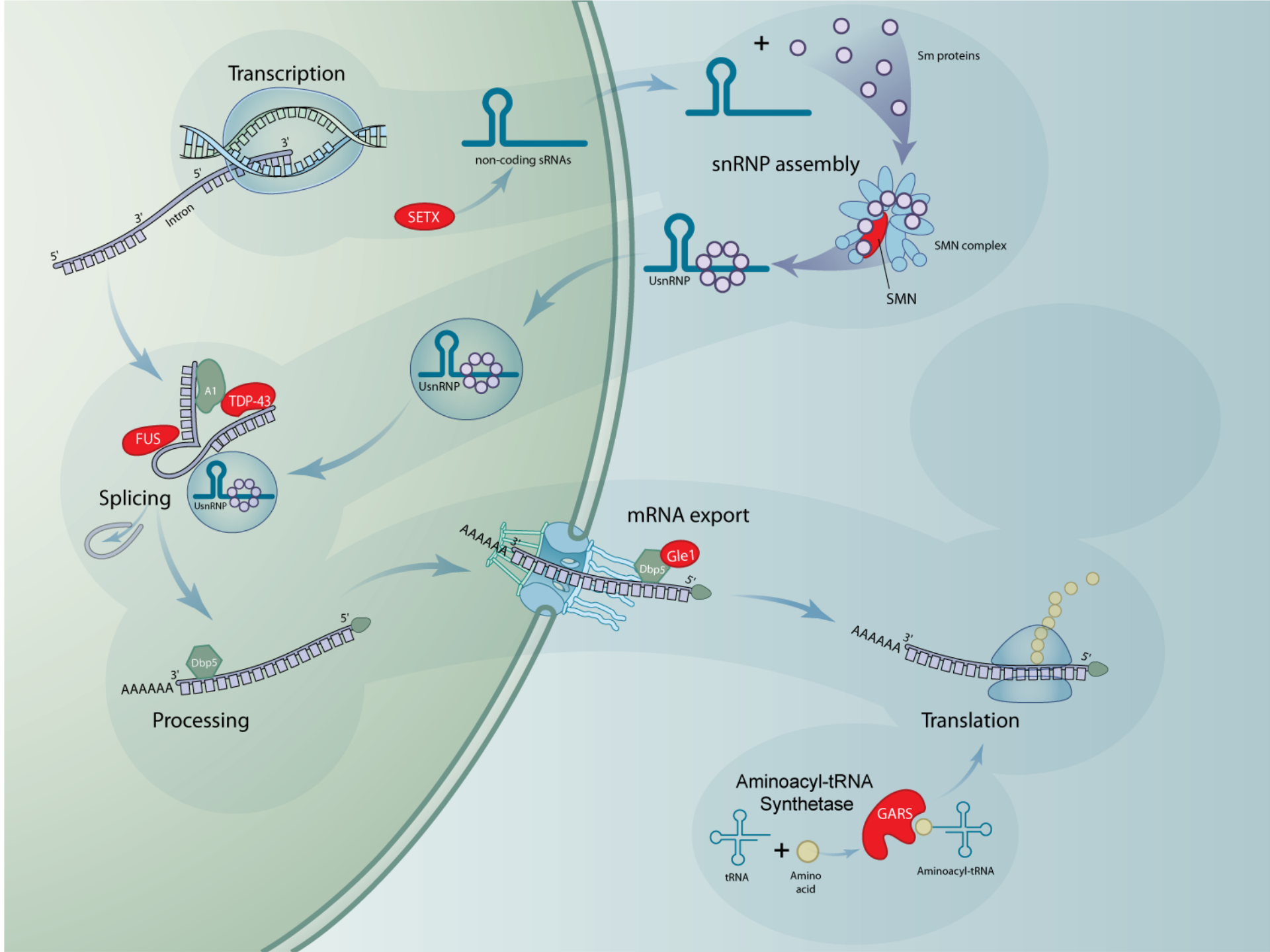

Transcription
3'
5'
3'
Intron
5'
non-coding sRNAs
SETX
+
Sm proteins
snRNP assembly
SMN complex
SMN
UsnRNP
Splicing
mRNA export
AAAAAA
3'
Dbp5
5'
5'
Dbp5
3'
AAAAAA
Processing
AAAAAA
3'
5'
Translation

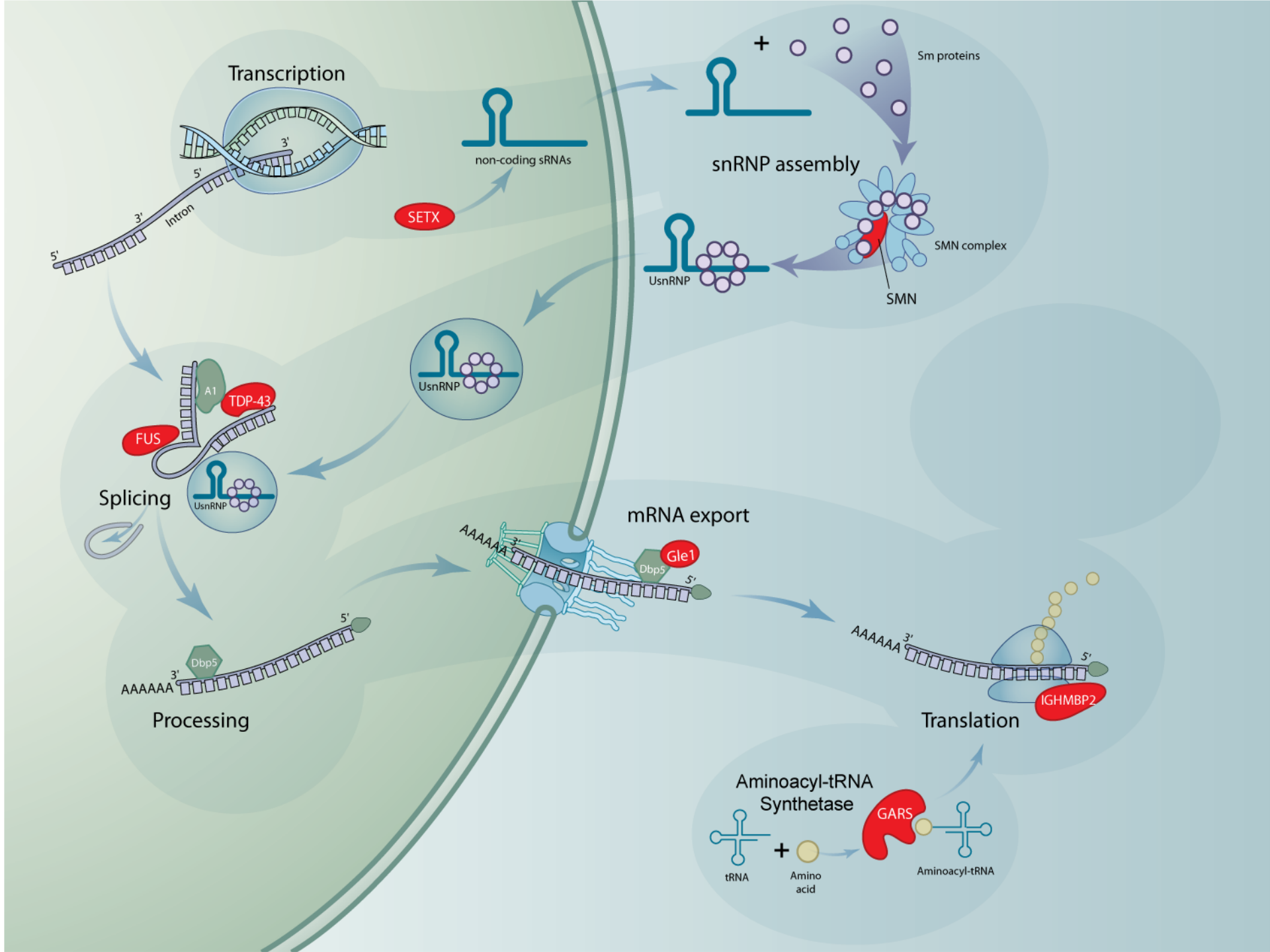

Transcription
3'
5'
3'
Intron
5'
non-coding sRNAs
SETX
+
Sm proteins
snRNP assembly
SMN complex
SMN
UsnRNP
UsnRNP
Splicing
mRNA export
AAAAAA
3'
Dbp5
5'
5'
Dbp5
3'
AAAAAA
Processing
AAAAAA
3'
5'
Translation

