

**Intranuclear (G₄C₂)_n repeat RNA foci,
transcribed from **C9orf72**
hexanucleotide expansion mutation
form **paraspeckle-like** structures**

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ENCALS 2017

C9orf72 mutation in ALS

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- The most common genetic cause for ALS – about 40% familial ALS,

SFPQ, NPM1, EF1 α 2, hnRNP-H and Prx-III bind to (G₄C₂)₄₈ RNA *in vitro*

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T7 GGGG CC GGGG CC GGGG CC GGGG CC S1

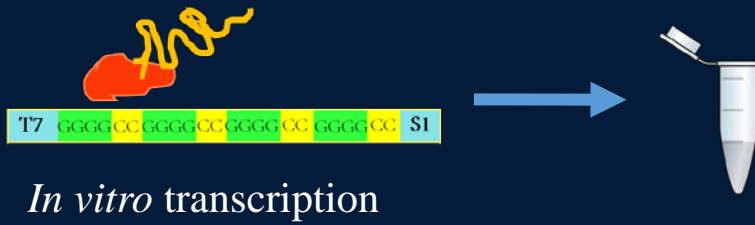
In vitro transcription

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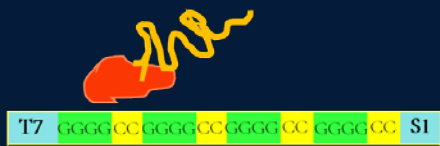


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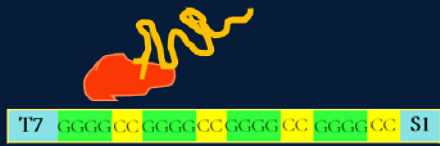


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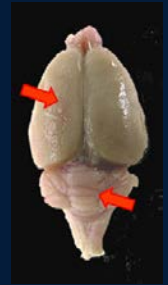


Rat cortex and cerebellum

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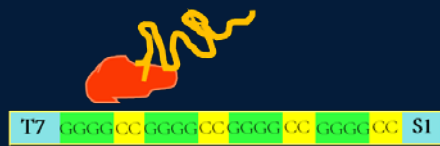


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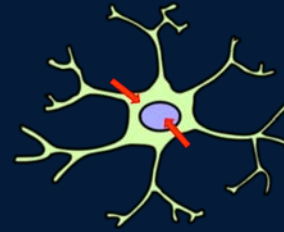


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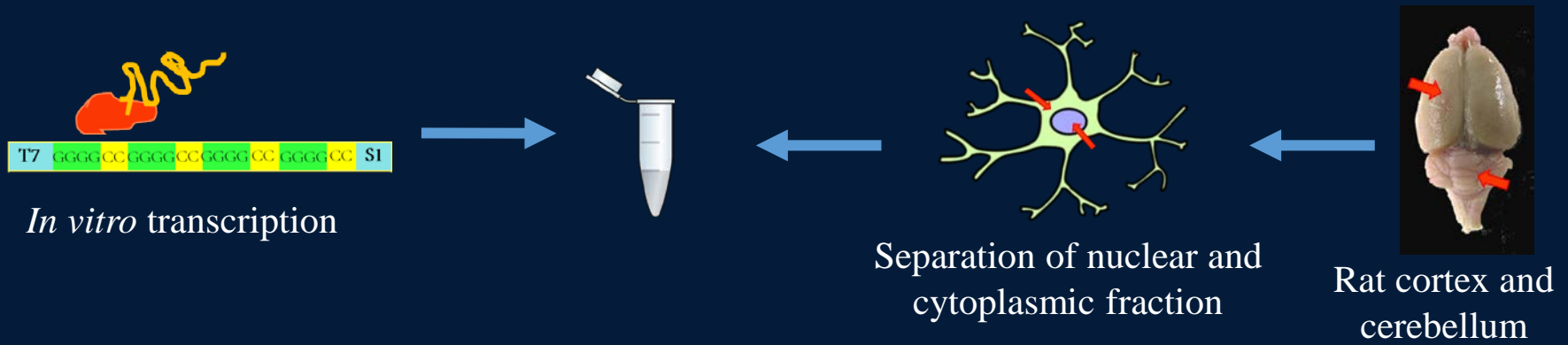


Separation of nuclear and cytoplasmic fraction

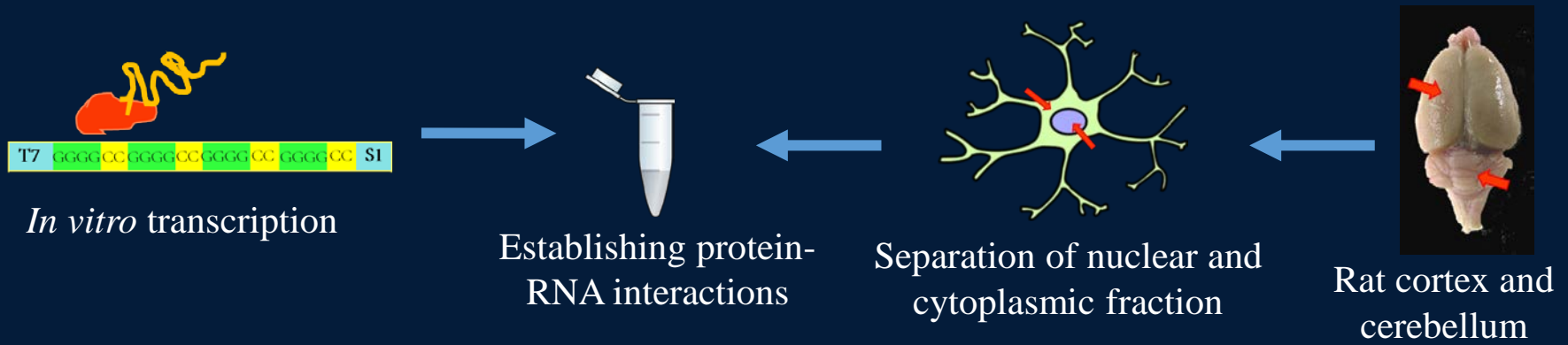


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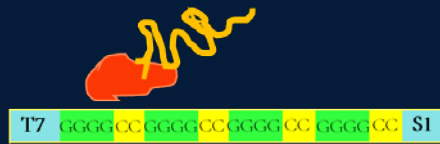
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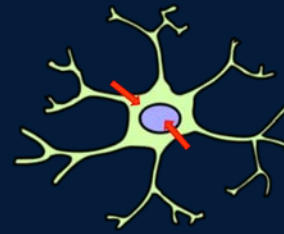
In vitro transcription



