Intranuclear \((G_4C_2)_n\) repeat RNA foci, transcribed from \textbf{C9orf72} hexanucleotide expansion mutation form \textbf{paraspeckle-like} structures
C9orf72 mutation in ALS
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Vatovec, *Neurobiol Ageing* 2014
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SFPQ co-localizes with G₄C₂ RNA foci

Hek 293 cells

Transfection, 24h incubation, fixation

FISH, immunofluorescence
Paraspeckles

- Mammalian-specific subnuclear bodies,
- Assembled on the backbone of the long non-coding RNA NEAT1,
- Proposed to modulate post-transcriptional processes by:
  - Sequestration of the RBP and
  - Nuclear retention of mRNAs.

- More than 40 proteins associate with paraspeckles,
- PSPC1 was the first identified protein,
- NONO and SFPQ are essential for the paraspeckle formation.
NONO and PSPC1 also co-localize with $G_4C_2$ RNA foci

Hek 293 cells
Transfection, 24h incubation, fixation
FISH, immunofluorescence
SFPQ and NONO co-localize with RNA foci in C9orf72 positive cerebellum

- \(G_4C_2\) foci are the **most abundant in cerebellum**,
- Post-mortem brain tissue analyzed using FISH and immunohistochemistry,
- 4.1% of \((G_4C_2)_n\) RNA foci **co-localize with SFPQ** and 4.0% co-localize with NONO.
Do $G_4C_2$ RNA nuclear foci associate with NEAT1 as well?

- Yes, 14%
- However, additional NEAT1-negative, SFPQ-stained subnuclear bodies in cells expressing $G_4C_2$ repeats.
The average number of SFPQ-stained subnuclear bodies per cell increased from 2.3 for mock transfected cells to 9.8 for cells expressing G₄C₂ repeats.

Could NEAT1 be replaced by G₄C₂ repeats as a structural backbone for the paraspeckles?
SFPQ colocalizes with $G_4C_2$ nuclear foci in NEAT1 knockdown HEK293T cells

- Localization of paraspeckle proteins in $G_4C_2$ foci is a NEAT1-independent event!
G₄C₂ RNA foci co-localize with hLincRNA-p21

- Transcripts of genes with inverted repeat Alu elements associate with paraspeckles.
- Human LincRNA-p21 has an IRAlu element leading to co-localization with paraspeckles.

Hek 293 cells Transfection, 24h incubation, fixation Double FISH, immunofluorescence

Paraspeckle structure, Hu et al. JCB, 2016
Conclusions

- $\text{G}_4\text{C}_2$ foci associate with paraspeckle proteins,
- The association is independent of NEAT1,
- $\text{G}_4\text{C}_2$ paraspeckle-like structures associate with Alu-repeat RNAs.
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