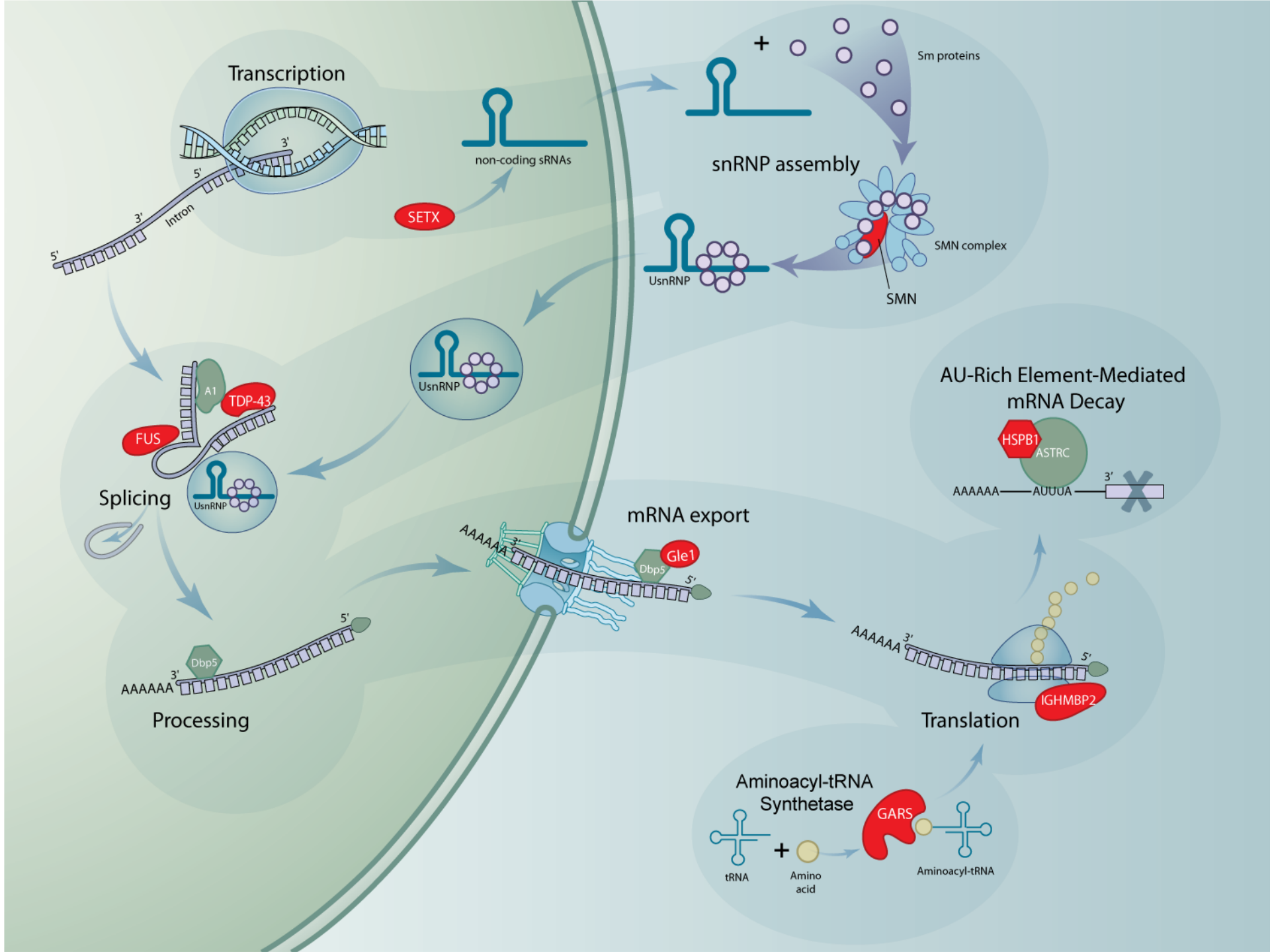

Transcription
3'
5'
3'
Intron
5'
non-coding sRNAs
SETX
+
Sm proteins
snRNP assembly
SMN complex
SMN
UsnRNP
UsnRNP
Splicing
UsnRNP
mRNA export
AAAAAA
3'
Dbp5
5'
5'
Dbp5
3'
AAAAAA
Processing
AAAAAA
3'
5'
Translation


Transcription
3'
5'
3'
Intron
5'
non-coding sRNAs
SETX
+
Sm proteins
snRNP assembly
SMN complex
UsnRNP
SMN
UsnRNP
A1
TDP-43
FUS
UsnRNP
Splicing
mRNA export
AAAAAA
3'
Dbp5
5'
5'
Dbp5
3'
AAAAAA
Processing
AAAAAA
3'
5'
Translation

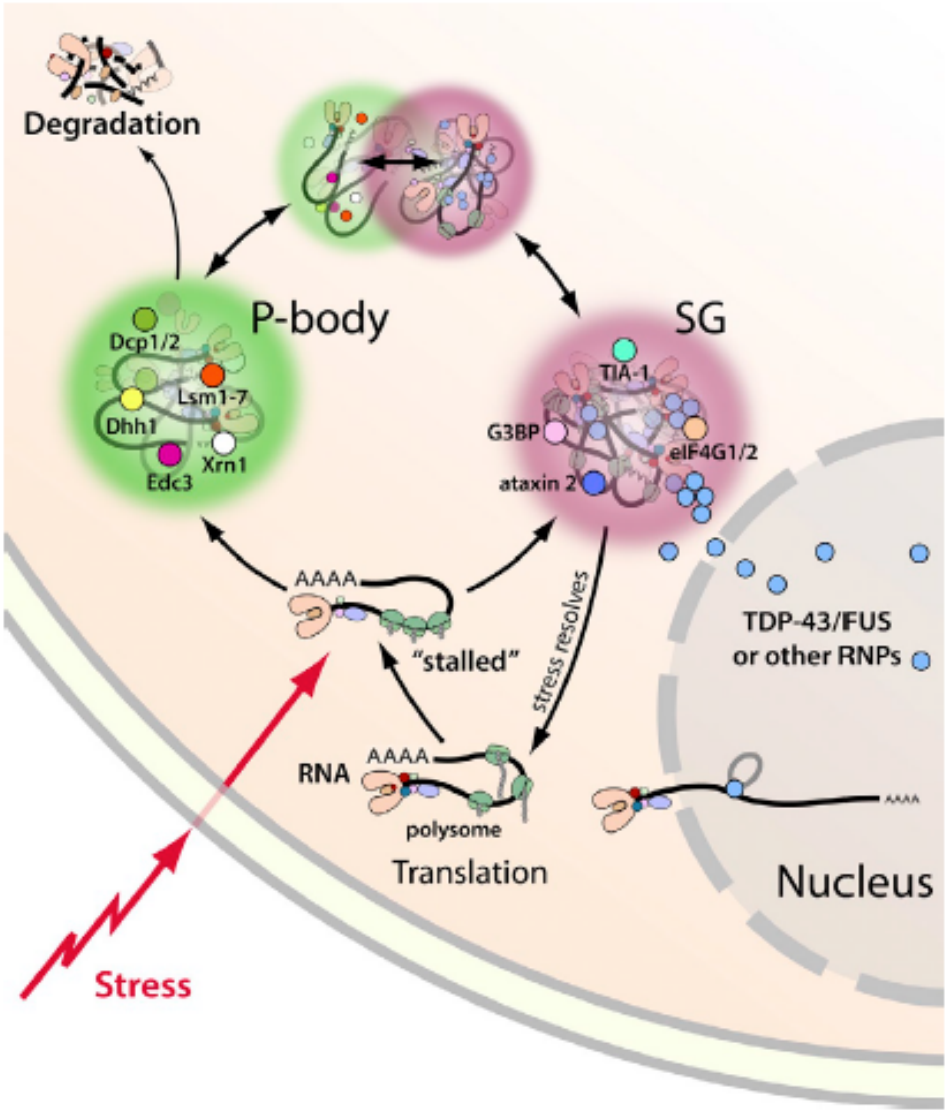

Transcription
3'
5'
3'
Intron
5'
non-coding sRNAs
SETX
+
Sm proteins
snRNP assembly
SMN complex
SMN
UsnRNP
UsnRNP
A1
TDP-43
FUS
UsnRNP
Splicing
mRNA export
AAAAAA
3'
Gle1
Dbp5
5'
5'
Dbp5
3'
AAAAAA
Processing
AAAAAA
3'
5'
Translation


Transcription
3'
5'
3'
Intron
5'
non-coding sRNAs
SETX
Sm proteins
snRNP assembly
SMN complex
SMN
UsnRNP
UsnRNP
A1
TDP-43
FUS
UsnRNP
Splicing
mRNA export
AAAAAA
3'
Gle1
Dbp5
5'
5'
Dbp5
3'
AAAAAA
Processing
AAAAAA
3'
5'
Translation
Aminoacyl-tRNA
Synthetase
GARS
tRNA
Amino
acid
Aminoacyl-tRNA


Transcription
3'
5'
3'
Intron
5'
non-coding sRNAs
SETX
Sm proteins
snRNP assembly
SMN complex
UsnRNP
SMN
UsnRNP
A1
TDP-43
FUS
UsnRNP
Splicing
mRNA export
AAAAAA
3'
Gle1
Dbp5
5'
5'
Dbp5
3'
AAAAAA
Processing
AAAAAA
3'
5'
IGHMBP2
Translation
Aminoacyl-tRNA
Synthetase
GARS
tRNA
Amino
acid
Aminoacyl-tRNA


Transcription
3'
5'
3'
Intron
5'
non-coding sRNAs
SETX
+
Sm proteins
snRNP assembly
SMN complex
SMN
UsnRNP
UsnRNP
A1
TDP-43
FUS
UsnRNP
Splicing
AU-Rich Element-Mediated
mRNA Decay
HSPB1
ASTRC
AAAAAA
AUUUA
3'
mRNA export
AAAAAA
3'
Gle1
Dbp5
5'
5'
Dbp5
3'
AAAAAA
Processing
AAAAAA
3'
5'
IGHMBP2
Translation
Aminoacyl-tRNA
Synthetase
GARS
+
tRNA
Amino
acid
Aminoacyl-tRNA

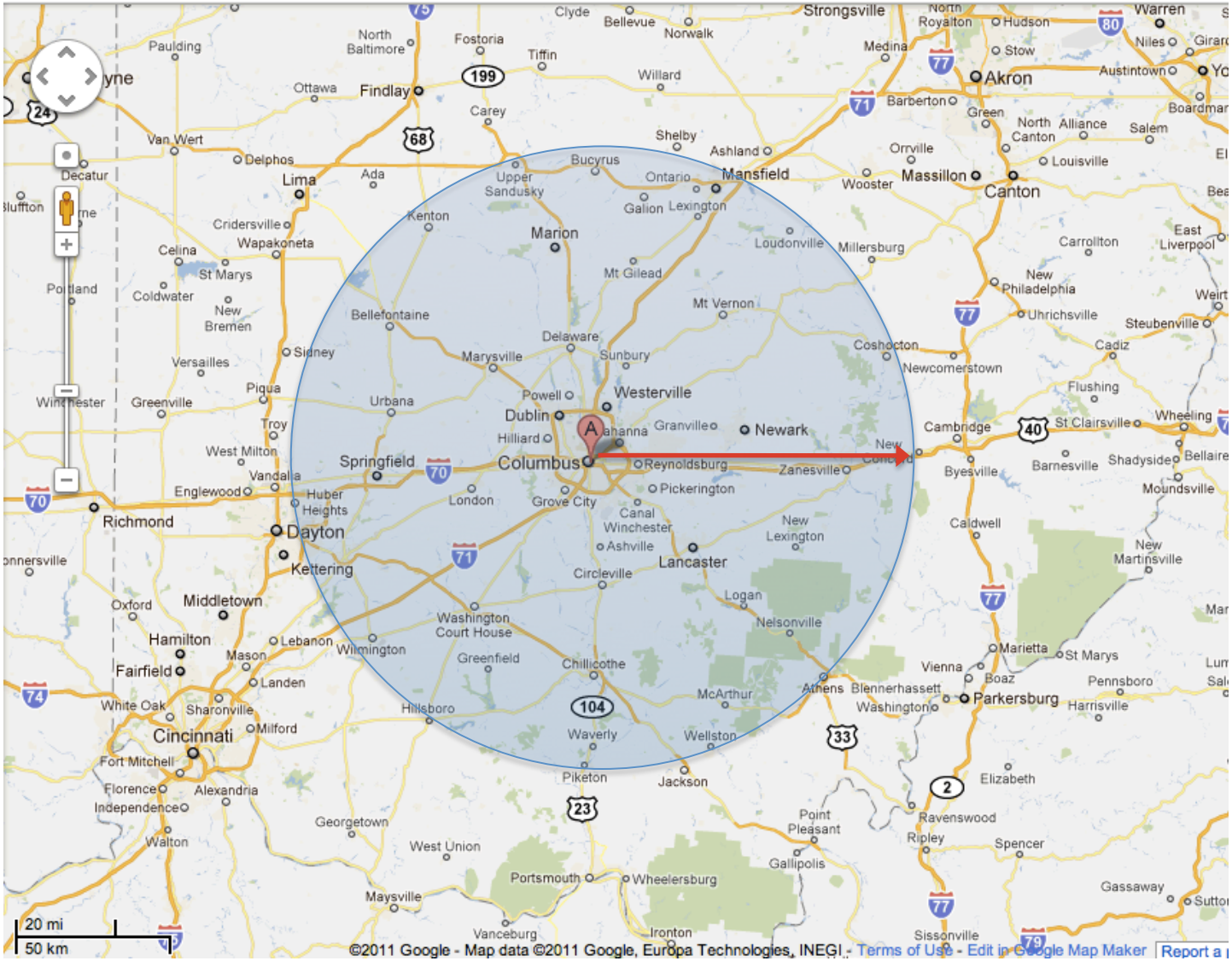
P-bodies and Stress Granules



- Decapping and degradation of unwanted mRNAs
- Storing of mRNA until needed
- Involved in translational suppresion by miRNAs
- Involved in the transport of mRNAs in neurons in response to stimulation