Establishing protein-RNA interactions



Separation of nuclear and cytoplasmic fraction

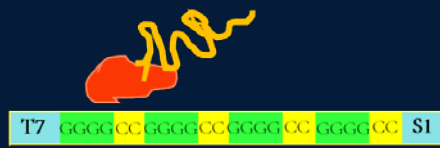


Rat cortex and cerebellum



Specific proteins bound to RNA

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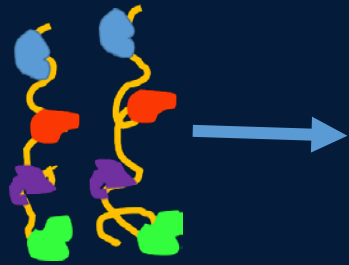
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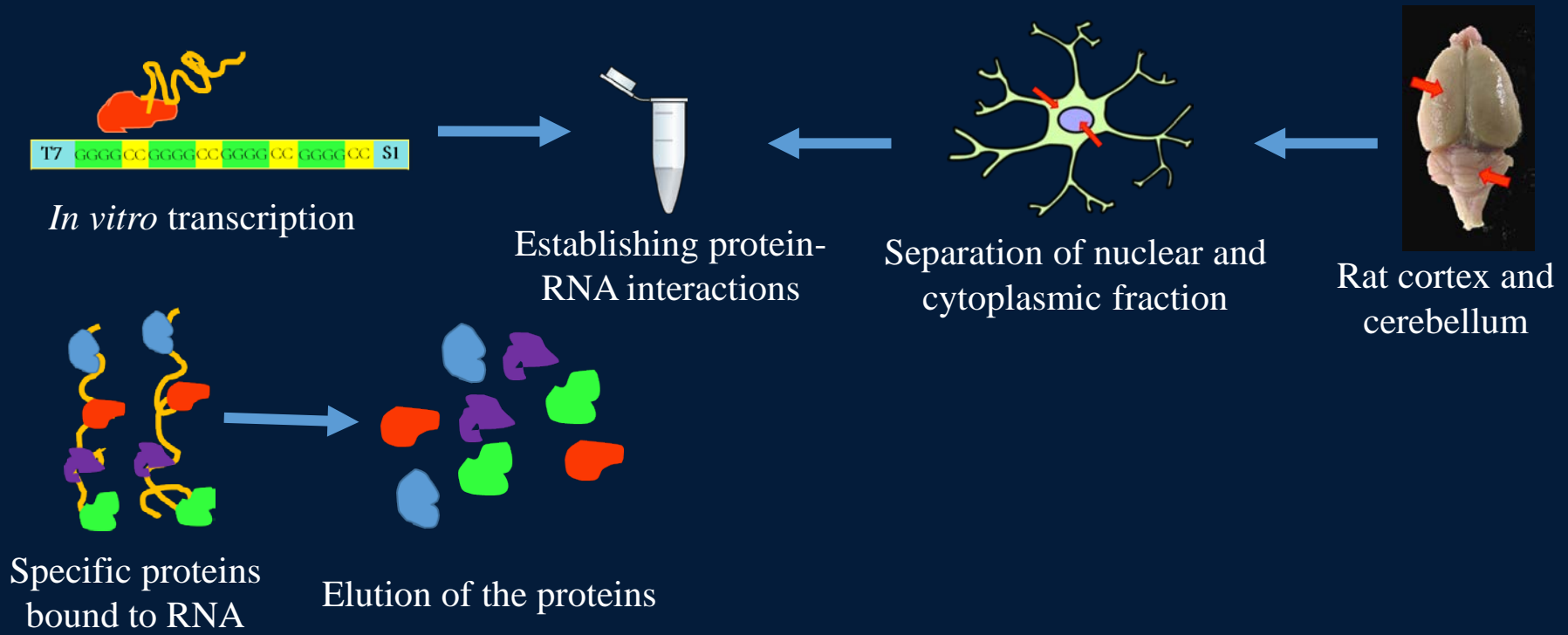


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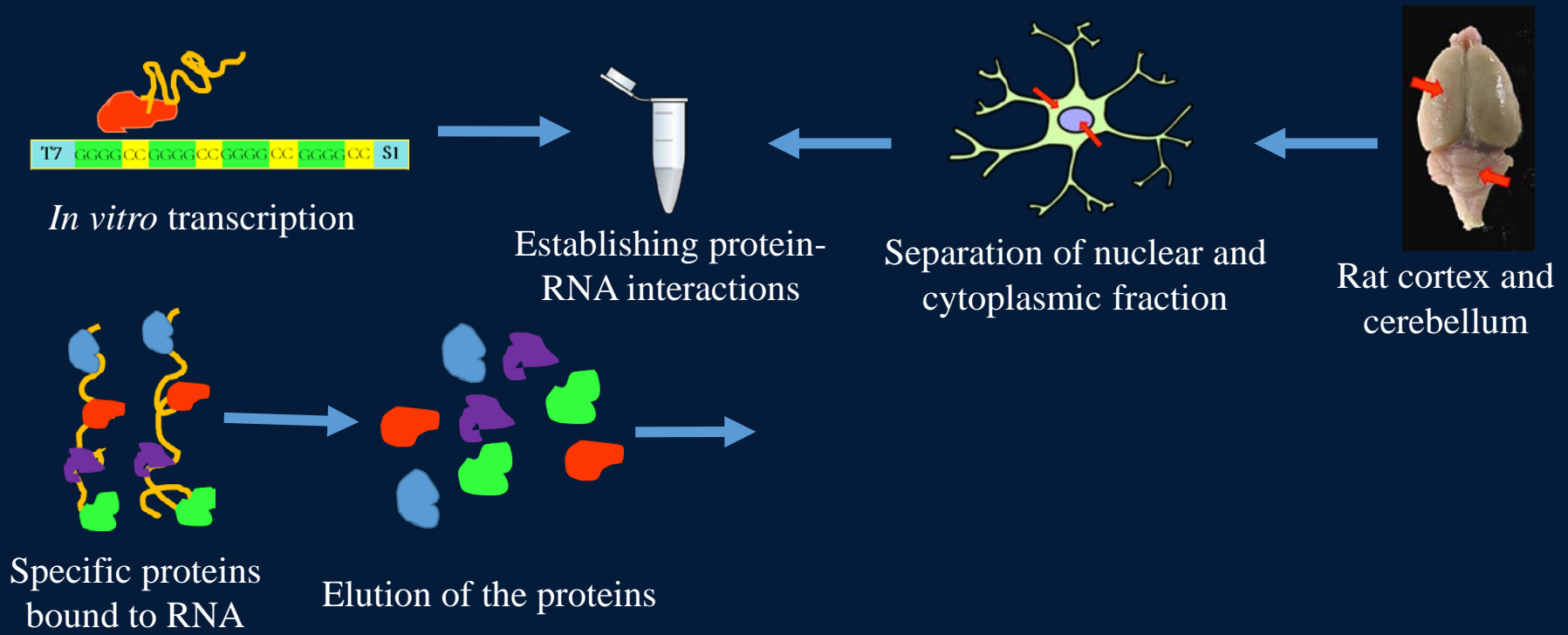


