Can a single celled Yeast be a model organism for neurodegenerative disease?

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Overview

Motor Neuron Disease (MND) is a neurodegenerative disease. MND affects 1:400 people in the UK. Pathogenicity manifests as an initial loss of muscle strength in the limbs, before an overall loss of motor functions resulting in progressive paralysis and death. Investigating the molecular mechanisms behind this disease may allow us to identify therapeutic target.

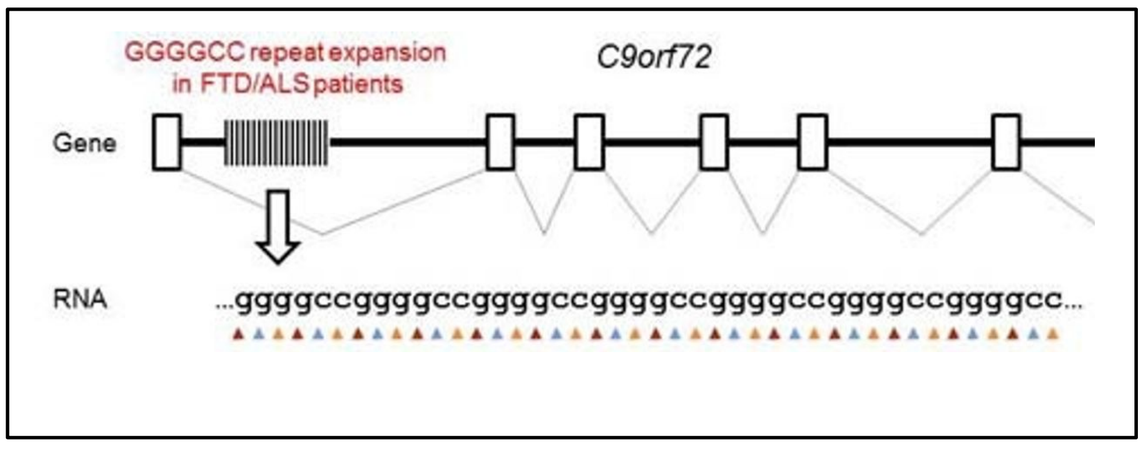
Aims

- Can Yeast be used as a model organism for MND?
- Does insertion of the MND mutation into yeast cells result in the production of neurodegenerative causing agents, short polypeptides known as Dipeptide Repeats?
- Can we rescue MND yeast to identify a novel therapeutic target?

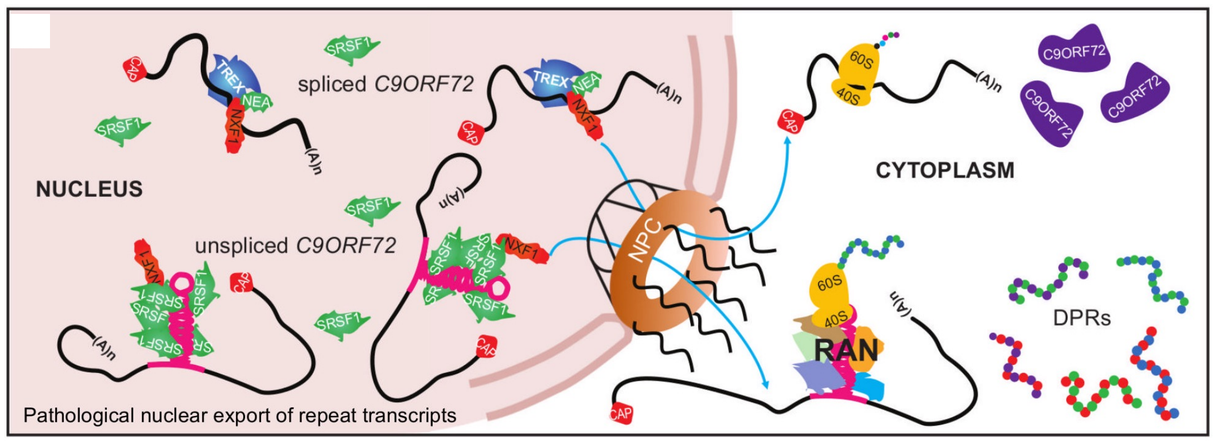
Scanning Electron Micrograph of Saccharomyces cerevisiae- yeast.

Background information

C9ORF72 has been widely associated with MND. This is a gene of unknown function located on chromosome 9. Within Intron 1 of C9ORF72 there is a repeat sequence GGGGCC- (G4C2). In MND patients this sequence can be repeated up to 1000 times compared to non-MND patients with 3-6 repeats.