Li et al. J. Cell Bio. 2013;201, 361

Axon length is 20,000 x soma diameter

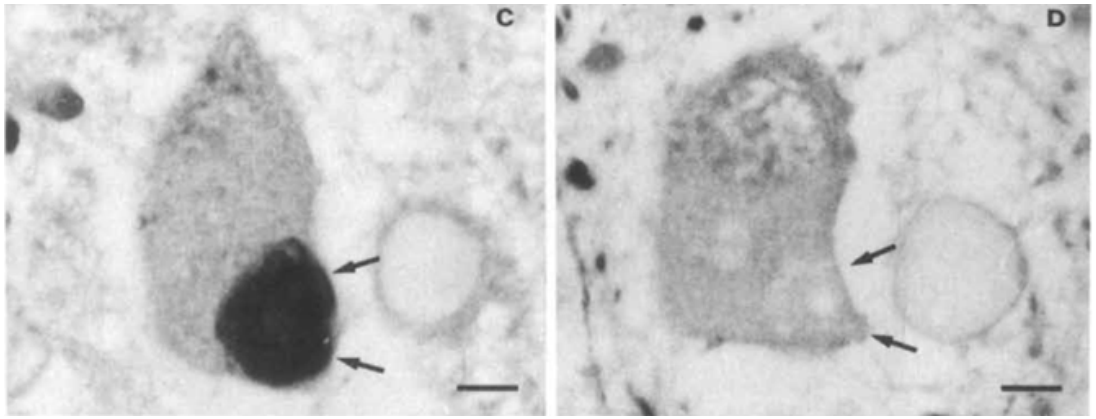


Gene Variants in ALS genes (Feb, 2015)

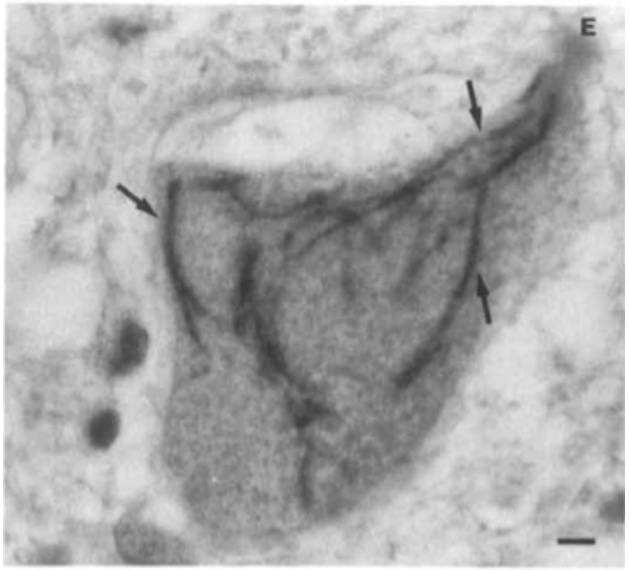
Gene	Reported inheritance model	Reported FALS explained	Reported SALS explained	Best model with case enrichment in present study (p-value)	Cases with variant in best model	Controls with variant in best model	Potential ALS cases explained
TBK1	N/A	N/A	N/A	Dom not benign (D = 1.13×10^{-5}; R = 5.78×10^{-7}; C = 3.63×10^{-11})	D = 23 (0.8%); R = 23 (1.745%); C = 46 (1.097%)	D = 12 (0.187%); R = 5 (0.211%); C = 17 (0.194%)	0.904%
NEK1	N/A	N/A	N/A	Dom LoF (D = 1.08×10^{-6}; R = 0.001; C = 3.20×10^{-9})	D = 25 (0.870%); R = 10 (0.759%); C = 35 (0.835%)	D = 6 (0.094%); R = 2 (0.084%); C = 8 (0.091%)	0.744%
SOD1	AR/AD	12%	1.50%	Dom coding (7.23×10^{-8})	25 (0.870%)	5 (0.078%)	0.792%
TARDBP	AD	4%	1%	Dom coding (2.97×10^{-6})	19 (0.661%)	6 (0.094%)	0.567%
OPTN	AR/AD	<1%	<1%	Dom not benign (D = 0.023; R = 0.002; C = 0.002)	D = 18 (0.626%); R = 8 (0.607%); C = 26 (0.620%)	D = 16 (0.25%); R = 4 (0.169%); C = 20 (0.228%)	0.392%
SPG11	AR	<1%	<1%	Dom LoF (D = 0.015; R = 0.183; C = 0.017)	D = 21 (0.731%); R = 5 (0.379%); C = 26 (0.620%)	D = 20 (0.312%); R = 7 (0.295%); C = 27 (0.308%)	0.313%
VCP	AD	1%	1%	Dom coding (0.022)	8 (0.278%)	4 (0.062%)	0.216%
HNRNPA1	AD	<1%	<1%	Dom coding (0.103)	6 (0.209%)	5 (0.078%)	0.131%
*ATXN2**	AD	<1%	<1%	Rec coding (0.206)	4 (0.139%)	2 (0.031%)	0.108%
ANG	AD	<1%	<1%	Dom LoF (0.217)	2 (0.070%)	1 (0.016%)	0.054%
CHCHD10	AD	<1%	<1%	Dom coding (0.226)	2 (0.070%)	0 (0%)	0.070%
SIGMAR1	AR	<1%	<1%	Dom LoF (0.226)	1 (0.035%)	0 (0%)	0.035%
FIG4	AR/AD	<1%	<1%	Dom LoF (0.233)	9 (0.313%)	12 (0.187%)	0.126%
SS18L1	AD	<1%	<1%	Dom LoF (0.241)	1 (0.035%)	0 (0%)	0.035%
GRN	AD	<1%	<1%	Dom not benign (0.357)	14 (0.487%)	24 (0.375%)	0.112%
SETX	AD	<1%	<1%	Rec not benign (0.380)	3 (0.104%)	4 (0.062%)	0.042%
HNRNPA2B1	AD	<1%	<1%	Dom not benign (0.423)	3 (0.104%)	4 (0.062%)	0.042%
SQSTM1	AD	1%	<1%	Dom LoF (0.546)	1 (0.035%)	2 (0.031%)	0.004%
TAF15	AR/AD	<1%	<1%	Rec not benign (0.555)	2 (0.070%)	1 (0.016%)	0.054%
FUS	AR/AD	4%	1%	Dom LoF (0.612)	2 (0.070%)	3 (0.047%)	0.023%
ALS2	AR	<1%	<1%	Rec coding (0.655)	2 (0.070%)	4 (0.062%)	0.007%
VAPB	AD	<1%	<1%	Dom not benign (0.688)	3 (0.104%)	5 (0.078%)	0.026%
NEFH	AD	<1%	<1%	Dom coding (0.777)	22 (0.765%)	37 (0.578%)	0.188%
*C9orf72**	AD	40%	7%	Dom not benign (1.000)	4 (0.139%)	7 (0.109%)	0.030%
CHMP2B	AD	<1%	<1%	Rec coding (1.000)	1 (0.035%)	1 (0.016%)	0.019%
MATR3	AD	<1%	<1%	Dom coding (1.000)	19 (0.661%)	35 (0.546%)	0.115%
PFN1	AD	<1%	<1%	Rec coding (1.000)	9 (0.313%)	15 (0.234%)	0.079%
PRPH	AD	<1%	<1%	Dom LoF (1.000)	1 (0.035%)	2 (0.031%)	0.004%
SPAST	AD	<1%	<1%	Dom coding (1.000)	6 (0.209%)	12 (0.187%)	0.021%
TUBA4A†	AD	1%	<1%	Dom coding (0.743)	3 (0.104%)	7 (0.109%)	0%
ELP3†	Allelic	<1%	<1%	Rec coding (1.000)	0 (0%)	0 (0%)	0%
DAO†	AD	<1%	<1%	Rec coding (1.000)	0 (0%)	0 (0%)	0%
DCTN1†	AD	<1%	<1%	Dom coding (0.668)	32 (1.113%)	76 (1.187%)	0%
EWSR1†	AD	<1%	<1%	Dom coding (0.375)	10 (0.348%)	28 (0.437%)	0%
GLE1†	AD	<1%	<1%	Rec LoF (1.000)	0 (0%)	0 (0%)	0%
UBQLN2†	XD	<1%	<1%	Dom LoF (1.000)	0 (0%)	0 (0%)	0%