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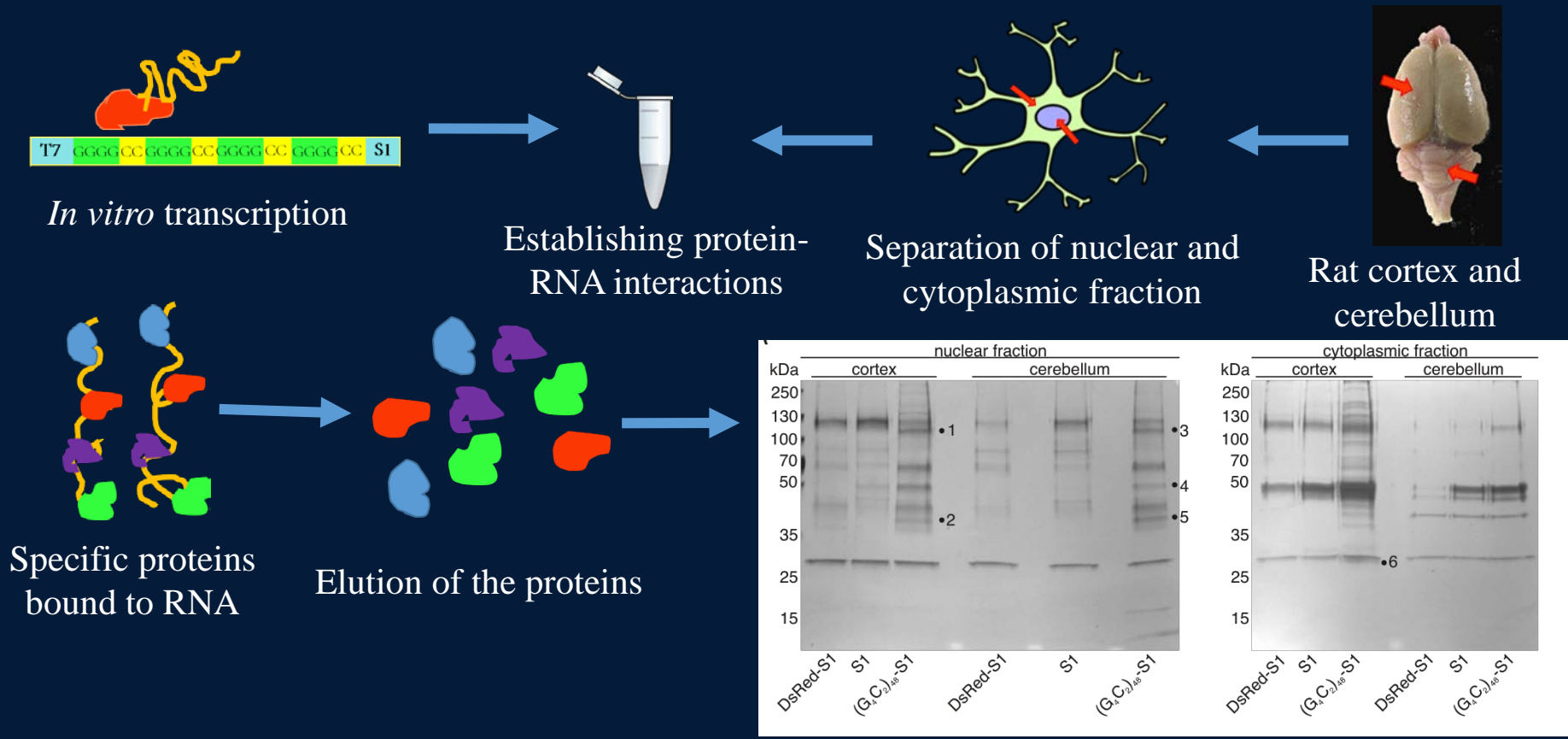
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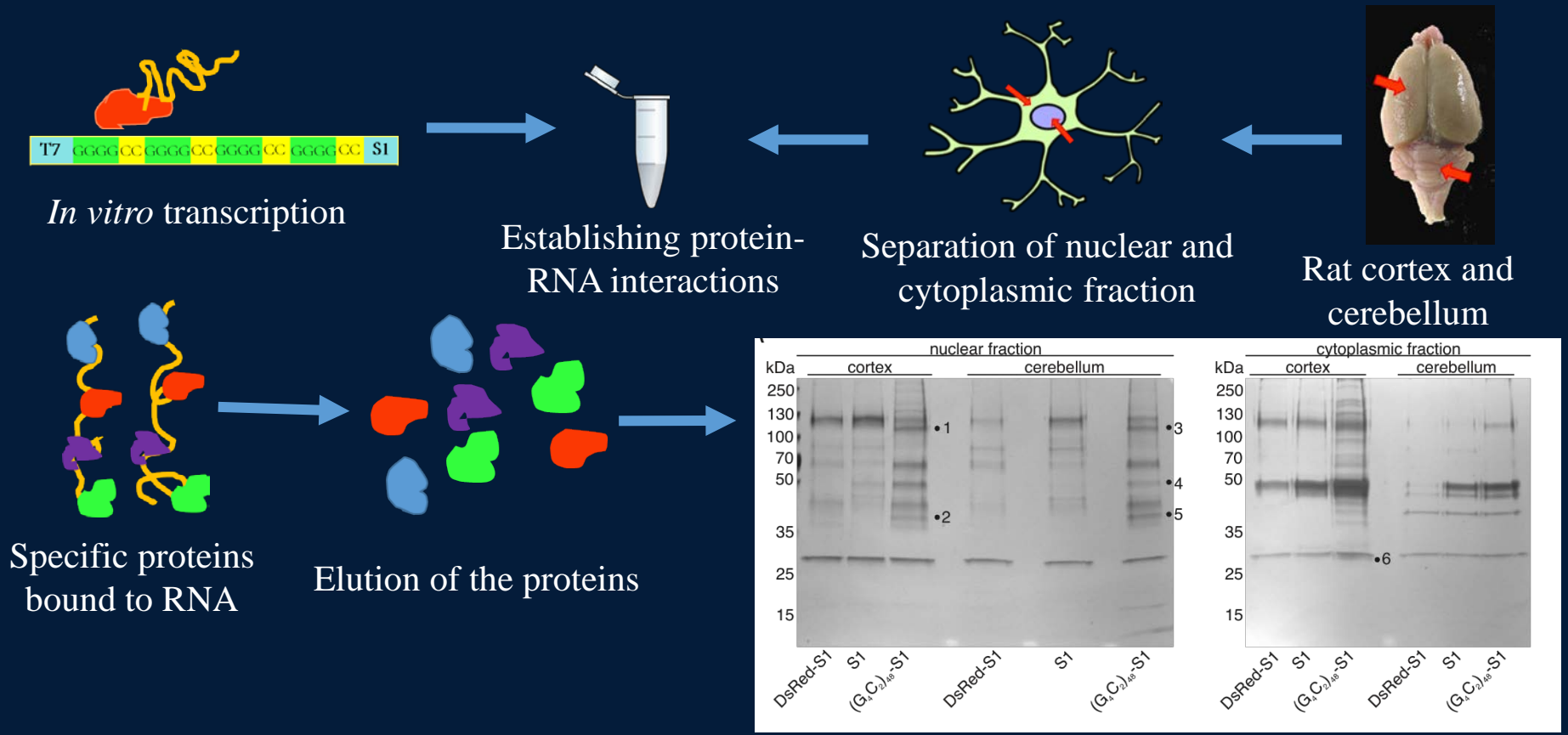
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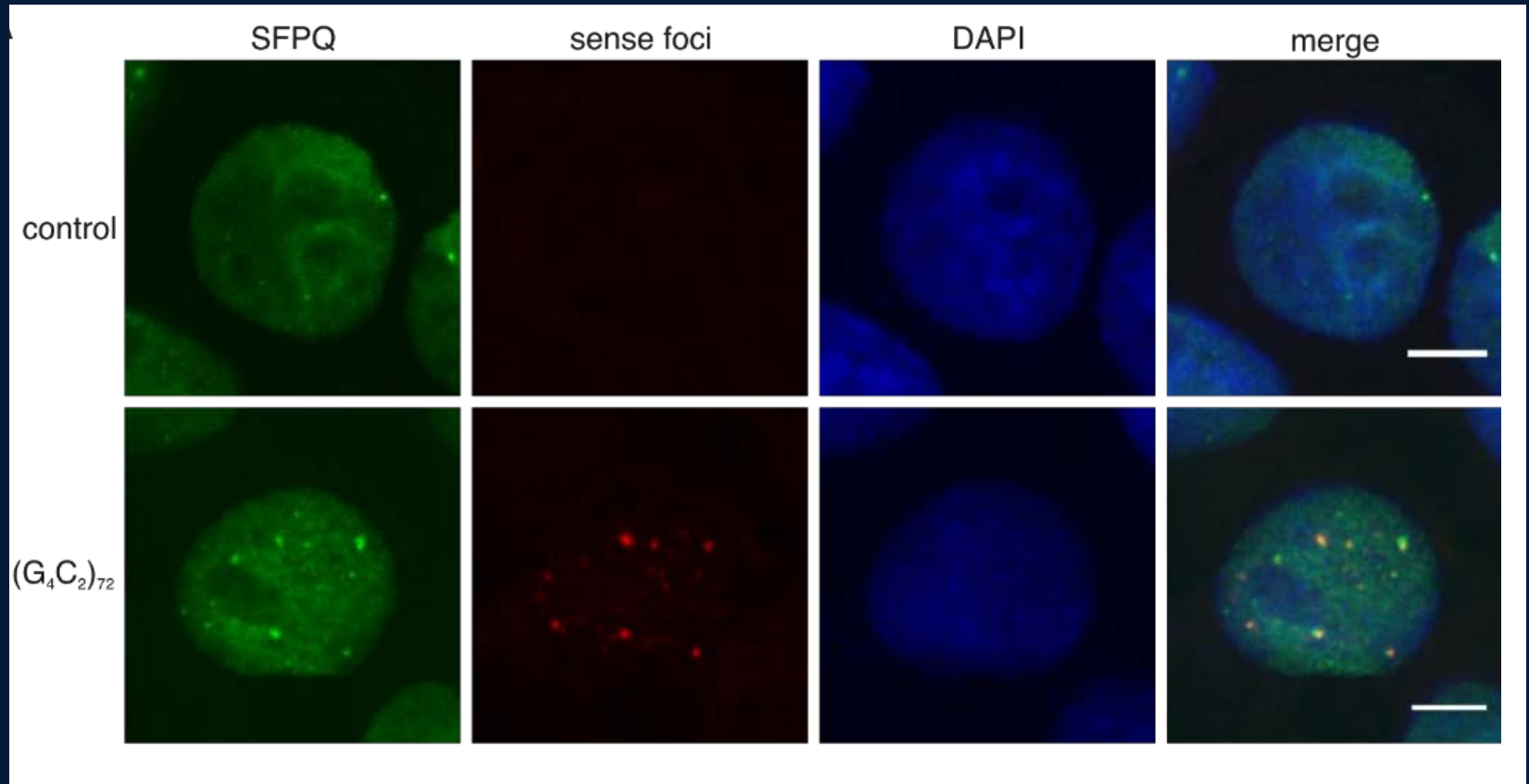
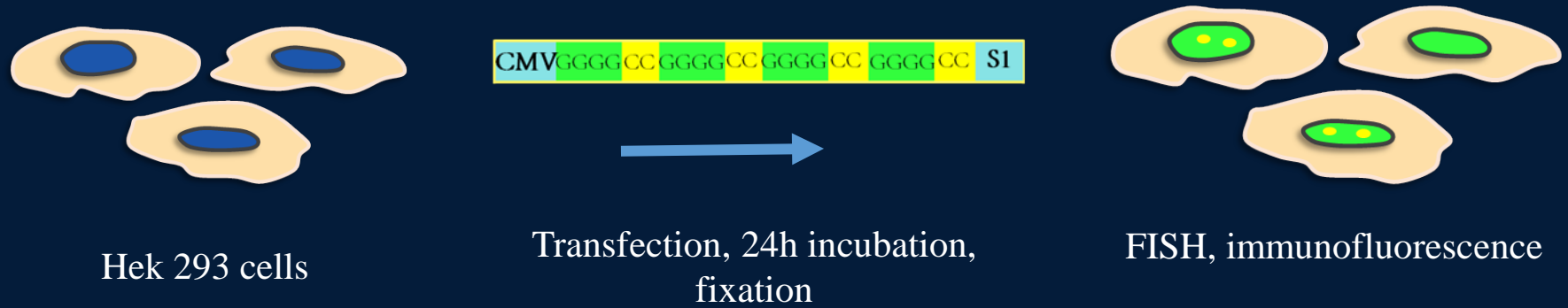


