Tricks that our cells use to express their genes in the brain, and how aging affects this

Jernej Ule, ulelab.info
How do genes get expressed?
How RNA processing affects gene expression?

Lu et al., WIREs RNA, 2012
Ribonucleoprotein complexes (RNPs)

Neuronal regulatory functions

Longest 3’UTRs in neurons up to 10000nt

mRNAs are transported to allow localised protein synthesis

Motor neuron disease (ALS)

No cure available
Lifetime risk ±1/400 in UK

Mutations in genes encoding TDP-43 and eight other RNA-binding proteins can cause ALS
Aggregates of TDP-43 are seen in the neurons of spinal cord in amyotrophic lateral sclerosis (ALS) and brain cortex in frontotemporal dementia (FTD)

Mackenzie et al., The Lancet Neurology. 2010
Mutations in RNA-binding proteins cause many diseases

IDR = intrinsically disordered region
Human induced pluripotent cells (iPSCs) as a disease model

Avior et al., Nat Rev MCB, 2016
RNA remodelling during motor neurogenesis in human iPS models of ALS

iPSC: Induced pluripotent stem cells
NPC: neural precursors
pMN: precursor motor neurons patterned for the ventral spinal cord
MN: post-mitotic but electrophysiologically immature motor neurons
mMN: electrophysiologically mature MNs
iPSC: Induced pluripotent stem cells
NPC: neural precursors
pMN: precursor motor neurons patterned for the ventral spinal cord
MN: post-mitotic but electrophysiologically immature motor neurons
mMN: electrophysiologically mature MNs
RNA remodelling during motor neurogenesis in human iPS models of ALS

iPSC: Induced pluripotent stem cells
NPC: neural precursors
pMN: precursor motor neurons patterned for the ventral spinal cord
MN: post-mitotic but electrophysiologically immature motor neurons
mMN: electrophysiologically mature MNs
Why might RNA processing be modified in ALS?

RNA:
Each RNA interacts with RNA-binding proteins (RBPs)

Ribonucleoprotein complex:
Protein-RNA binding studies

- UV crosslinking and immunoprecipitation (iCLIP, hiCLIP)
- Sequence and structural RNA motifs

Regulatory functions of RBPs

- Alternative splicing
- Alternative polyadenylation

Dynamics of ribonucleoprotein complexes in development, disease and evolution

- Position-dependent RNA maps
- Combinatorial regulation

Regulatory mechanisms

Neuronal RNA biology
iCLIP: nucleotide-resolution crosslinking and immunoprecipitation

1. In vivo protein-RNA crosslinking
2. Immunoprecipitation and RNA fragmentation
3. Oligonucleotide adapter ligation
4. SDS-PAGE purification and size-selection of protein-RNA complex
5. Digestion of RBP by proteinase K
6. Reverse transcription
7. Ligation of adapter to cDNA starts to allow amplification of truncated cDNAs
8. High-throughput sequencing

Konig et al., NSMB, 2010
Haberman et al, Gen biol, 2017
TDP-43 regulates splicing in a position-dependent manner

RNA splicing map:

regulated exons

enhanced

repressed

Tollervey et al., Nat Neuro, 2011
TDP-43 regulates splicing & alternative polyadenylation

Regulated exon

Binding to 3’ UTR

Tollervey et al., Nat Neuro, 2011
TDP-43 binding around the regulated polyA sites

Repressed targets (top 20) around proximal site (enhanced=143, repressed=152, controls=925)

Enhanced targets (top 20) around proximal site (enhanced=143, repressed=152, controls=925)

http://www.expressrna.org

Rot et al, Cell Reports, 2017
Comparison of TDP-43 binding in FTLD vs. healthy control brain tissue
TDP-43 binds to NEAT1, a long non-coding RNA (lncRNA)

Tollervey et al, Nat neurosci, 2011
Undifferentiated stem cells express only the short unstable NEAT1 isoform.

Paraspeckles are assembled by NEAT1 lncRNA.

wikipedia image, by Archa Fox
Protein-RNA complexes often form membraneless organelles.
Occurrence of NEAT1 ncRNA is increased in ALS motor neurons

Nishimoto et al, Mol Brain, 2013
We studied paraspeckle dynamics during stem cell differentiation.
NEAT1 and paraspeckles are induced by differentiation

Modic et al, in preparation
TDP-43 represses production of the full NEAT1 transcript

Modic et al, in preparation
Undifferentiated stem cells express only the short unstable NEAT1 isoform. TDP-43 binds close to the polyA site in the NEAT1 transcript. Modic et al, in preparation.
Undifferentiated stem cells express only the short unstable NEAT1 isoform