Cirulli et al. Science 2015;Feb 19. aaa3650

Ubiquitin-positive inclusions



Dense anti-ubiquitin positive deposit



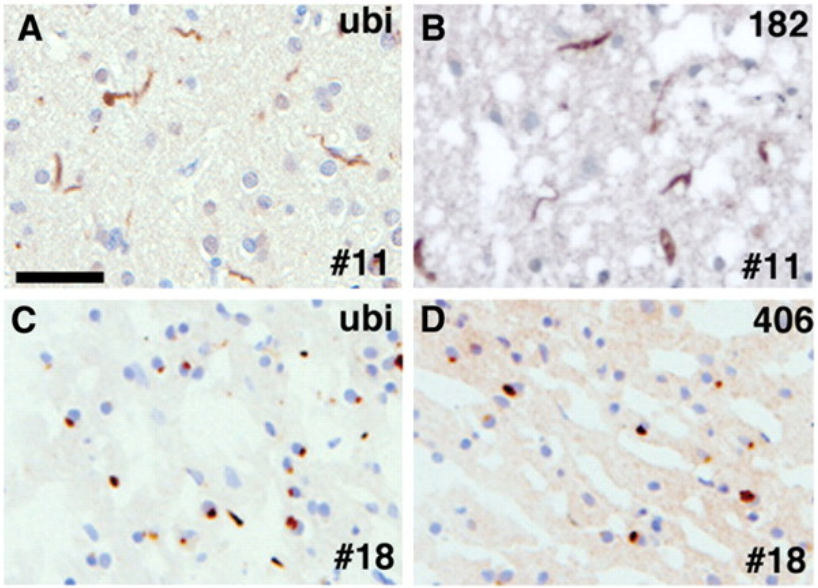
“Skein-like” filamentous arrays

Scale bars = 5 μm

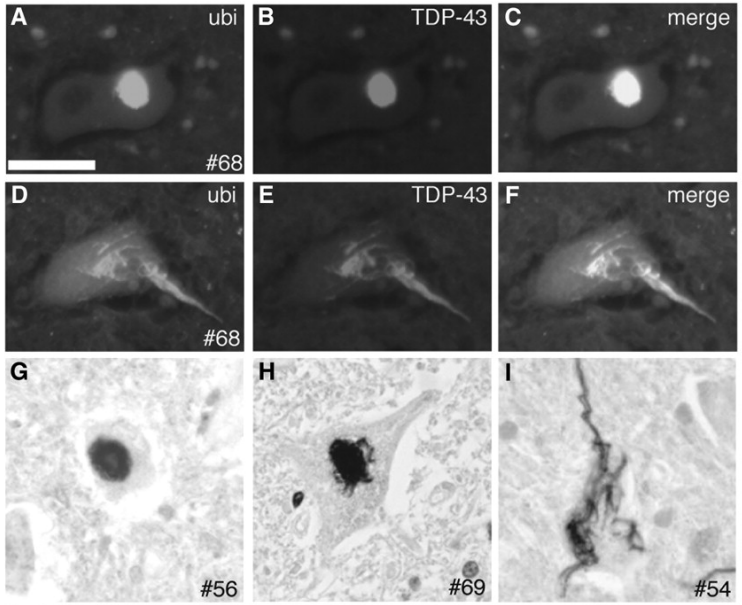
Neuroscience Letters, 1988: 93:197-203

TDP-43 inclusions in FTD and ALS

FTLD Ubiquitinated inclusions



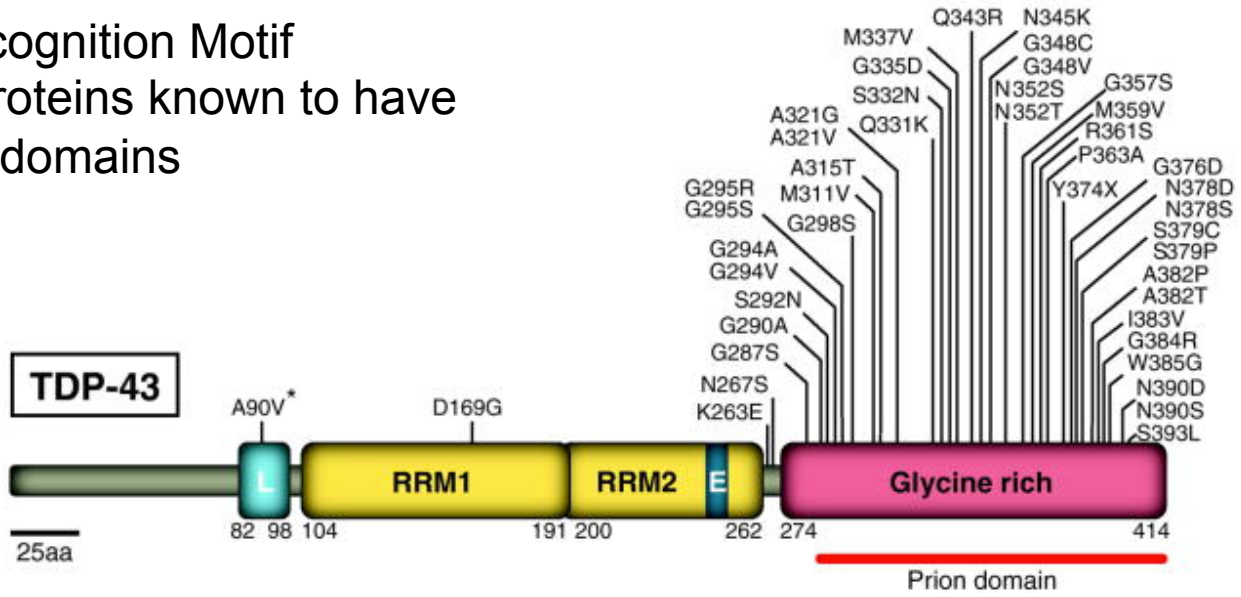
ALS Ubiquitinated inclusions



Manuela Neumann et al. Science 2006;314:130-133

Mutations in TDP-43 result in FTD and ALS

RNA Recognition Motif
- 210 proteins known to have these domains



29 RNA binding proteins contain prion-like domains!

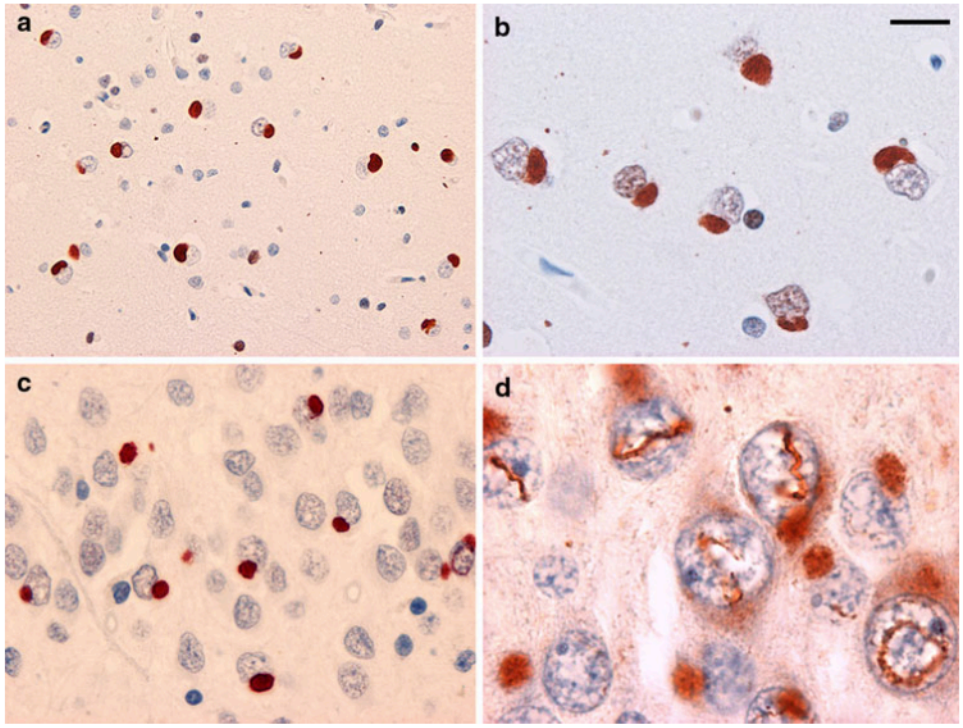
Prion-like domain:
- enriched in polar residues and glycin
- Confer “prionogenicity