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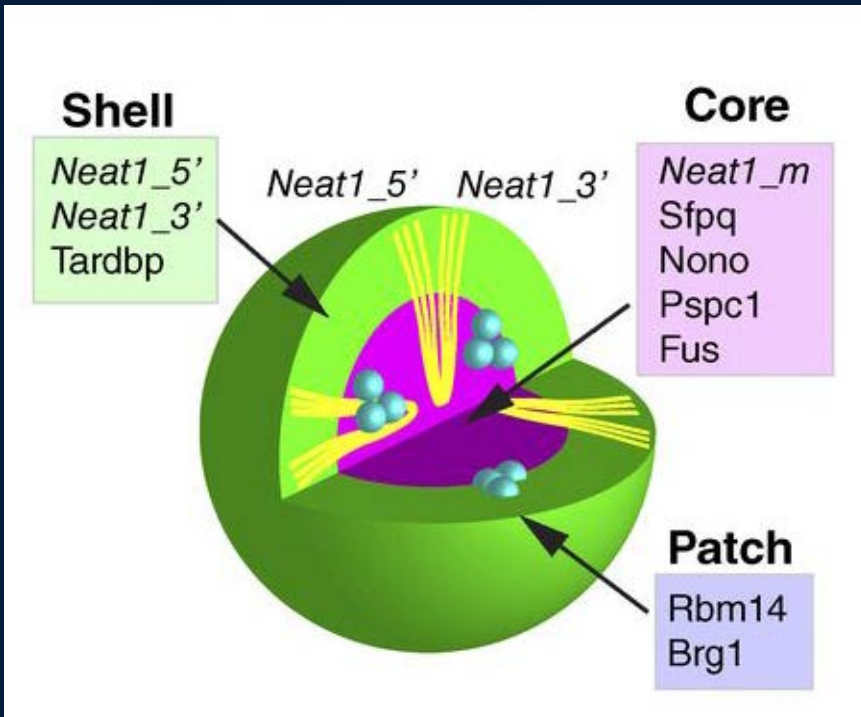
Band No.	Protein	Short name	NCBI accession No.	MW [kDa]	Identified peptides
1, 3	Splicing factor, proline- and glutamine-rich	SFPQ	23956214	75	25
2, 5	Nucleophosmin	NPM1	114763	33	6
4	Elongation factor 1-alpha 2	EF 1 α 2	50402096	50	41
4	Heterogenous nuclear ribonucleoprotein H	hnRNP H	120538378	49	2
6	Thioredoxin-dependent peroxide reductase, mitochondrial	Prx-III	11968132	28	2