TDP-43 promotes formation of the short NEAT1 transcript

Abundance of TDP-43 decreases upon spontaneous differentiation of mouse ES cells
Knockout of the internal polyA site allows production of $NEAT1v2$

Modic et al, in preparation
Knockdown of TDP-43 allows production of \textit{NEAT1v2}

\textbf{NEAT1v1}

\textbf{NEAT1v2}

**smFISH isoform quantification**

<table>
<thead>
<tr>
<th></th>
<th>CTR mESC</th>
<th>\textit{Neat1\textDelta pA} mESC</th>
<th>\textit{iTDPKO} mESC</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Neat1v1}</td>
<td>50</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>\textit{v2}</td>
<td>25</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

**smFISH isoform quantification**

<table>
<thead>
<tr>
<th></th>
<th>CTR hESC</th>
<th>\textit{NEAT1\textDelta pA} hESC</th>
<th>TDP-43 KD hESC</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Neat1v1}</td>
<td>50</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>\textit{v2}</td>
<td>25</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

\textit{Modic et al, in preparation}
Paraspeckles partially sequester TDP-43 away from mRNAs

Modic et al, in preparation
Cells overexpressing TDP-43 exhibit a developmental delay

SSEA1 is a marker for murine pluripotent stem cells

Modic et al, in preparation
Cells lacking NEAT1 exhibit a developmental delay

SSEA1 is a marker for murine pluripotent stem cells

flow-cytometry analysis after 48h of spontaneous differentiation of mouse iPS cells

SSEA1 is a marker for murine pluripotent stem cells

Modic et al, in preparation
TDP-43 knockdown induces the ‘differentiated’ pattern of 3’ end mRNA processing

Comparison of TDP-43 knockdown and spontaneous differentiation of mouse ES cells

Modic et al, in preparation
TDP-43 regulates 3’end processing of SOX2 pre-mRNA
TDP-43 is required for expression of SOX2 protein in pluripotent cells

Fatica and Bozzoni, Nat Rev Genet, 2014
Cross-regulation of TDP-43 and paraspeckles promote a bistable switch

Partial sequestration of TDP-43 away from mRNAs

Paraspeckles

Partial sequestration of multiple RBPs

Repression of long NEAT1 RNA

Production of SOX2 and other proteins that promote pluripotent state
How are neurodegenerative diseases linked to brain aging?

![Diagram showing the link between neurodegenerative diseases and brain aging through processes such as loss of protein homeostasis, DNA damage, lysosomal dysfunction, epigenetic changes, and immune dysregulation.]

Wyss-Coray et al., Nature, 2016
Splicing of TDP-43-regulated alternative exons in human brain

Tollervey et al., Genome Res, 2013
Alternative splicing affected by aging and/or disease in human brain

Tollervey et al., Genome Res, 2013
Changes in cell-type specific gene expression programmes

Tollervey et al., Genome Res, 2013
Transcriptional hallmarks of human brain aging

RNA expression data sets

UKBEC samples
N = 1,231

NABEC samples
N = 607

Mouse cell-types
N = 12 x 7

Human cell-types
N ≥ 4 x 24

Lilach Soreq

Soreq et al., Cell Rep, 2017
Analysis of cell-type specific gene expression upon aging

Soreq et al., Cell Rep, 2017
Neuroinflammation in neurodegeneration

Schwartz et al., Trends Immun, 2016
Immunohistochemistry of NeuN-positive cells in the frontal cortex

NeuN staining

Soreq et al., Cell Rep, 2017
NeuN-stained image analysis

Soreq et al., Cell Rep, 2017
Analysis of astrocyte and oligodendrocyte-specific genes

Soreq et al., Cell Rep, 2017
Immunohistochemistry of Olig2-positive cells in the frontal cortex

Olig2 stain N=9k High Resolution slides

Immunohistochemistry Imaging

~0.6mm

~0.46mm

~24μm

~24μm

Soreq et al., Cell Rep, 2017
Quantification Olig2-stained images

Soreq et al., Cell Rep, 2017
Hypothesis:

The compensatory mechanisms are diminished upon aging.

Defective RNA granules disrupt the cell fate choices, leading to decreased glial support, and thus propagation of stressful signals that can lead to neuronal death.
Aging & TDP-43 in ALS

Collaborators

Lilach Soreq
Martina Halleger
Raphaëlle Luisier
Rickie Patani

TDP-43 and paraspeckles

Miha Modic
Helmholtz Munich
Zhen Wang
Gregor Rot
University of Zurich

Nick Luscombe
The Crick
Micha Drukker
Helmholtz Munich
Tomaž Curk
U. of Ljubljana
Cells overexpressing TDP-43 have improved reprogramming

Modic et al, in preparation
TDP-43 regulates 3’ end processing of SMC1A pre-mRNA.