King et al. Brain Res. 2012;1462:61

Da Cruz et al. Curr. Opin. Neurobiol. 2011;21:904

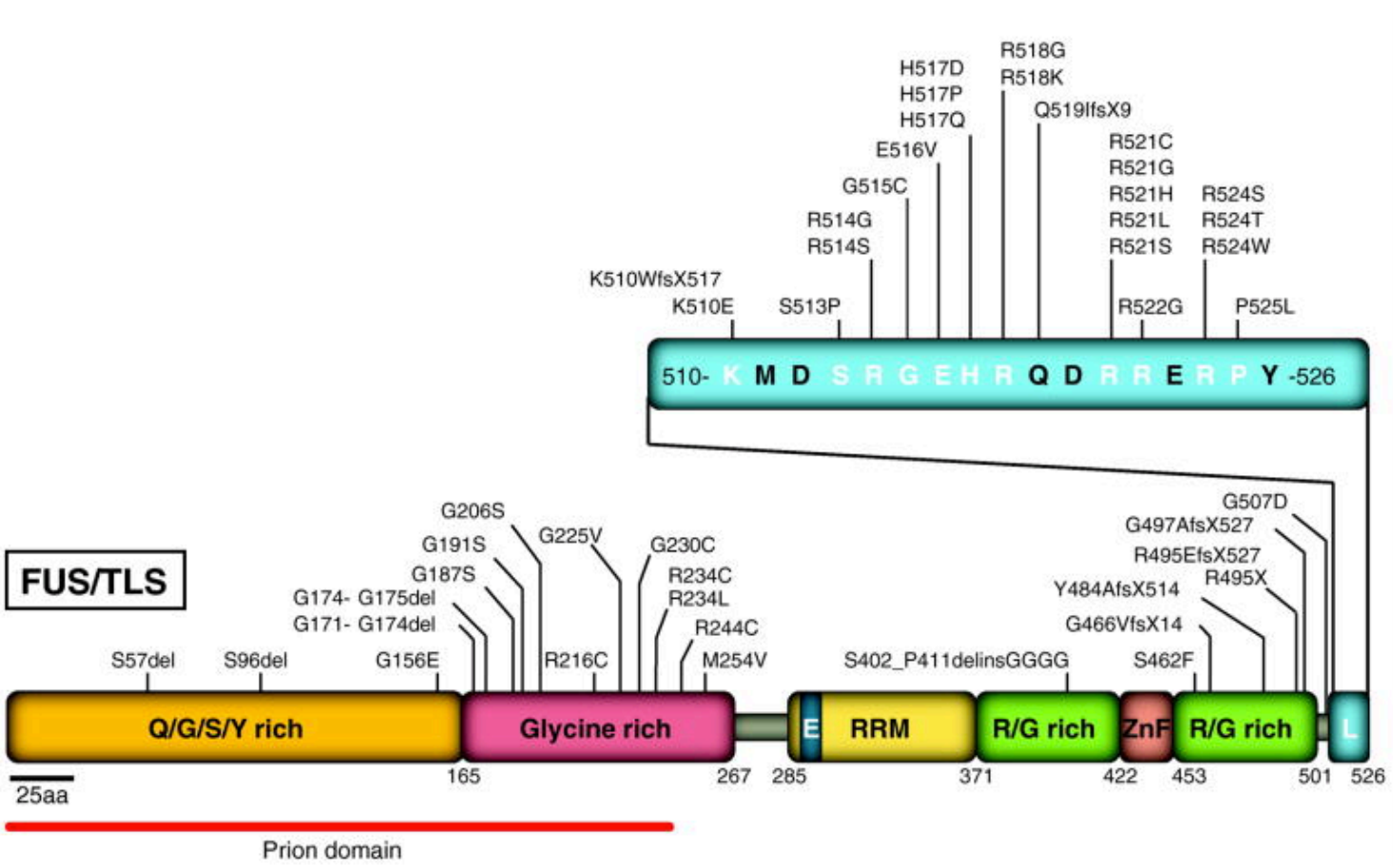
FUS/TLS inclusions in FTD

FTLD Ubiquitinated inclusions



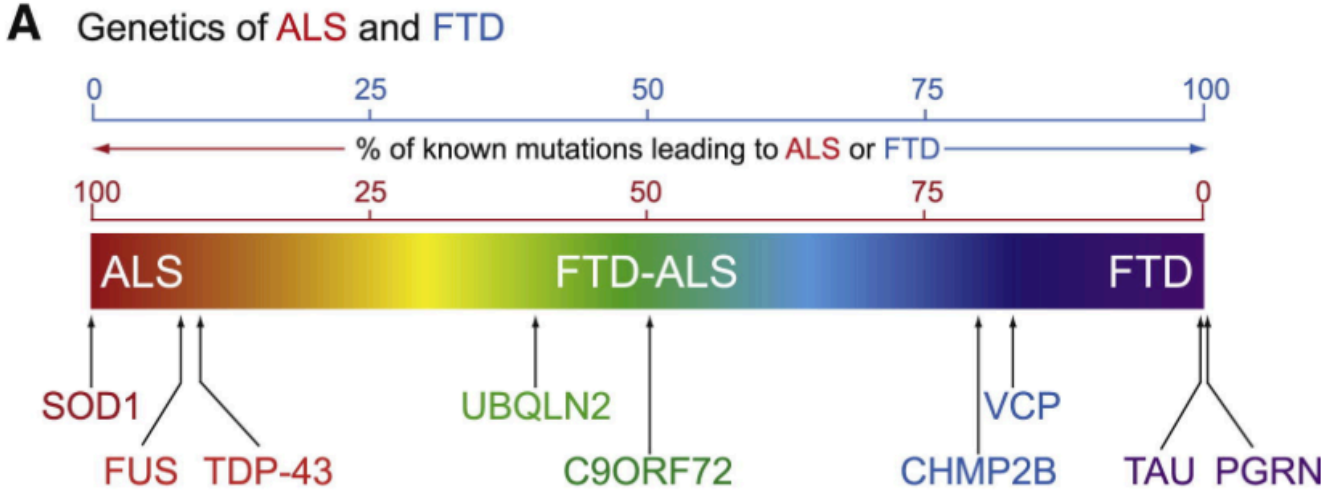
Mackenzie et al. Acta Neuropathol 2011;121, 207-218

Mutations in FUS/TLS result in FTD and ALS



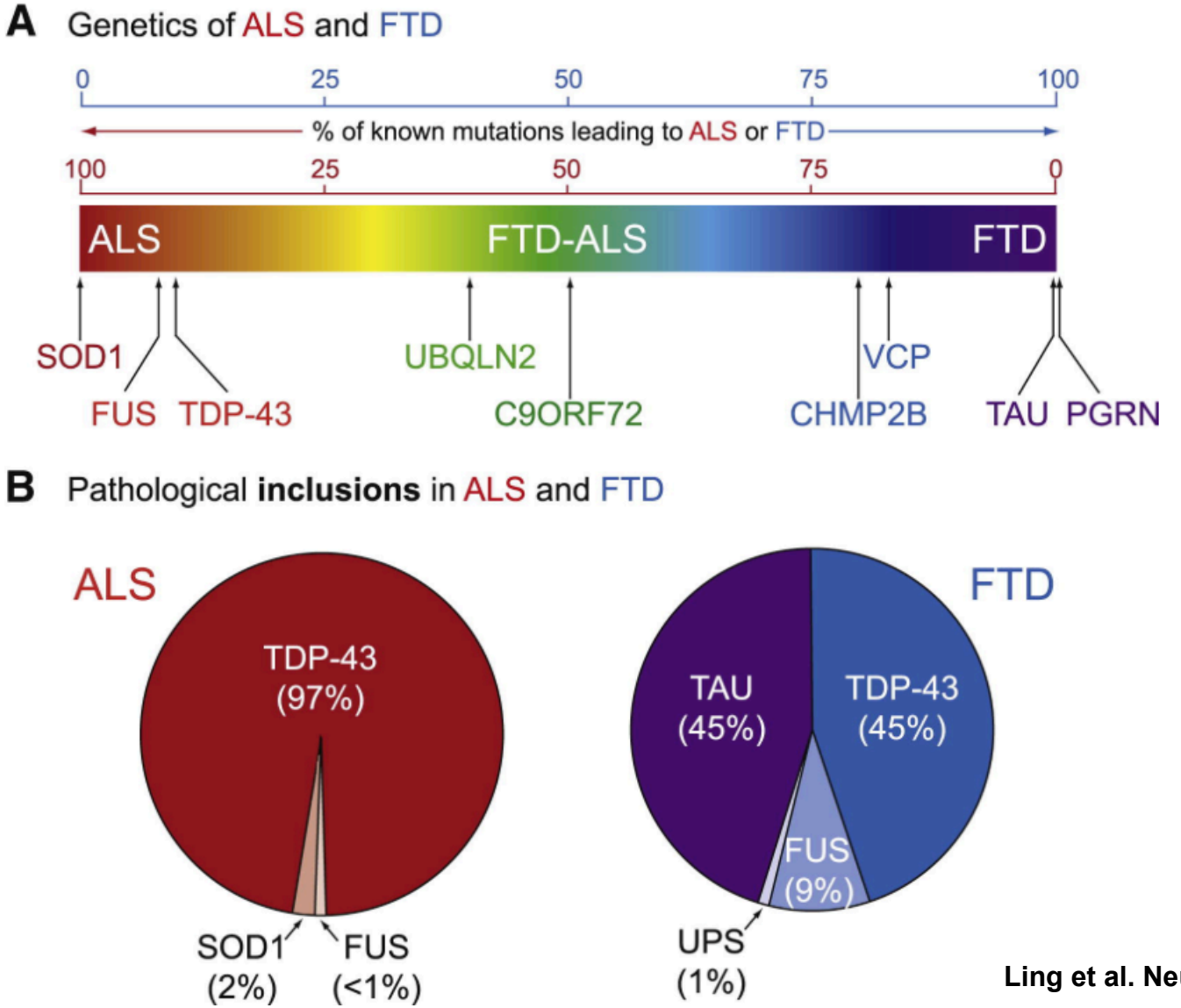
Da Cruz et al. Curr. Opin. Neurobiol. 2011;21:904