SFPQ co-localizes with G₄C₂ RNA foci



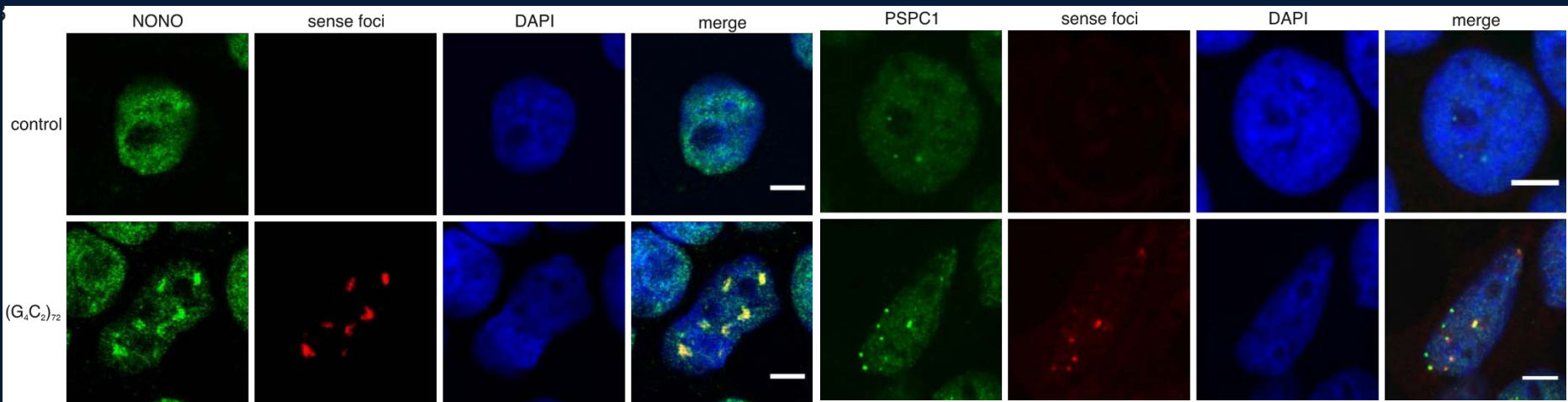
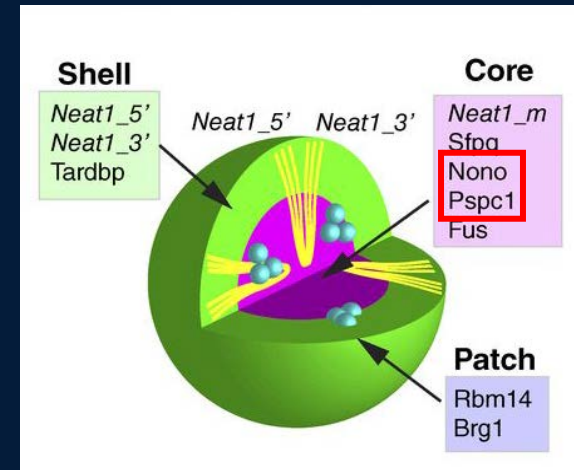
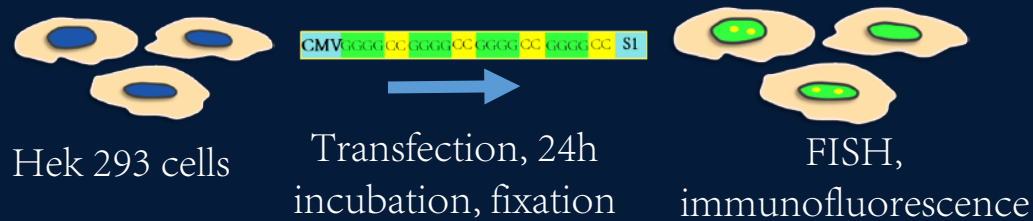
Paraspeckles