C9orf72- showing the MND associated G4C2 repeat expansion in intron 1; Image taken from Neurowiki 2014



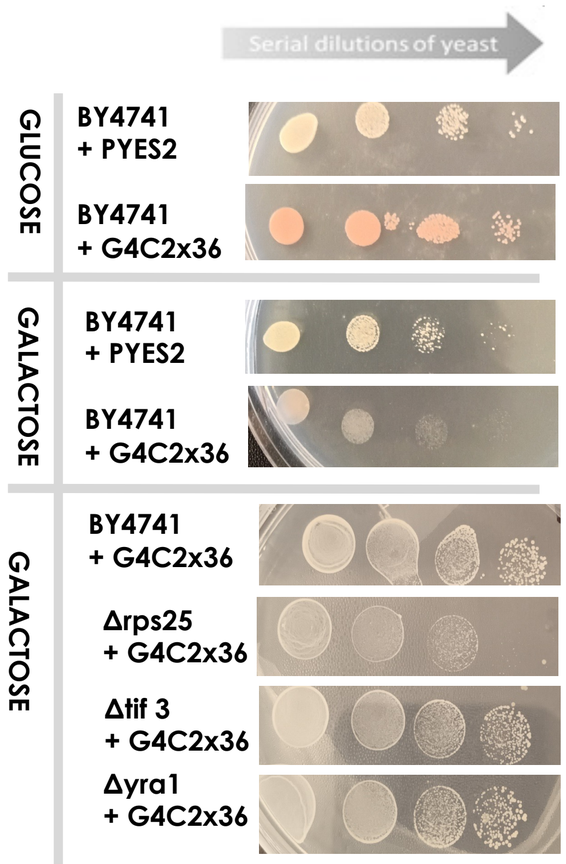
Mechanism for the mislocalisation of C9orf72 mRNA by SRSF1 into cytoplasm and subsequent translation of the MND causing DPRs (1).

The accepted mechanism for MND pathogenicity is the mislocalisation of this gene's RNA to the cytoplasm and subsequent RAN translation into short cytotoxic DPRs. The laboratory of G. Hautebergue (2017) have characterised a nuclear export factor SRSF1 in this process. Removal of SRSF1 from cells and in insect models rescues the detrimental DPR phenotype in these systems.

Results:

DPR toxicity phenotype in the presence of the MND mutation.

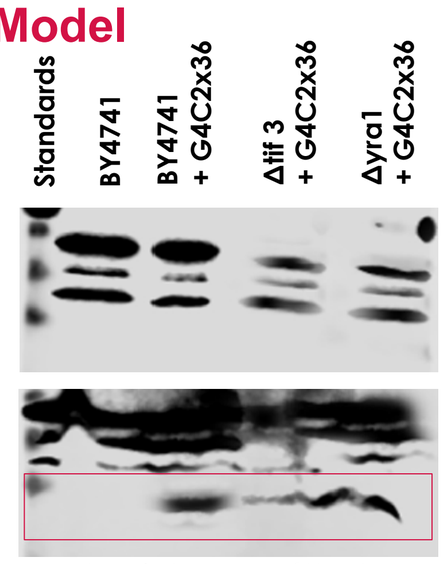
The G4C2x36 DNA sequence was inserted into yeast using the GAL promoter controlled -pYES2 expression plasmid. A reduced growth phenotype upon expression of the MND mutation in the wild type (WT) strain BY4741 demonstrates yeast as a viable model for MND. Yeast strains deleted for Nuclear shuttling proteins TIF3 and YRA1 as well as RPS25 (control) [2] were transformed with the G4C2x36 MND sequence, however analysis by growth phenotype was not possible.



Yeast growth assays demonstrating a reduced growth phenotype in the strains expressing MND repeat

DPRs detectable in Yeast Model

Yeast transformants were grown in Galactose to induce transcription of MND RNA. Protein was extracted and separated on a gel. Antibodies were used to detect the V5 tag included in the G4C2x36 DNA sequence.



Western Blot analysis of Yeast cell lysates, Anti V5 Tag antibody.

Despite the background signal in cell lysate DPRS were detected in the cell pellet (lower panel red box) but absent in the WT strain not expressing MND mutation.

Conclusion

We have demonstrated that Yeast can be a model for MND, using this yeast expression system for the MND repeat.

Our next steps are to perform the same investigation in the background of other nuclear export factor delete strains looking for the loss of the DPR and identifying a potential therapeutic target.

References

1) Hautbergue, G., Castelli, L., Ferraiuolo, L. et al. SRSF1-dependent nuclear export inhibition of C9ORF72 repeat transcripts prevents neurodegeneration and associated motor deficits. Nat Commun 8, 16063 (2017).

2) Yamada SB, Gendron TF, Niccoli T, et al. RPS25 is required for efficient RAN translation of C9orf72 and other neurodegenerative disease-associated nucleotide repeats. Nat Neurosci 22, 13383 (2019)4C2x36 DNA sequence was inserted in to yeast using the GAL promoter controlled.

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