Link between RNA metabolism and Pathological Inclusions



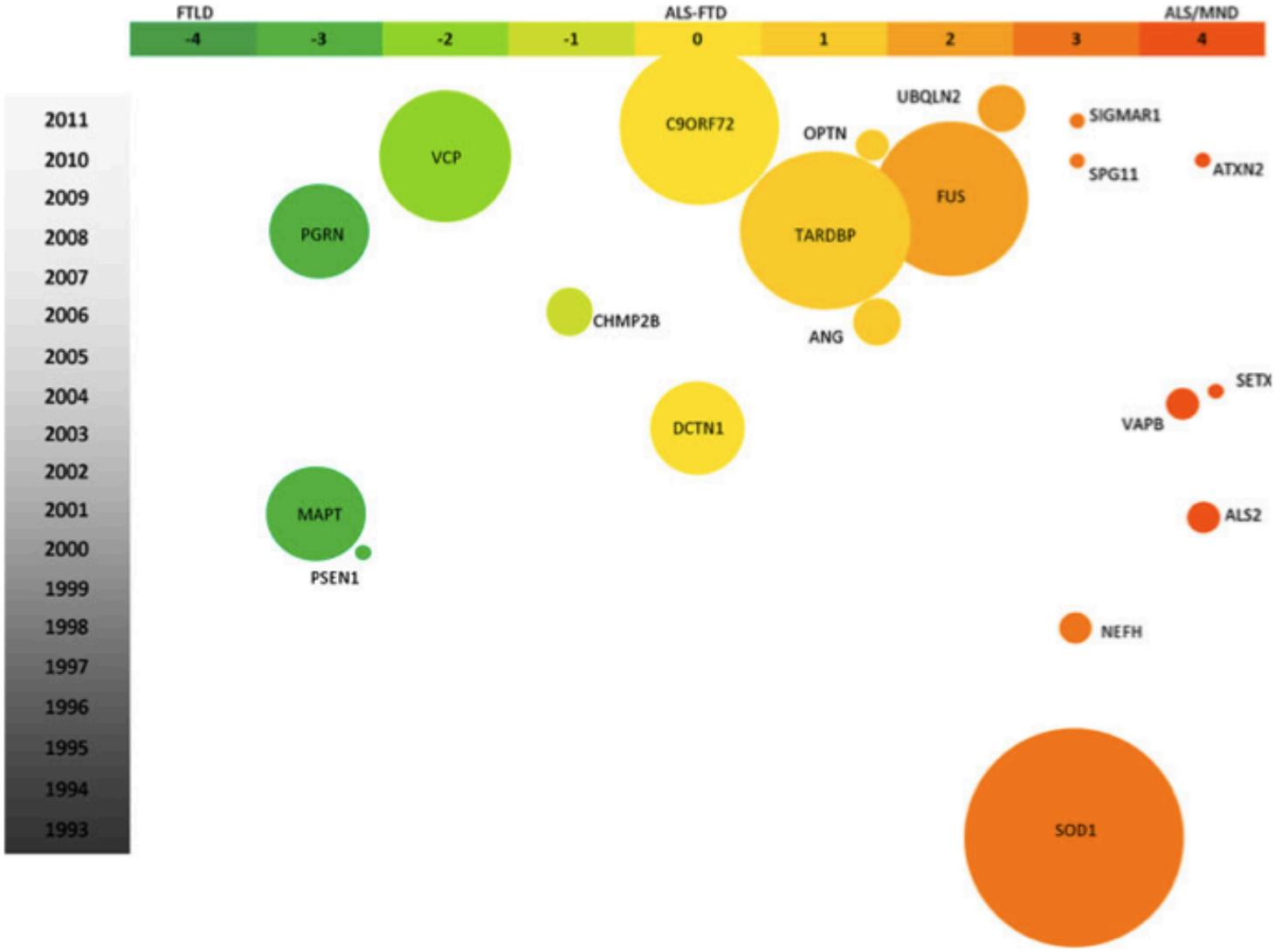
Ling et al. Neuron 2013;79:416

Link between RNA metabolism and Pathological Inclusions



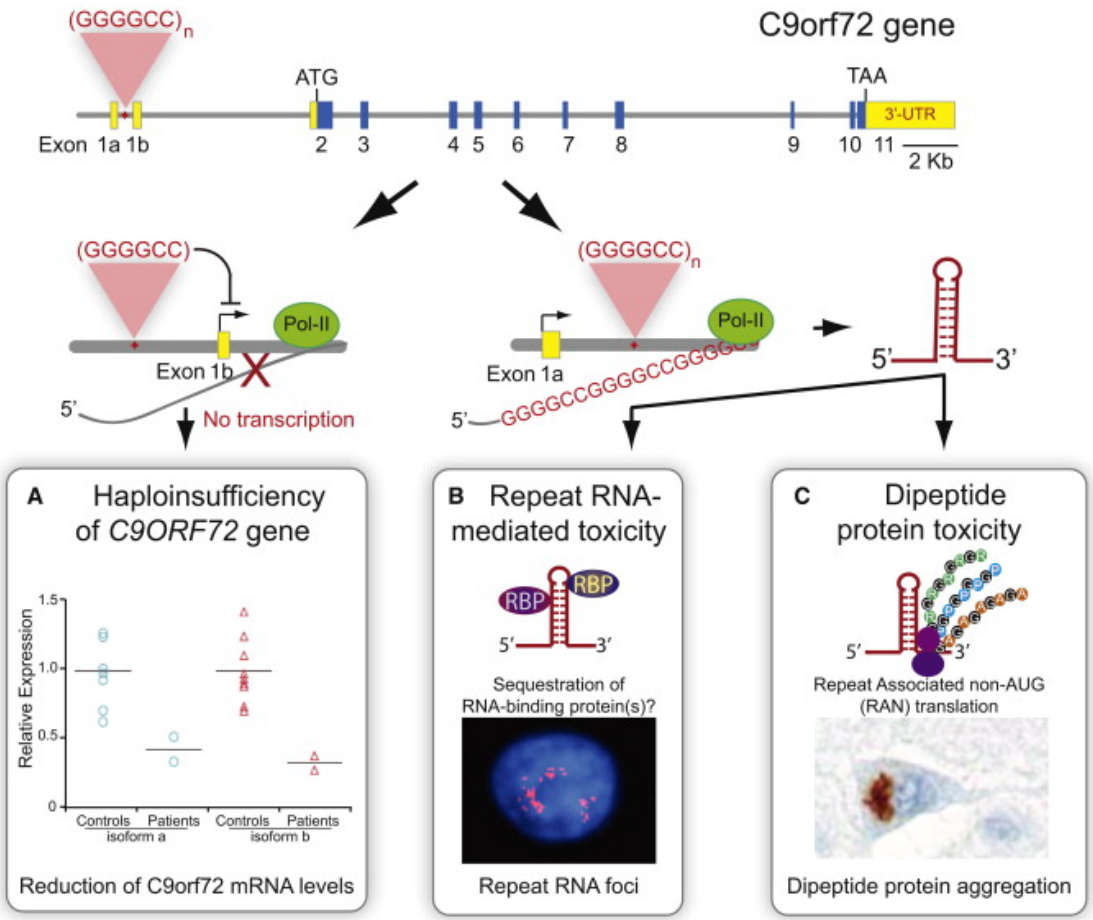
Ling et al. Neuron 2013;79:416

Clinical - Genetic Spectrum of ALS-FTD



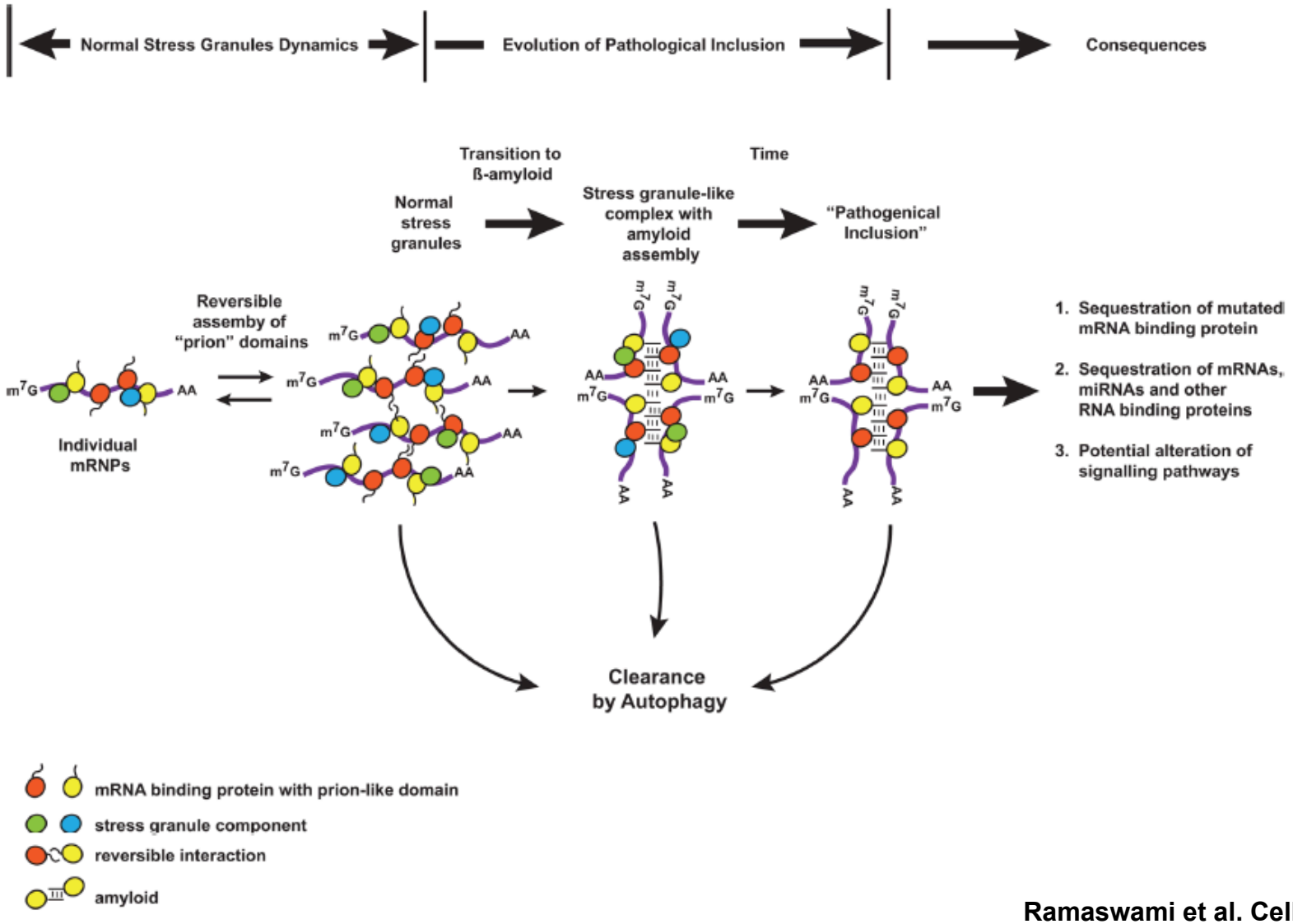
Acta Neuropathol, 2012: 124: 339-352

Another Link between FTD and ALS



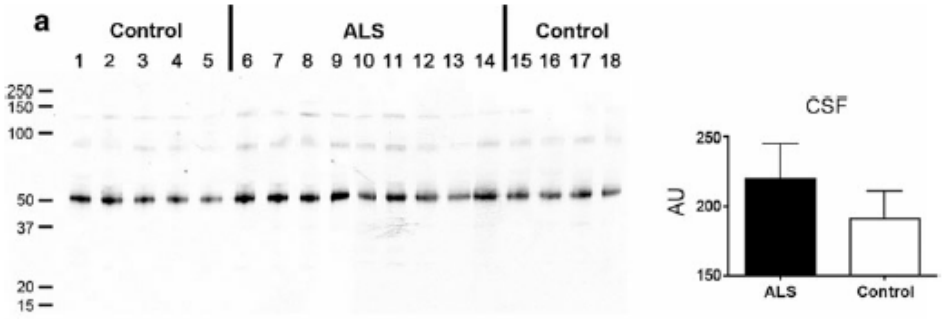
Ling et al. Neuron 2013;79:416

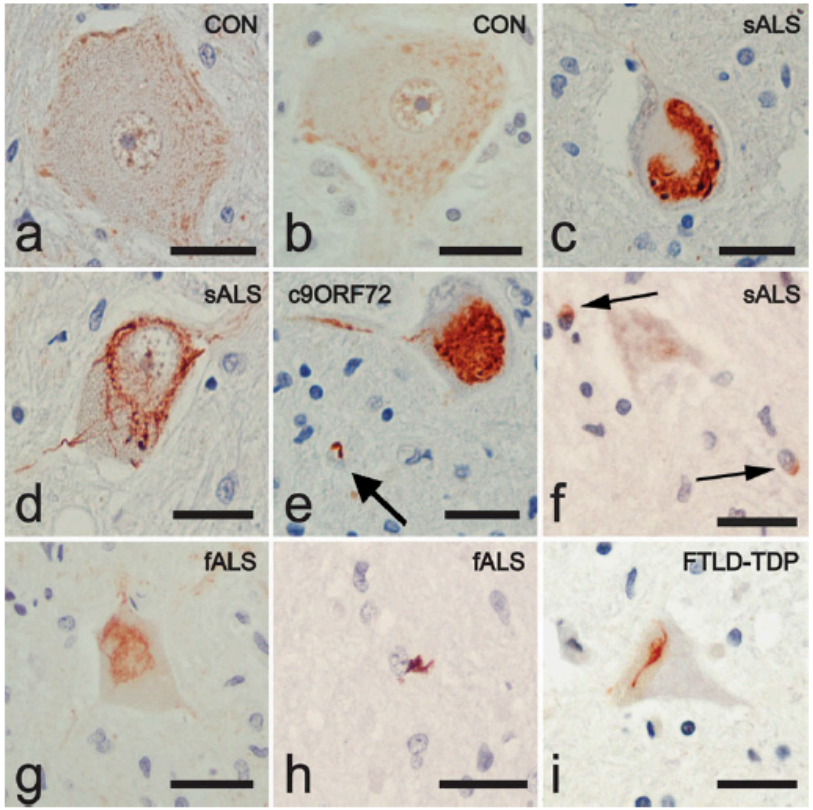
Why prion-like domains in RNA-binding proteins?