- 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.**



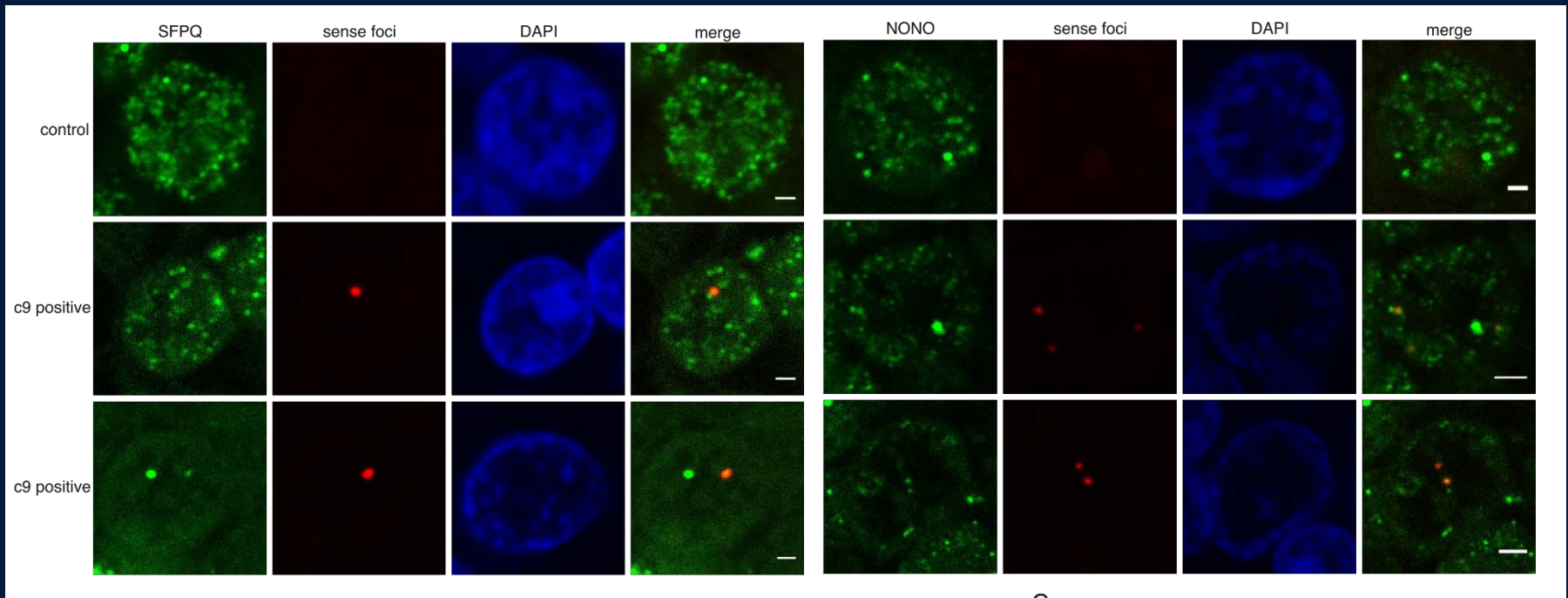
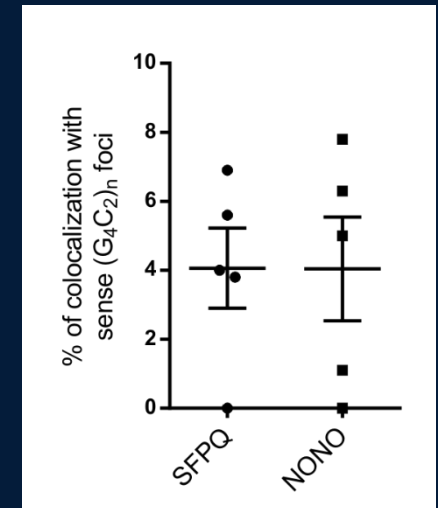
- More than **40 proteins** associate with paraspeckles,
- PSPC1 was the first identified protein,
- NONO and SFPQ are essential for the paraspeckle formation.

NONO and PSPC1 also co-localize with G₄C₂ RNA foci

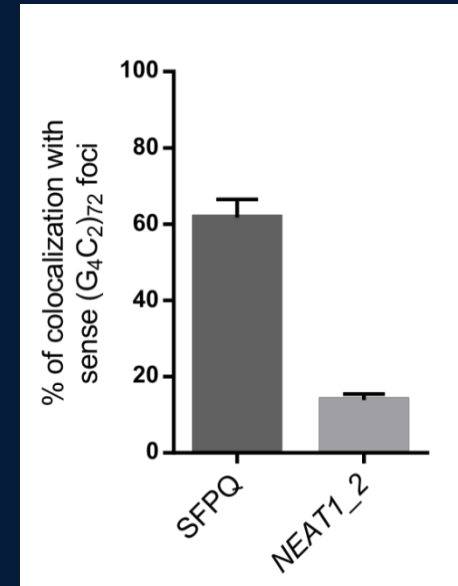
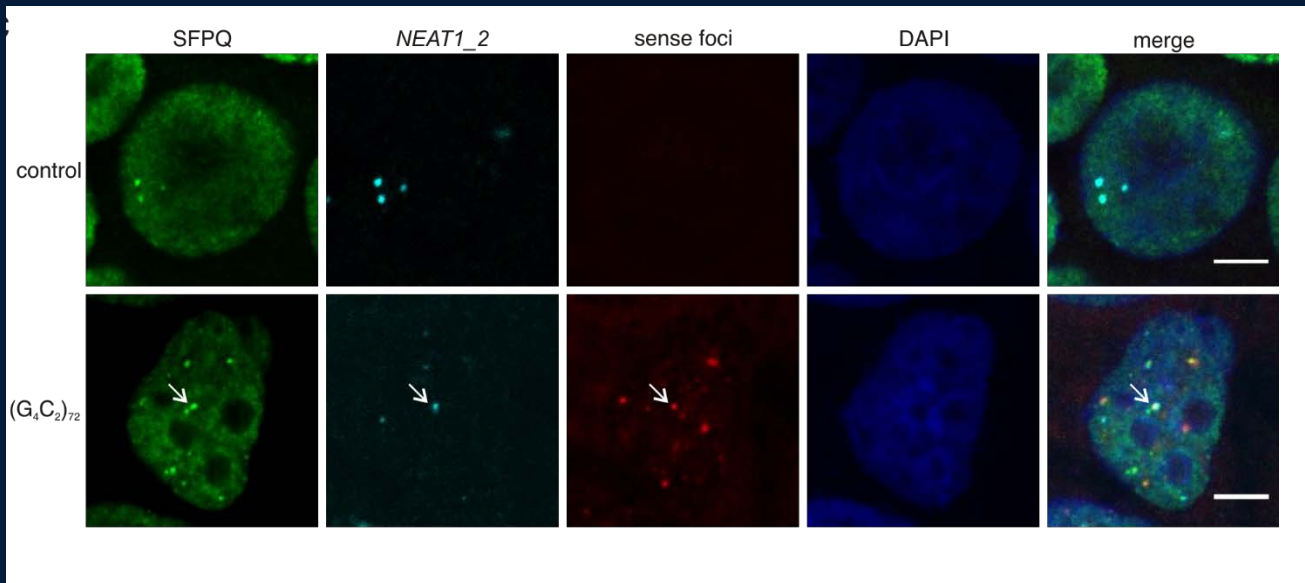
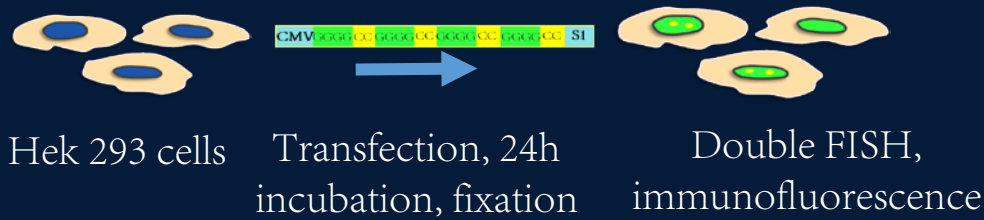


SFPQ and NONO co-localize with RNA foci in C9orf72 positive cerebellum

- 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**.