TDP-43 cross-linking in SMC1A RNA as determined by iCLIP

Two different mRNA isoforms in the SMC1A result from alternative 3’ end processing

TGTTGTGTGGTATGATGTGTGTAATGATATGATCCGATGTGTGATGTGAGTATGTTTGCAAAAAATAAAGGGATATTGGAGACCTGTTT

WT SMC1A

polyA signal

proximal polyA site

Rot et al, Cell Reports, 2017
Identification of factors regulating pluripotency breakdown

Bistable switches promote cell fate transitions

Morris et al., Nat Rev Gen, 2016
Decreased of neuron-specific gene expression is a hallmark of neurodegeneration (as expected)

More complex cell type-specific expression changes are the hallmark of aging
- Microglia, endothelial cells: increased expression
- Astrocyte, oligodendrocytes: decreased and shifted regional expression patterns.

The number of oligodendrocytes and largest neurons decreases in the aging frontal cortex

iPSCs from ALS patients uncover changes in the timing of RNA processing events during differentiation of pluripotent cells into motor neurons.

Paraspeckles and TDP-43 form a molecular switch that increases the efficiency of cell differentiation.
A computational pipeline to monitor the dynamic functions of TDP-43

REGULATORY FUNCTION

3'-end sequencing
Manage data
Align to reference

Expression atlas
Compare control vs. test

Alternative polyadenylation

Alternative splicing

BINDING ANALYSIS

RNA-protein binding (iCLIP)

analysis of clustered motifs

POSITION DEPENDENT REGULATION

RNAmotifs: combinatorial motif discovery

RNA maps

Rot et al, in press, Cell Reports
Analysis of a standard immunohistochemistry image

Initial crop: 6Kx6K pixel
1st zoom: 1Kx1K pixel
2nd zoom: 200x200 pixel
3rd zoom: 50x50 pixel
4th zoom: 10x10 pixel
TDP-43 regulates splicing via UG repeat sequences

Pentamer enrichment in replicate experiments:

\[ r = 0.91 \]

Tollervey et al., Nat Neuro, 2011

data at http://icount.biolab.si
Cross-regulation of TDP-43 and paraspeckles promote a bistable switch

Courchaine et al, EMBO J, 2016
Cross-regulation of TDP-43 and paraspeckles promote a bistable switch

Courchaine et al, EMBO J, 2016
Staging of ALS motor neurons according to TDP-43 distribution

Pathological staging of motor neurons in ALS according to TDP-43 distribution

<table>
<thead>
<tr>
<th>Stage</th>
<th>TDP-43 distribution</th>
<th>nuclear membrane</th>
<th>plasma membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in the nucleus</td>
<td>in the cytoplasm</td>
<td></td>
</tr>
<tr>
<td>Stage 0</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Stage I</td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Stage II</td>
<td>ND</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Stage III</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
</tr>
</tbody>
</table>

Nishimoto et al, Mol Brain, 2013
Paraspeckles form in the early stage of ALS (stage 0)

Nishimoto et al, Mol Brain, 2013
TDP-43 inhibits formation of paraspeckles in pluripotent cells

modified from Ayalla et al
How does NEAT1 promote differentiation?

modified from Ayalla et al
TDP-43 binds its own mRNA to regulate alternative polyadenylation
UG-rich motifs bind to the core factors of 3’ end processing-machinery
QuantSeq 3'mRNA Sequencing (Lexogen) is used to prepare cDNA libraries. After soft clipping the adapter, reads are mapped to the genome, identifying polyA sites with nucleotide resolution.

- **Proximal site** (expression=4)
- **Distal site** (expression=7)
RNAmap shows enrichment of TDP-43 crosslinking around pA sites in ES cells. Proximal TDP-43 binding as defined by iCLIP: enhanced proximal mRNA repressed distal enhanced repressed. TDP-43 binding as defined by UG-rich motifs: proximal distal enhanced repressed.
TDP-43 is required for expression of SOX2 protein in pluripotent cells
Cross-regulation of TDP-43 and paraspeckles promote a bistable switch

Modic et al, in preparation
Disease-causing mutations are in the low-complexity (LC) region of TDP-43.
P045: The function and mechanisms of the low complexity domain of TDP-43, presented by Martina Hallegger

iCLIP: RNA interactions

Mass spec: protein interactions
Analysis of microglia and neuron-specific genes

Soreq et al., Cell Rep, 2017
Assessing how paraspeckles affect the mRNPome

Modic et al, in preparation
Two neurodegenerative diseases: ALS and FTLD

A. Genetics of ALS and FTD

B. Pathological inclusions in ALS and FTD

Ling et al., Neuron, 2013