Ramaswami et al. Cell 2013;154

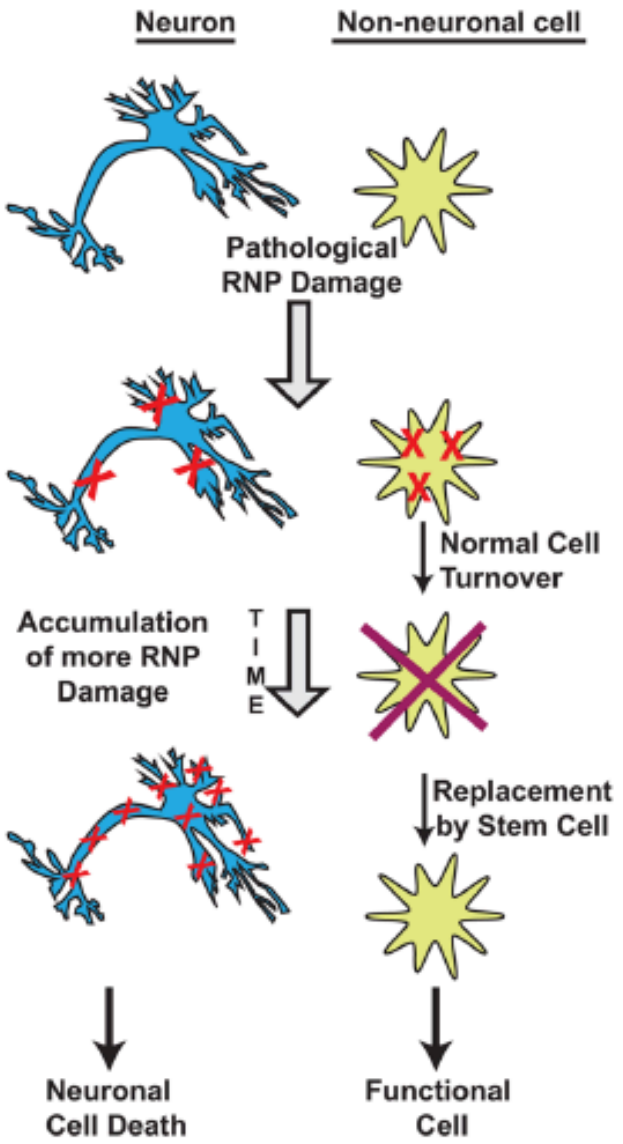
RNA - binding motif 45 accumulates with TDP-43 inclusions





Collins et al. Acta Neuropathol 2012;124:717

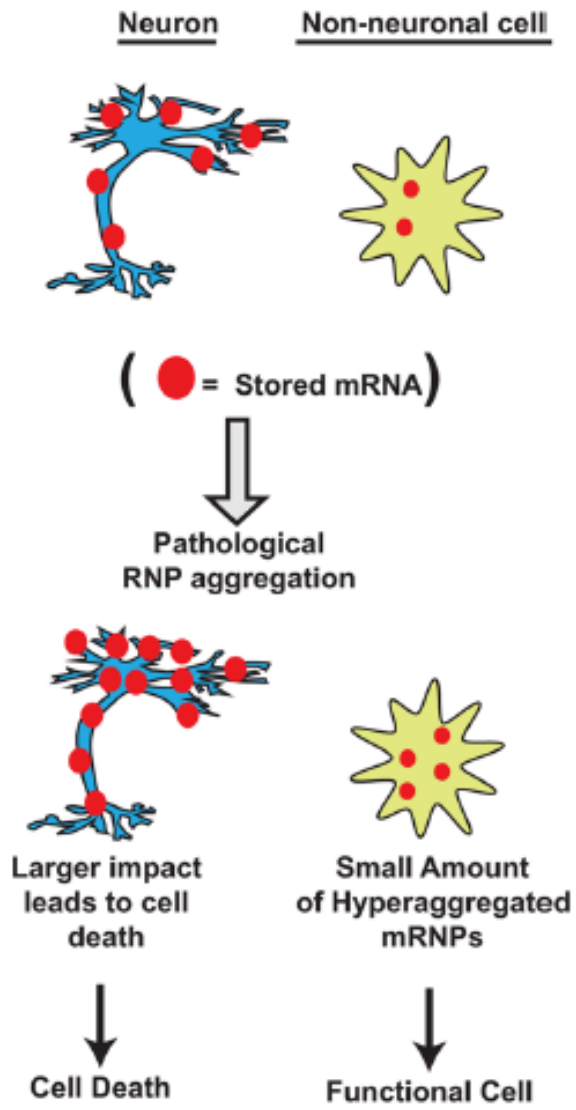
Why neurons? Why motor neurons?



Longevity of neurons allows accumulation of more damage

Ramaswami et al. Cell 2013;154

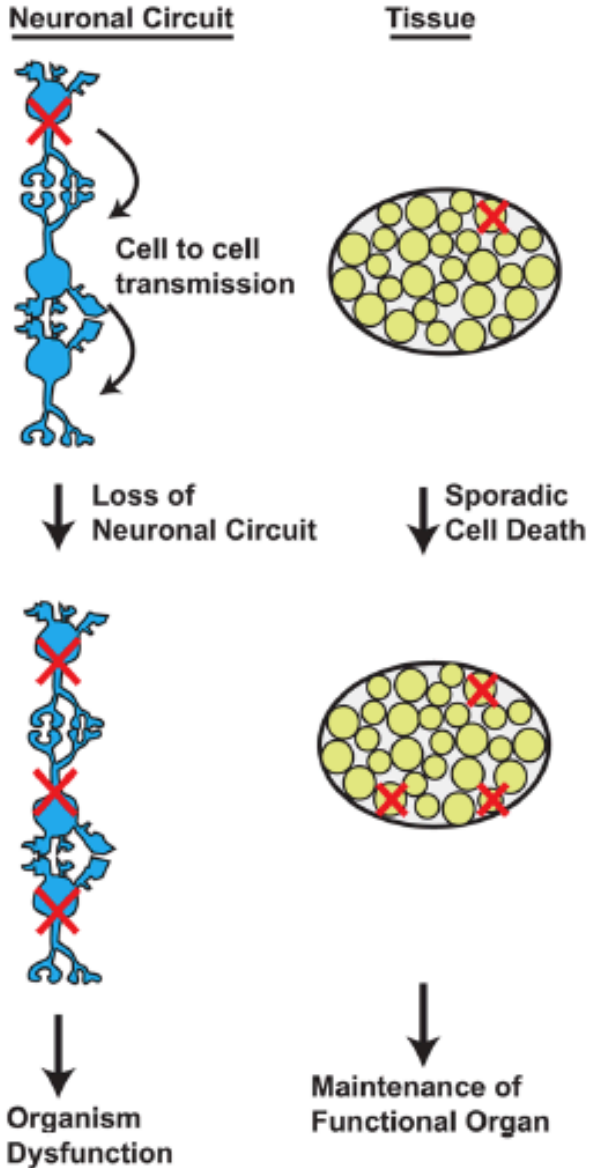
Why neurons? Why motor neurons?



Higher amount of stored mRNA increases sensitivity to hyperaggregation

Ramaswami et al. Cell 2013;154

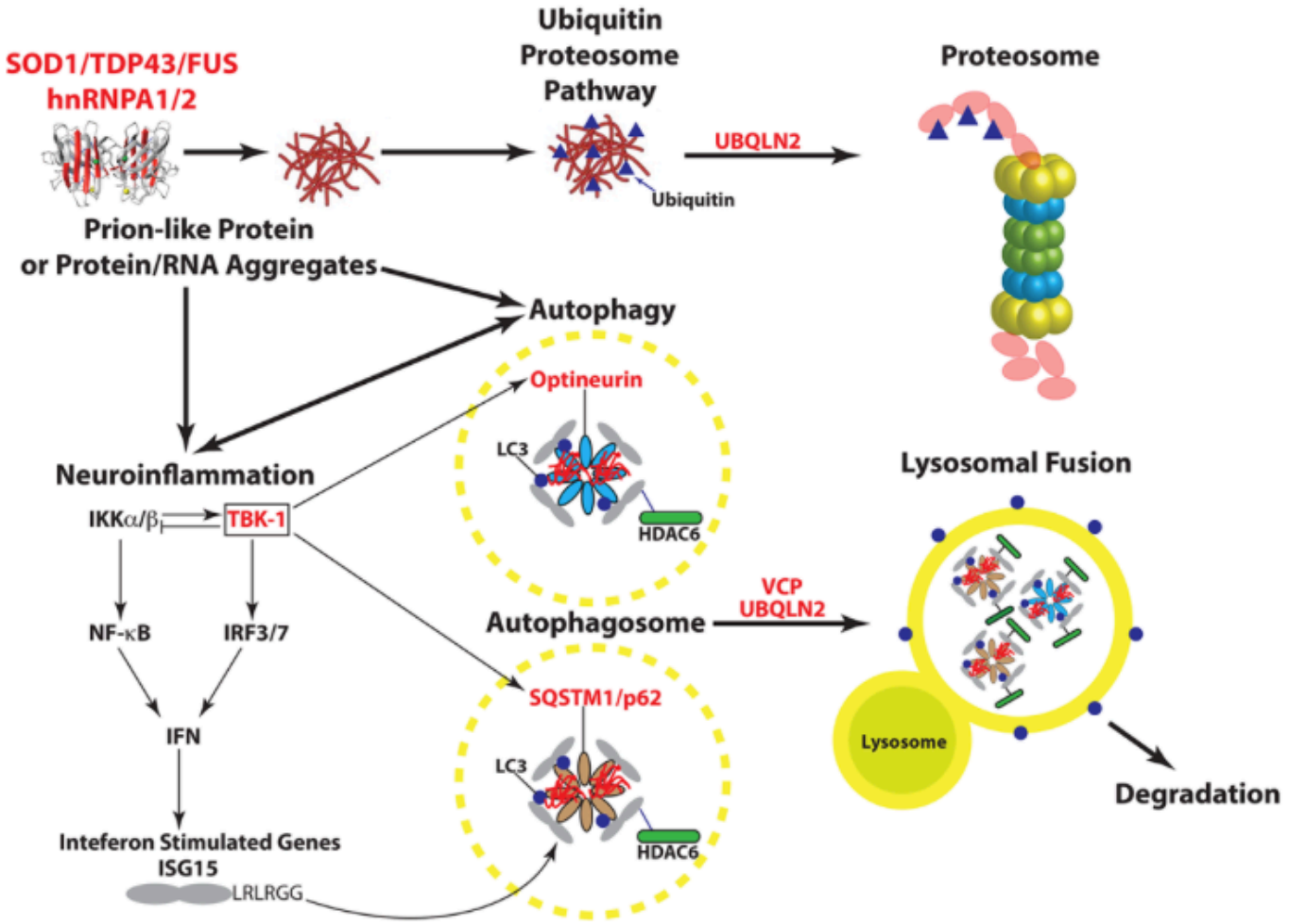
Why neurons? Why motor neurons?