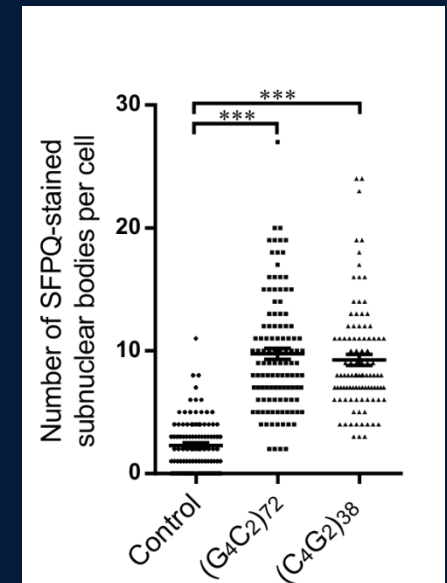
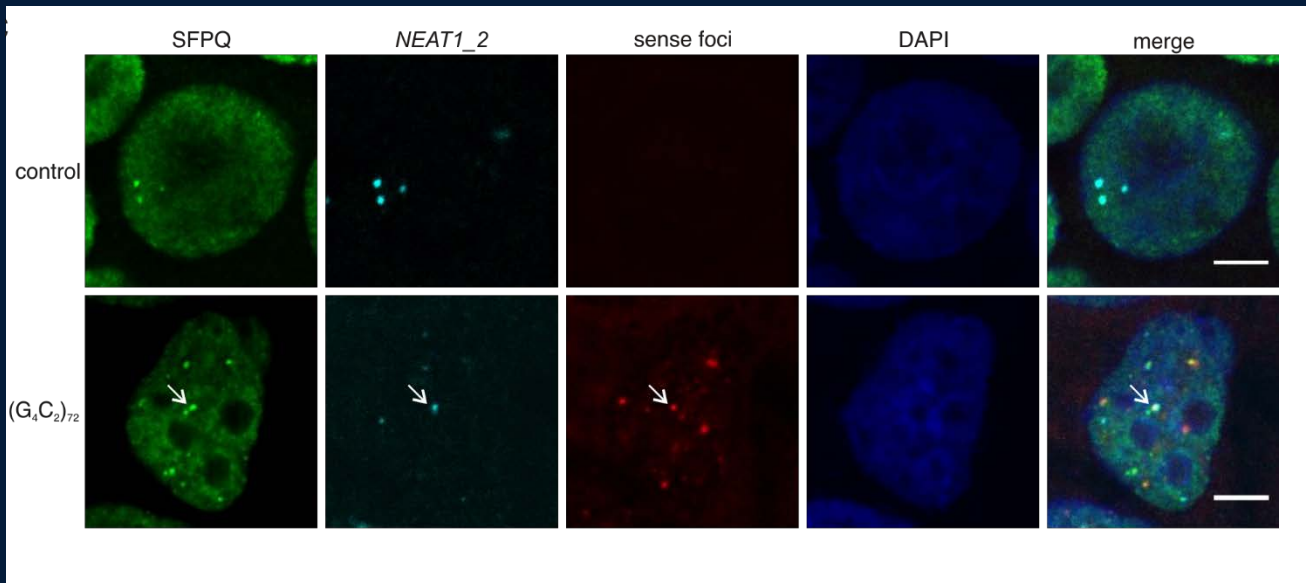


Do G_4C_2 RNA nuclear foci associate with NEAT1 as well?



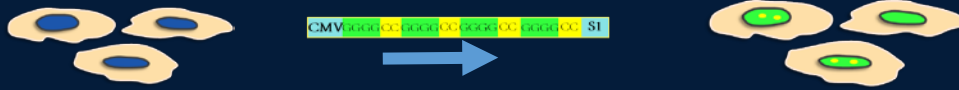
- Yes, 14%
- However, additional NEAT1-negative, SFPQ-stained subnuclear bodies in cells expressing G_4C_2 repeats.

Increase in SFPQ-stained subnuclear bodies in G₄C₂ cells

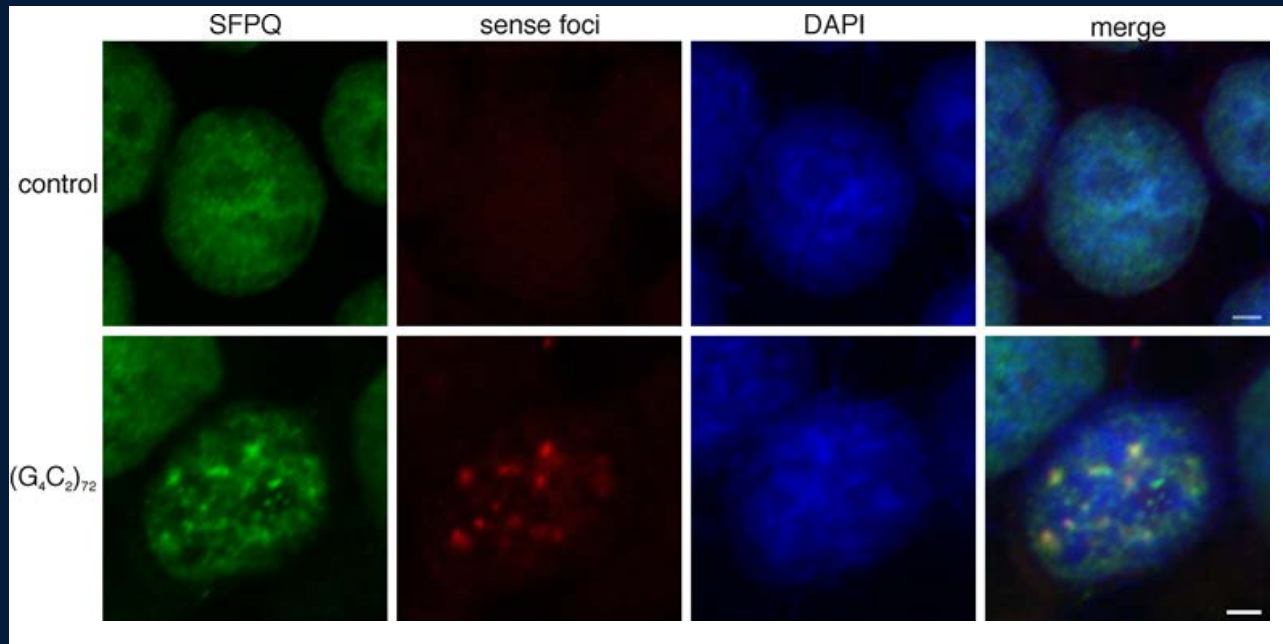
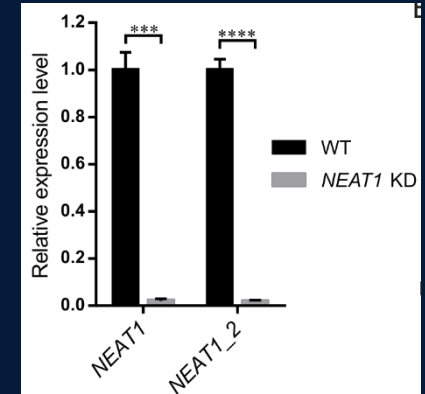


- The average number of SFPQ-stained subnuclear bodies per cell increased from 2.3 for mock transfected cells to 9.8 for cells expressing G₄C₂ repeats,
- Could NEAT1 be replaced by G₄C₂ repeats as a structural backbone for the paraspeckles?