Connectivity of neurons causes systemic spread and functional failure

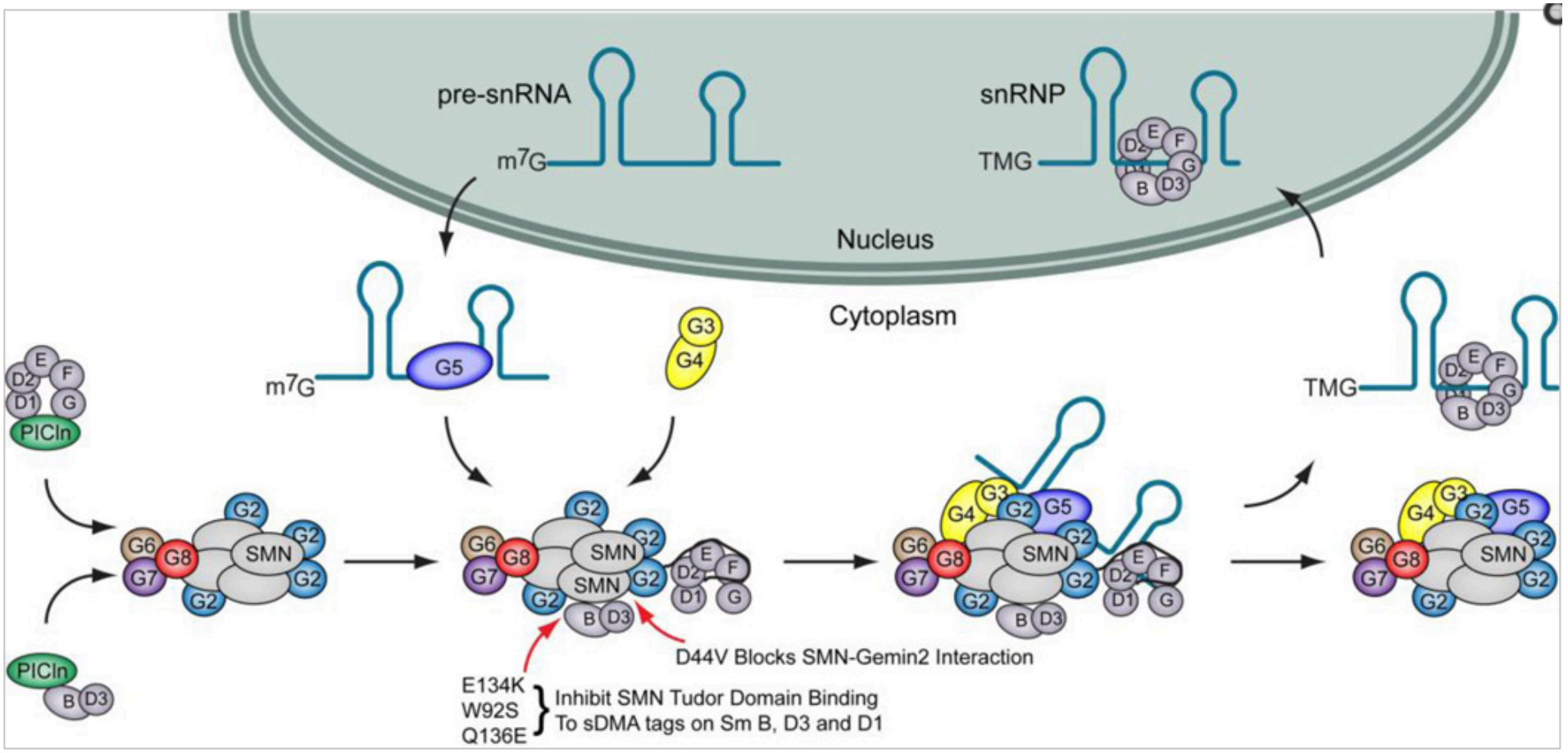
Ramaswami et al. Cell 2013;154

Putative downstream consequences of aberrant RNP formation



Cirulli et al. Science 2015;Feb 19. aaa3650

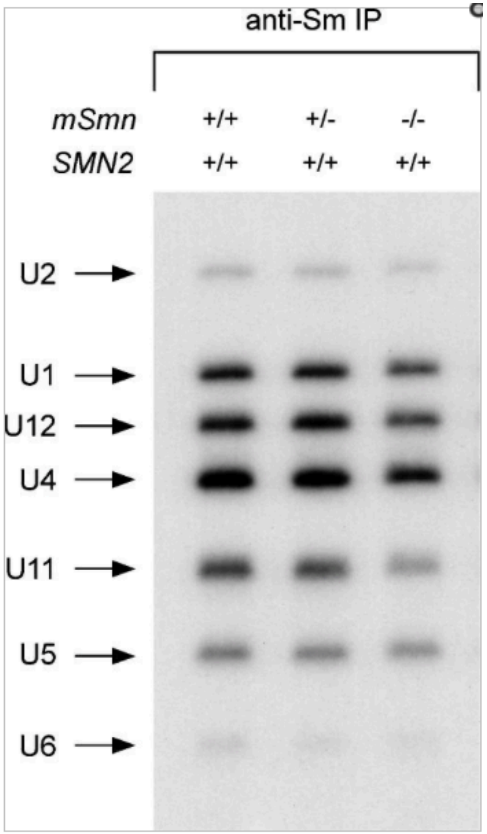
The example of Spinal Muscular Atrophy

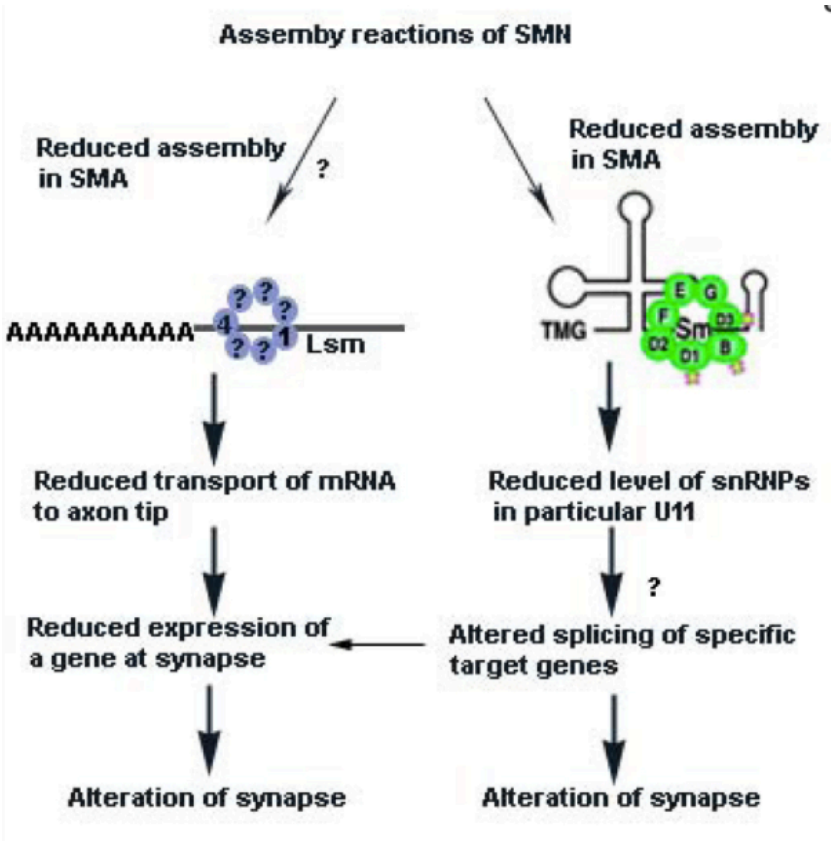


Workman et al. Brain Res 2012;1462, 93

The example of Spinal Muscular Atrophy

Reduced snRNP Assembly

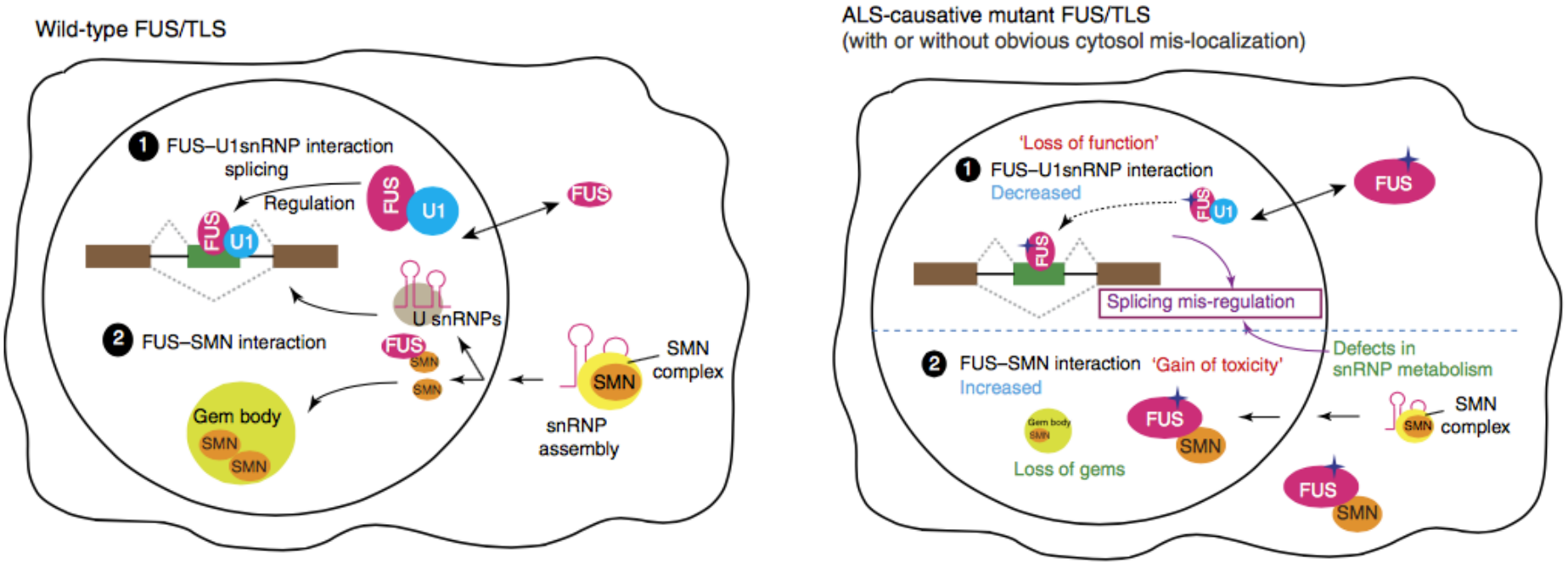




Gabenella et al. PlosOne 2007;2, e921

Burghes and Beattie Nat. Rev. Neurosci. 2009;10, 597

Another example of Molecular Convergence



Sun et al. Nature Communication 2015;6:6171

“Final Common Pathway?”

- How best to study RNA processing events in neurons and relevant non-neuronal cells?
- What is the molecular “tipping point” for neuronal death?
- Why is there a precipitous “spread” of neuronal death once the “tipping point” is realized?
  - Multiple types of therapy?
- What type of biomarkers can we develop that are based upon RNA processing defects?
- What are the therapeutic targets?

Charles Scott Sherrington 1857-1952

What is the molecular basis of motor neuron vulnerability?

“Let us keep looking, in spite of everything. Let us keep searching. It is indeed the best method of finding, and perhaps thanks to our efforts, the verdict we will give such a patient tomorrow will not be the same we must give this man today.”

Charcot (1889)