SFPQ colocalizes with G_4C_2 nuclear foci in NEAT1 knockdown HEK293T cells



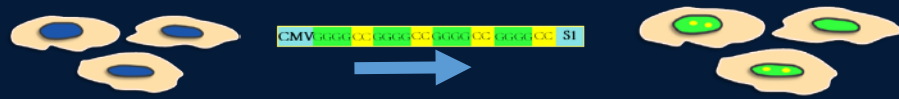
NEAT1 knockdown Transfection, 24h
Hek 293 cells incubation, fixation FISH, immunofluorescence



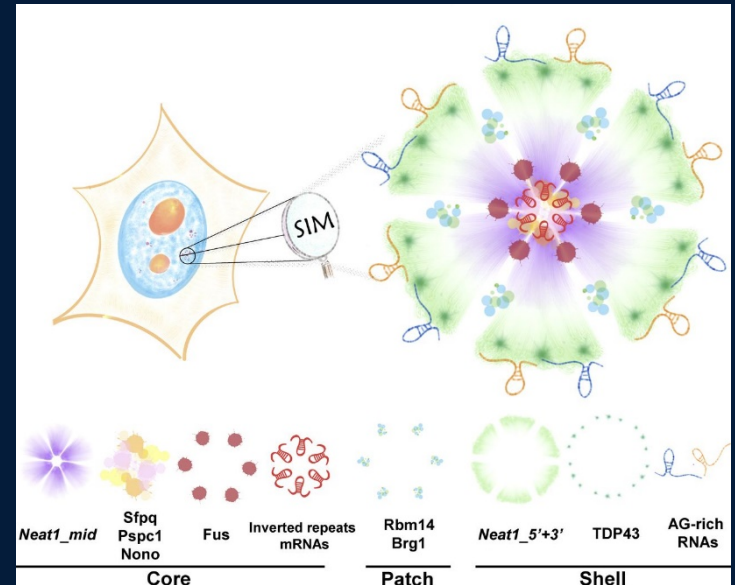
- Localization of paraspeckle proteins in G_4C_2 foci is a **NEAT1-independent event!**

G₄C₂ RNA foci co-localize with hLincRNA-p21

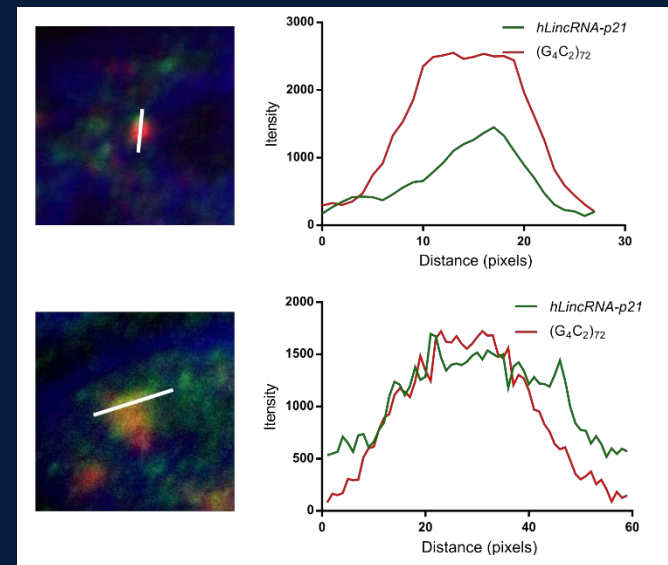
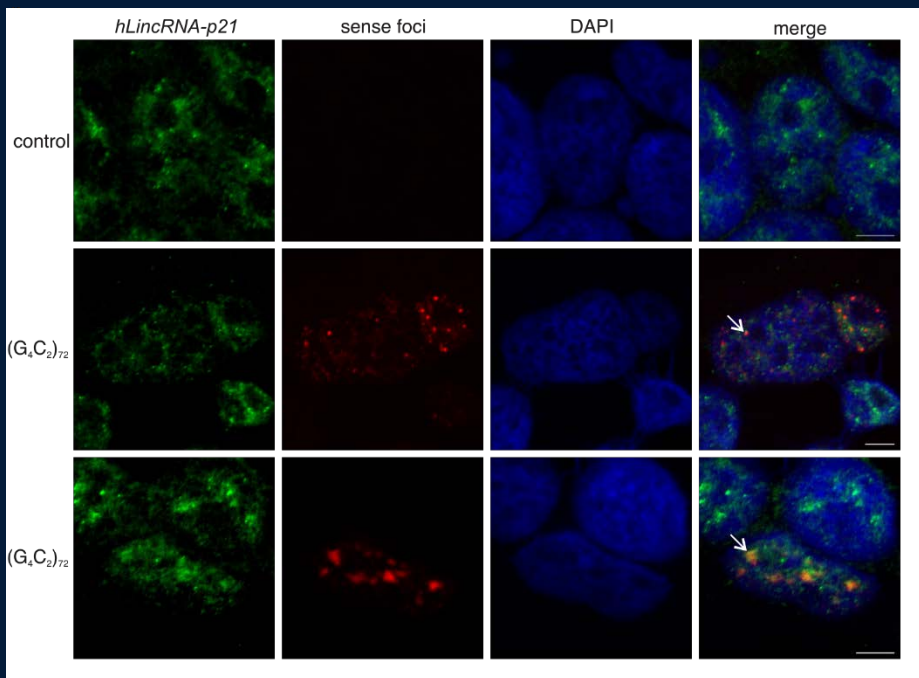
- Transcripts of genes with **inverted repeat** Alu elements associate with paraspeckles,
- Human LincRNA-p21 has an IRLu element leading to **co-localization with paraspeckles**.



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



Paraspeckle structure, Hu *et al. JCB*,
2016



Conclusions

- G_4C_2 foci associate with paraspeckle proteins,
- The association is independent of NEAT1,
- G_4C_2 paraspeckle-like structures associate with Alu-repeat RNAs.

Acknowledgements

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